GREWIA POLYSACCHARIDE GUM AS A PHARMACEUTICAL EXCIPIENT

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Thesis Summary

Grewia gum is obtained from the inner stem bark of the edible plant *Grewia mollis* Juss (Fam. Tiliaceae) which grows widely in the middle belt region of Nigeria, and is also cultivated. The dried and pulverised inner stem bark is used as a thickening agent in some food delicacies in that region of the country. This ability of the material to increase solution viscosity has generated a lot of interest and is the catalysing momentum for this research. Such materials have been used as stabilizers or suspending agents in cosmetics, foods and liquid medications, and as mucoadhesives and controlled release polymeric matrices in solid dosage forms.

The physicochemical characterization of candidate excipients forms an essential step towards establishing suitability for pharmaceutical application. For natural gums, this usually requires isolation of the gum from the storage site by extraction processes. Grewia polysaccharide gum was extracted and dried using techniques such as air-drying, freeze-drying or spray-drying.

Component analysis of the gum showed that it contains five neutral sugars: glucose, galactose, rhamnose, arabinose and xylose. The gum contains traces of elements such as zinc, magnesium, calcium and phosphorus. At low substance weight, the gum hydrates in aqueous medium swelling and dispersing to give a highly viscous dispersion with pseudoplasmic flow behaviour.

The method by which drying is achieved can have significant effect on some physicochemical properties of the gum. Consequently, the intrinsic viscosity and molecular weight, and parameters of powder flow were shown to differ with the method of drying. The gum has good thermal stability. In comparison with established excipients, grewia gum may be preferable to gum Arabic or sodium carboxymethylcellulose as a suspending agent in ibuprofen suspension formulations. The release retardant property of the gum was superior to guar and Metolose® in ibuprofen matrices. Similarly, carboxy methylcellulose, Methocel®, gum Arabic or Metolose® may not be preferable to grewia gum when controlled release of a soluble drug like cimetidine is indicated. The mucoadhesive performance of the gum compared favourably with excellent mucoadhesives such as hydroxypropyl methylcellulose, carboxymethylcellulose, guar and carbopol 971P.

Key words: *Grewia gum, suspending agent, mucoadhesive, release-retardant*

To my children

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List of Abbreviations

AAS	Atomic Absorption Spectroscopy
ACA	Acacia gum (gum Arabic)
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
API	Active pharmaceutical ingredient
BP	British Pharmacopoeiea
CBP	Carbopol
CMC	Carboxymethyl cellulose
COSY	Homonuclear Correlated Spectroscopy
COX	Cyclooxygenase
DSC	Differential scanning calorimetry
ESCA	Electron Spectroscopy for Chemical Analysis
FAB-MS	Fast Atom Bombardment Mass Spectroscopy
FDA	Food and Drug Administration
FDGG	Freeze-dried grewia gum
FT-IR	Fourier transformed IR
GC	Gas Chromatography
GFC	Gel Filtration Chromatography
GPC	Gel Permeation Chromatography
HBS	Hydrodynamically balanced systems
HPLC	High Performance Liquid Chromatography
HPMC	Hydroxypropyl methylcellulose
IR	Infra-red Spectroscopy
NMR	Nuclear Magnetic Resonance
NSAIDS	Non-steroidal anti-inflamatory drugs
MC	Methylcellulose
MCC	Microcrystalline cellulose
MD	Multi-dimentional
Mn	Number average molecular weight
Mv	Viscosity average molecular weight
Mw	Weight average molecular weight
Mz	Z-average molecular weight

MALDI-MS	Matrix-assisted Laser Desorption Ionization Mass spectroscopy
MALDI-TOF	Matrix-assisted laser Desorption Ionization Time of Flight
MW	Molecular weight
PEG	Polyethylene glycol
PGH_2	Prostaglandin H_2
PVDF	Polyvinylidene fluoride
pKa	Dissociation constant
RF	Response Factor
SCMC	Sodium carboxymethylcellulose
SDGG	Spray-dried grewia gum
SEC	Size Exclusion Chromatography
SEM	Scanning Electron Microscopy
TFA	Trifluoroacetic acid
Tg	Glass Transition Temperature
TGA	Thermogravimetric Analysis
TMS	Trimethylsilyl
UPGG	Unprocessed grewia gum
USP	United States Pharmacopoeiea
UV	Ultra-violet
VNMRS	Varian NMR Spectrometer
XAN	Xanthan gum
XPS	X-ray Photoelectron Spectroscopy

CHAPTER ONE

INTRODUCTION

1.1 POLYSACCHARIDES AND NATURAL GUMS

"Polysaccharide" is a general term given to high molecular weight substances which contain a large number of structural units called monomers or glycoses (monosaccharides) joined by O-glycosidic linkages (Yalpani,1988). The monomers joined by linkages often form into long repeating units or polymer chains.

The polysaccharides are also known as carbohydrate polymers. They have been described as the most abundant, versatile and diverse class of organic compounds occurring in nature (Yalpani, 1988; Izydorczyk, 2005). Polysaccharides or their derivatives which, when dispersed in hot or cold water at low dry-substance content, swell to produce gels, highly viscous dispersions or solutions, have been classified as gums (Glicksman and Schachat, 1959; Wallis, 1967; Mozumder *et al.*, 2004). These include the water-insoluble or water-swellable derivatives of cellulose and the derivatives and modifications of other polysaccharides which in their natural forms are insoluble. They also include polysaccharides and their derivatives which are slimy or mucilaginous.

Natural gums are also categorised as high molecular weight plant carbohydrates (Izydorczyk, 2005). They are normal products of cell metabolism laid down in the same fashion as hemicellulose. While plant gums could be intracellular in origin (Wallis, 1967), changes in the existing cell wall due to stress, including physical injury or unfavourable conditions of growth such as fungal attack, also result in gum formation as exudates through a process called gummosis.

1.2 SOURCES OF NATURAL OR POLYSACCHARIDE GUMS

The natural or polysaccharide gums occur in nature as storage materials, cell wall components or exudates from shrubs, trees or low-growing plants and as extracellular substances from plants or microorganisms (Whistler, 1959; Izydorczyk *et al.*, 2005).

1.2.1 Exudate gums

Plants, which form the sources of exudate gums, when cut give a viscous, sticky fluid which exudes from the incision and tends to cover and seal the opening. Gum exudates are therefore believed to be produced by plants in order to seal-off infected sections of the plant and prevent loss of moisture due to physical injury or fungal attack (Izydorczyk *et al.*, 2005). The fluid eventually dries to a brittle, translucent, glassy, hard mass. These gum exudates are secreted by various organs of the plant.

Tragacanth gum is obtained as an exudate from the root of the plant *Astragallus gummifer* (Fam. Leguminosae). Acacia gum or gum Arabic is the dried gummy exudate from the stems and branches of *Acacia senegal* and other related African species of acacia (Fam. Leguminosae). Other sources of exudate gums include gum karaya from *Sterculia urens* tree (Fam. Sterculiaceae) and gum ghatti which forms an amorphous transluscent exudate from *Anogeissus latifolia* tree (Fam. Combretaceae) grown in India.

1.2.2 Mucilage gums

Some gums are not exuded from the plant upon incision but rather are obtained by extraction from various plant parts such as the seeds or soft stems of the plant. Such plant gums are described as intracellular or mucilage gums (Izydorczyk *et al.*, 2005).

Many seeds contain polysaccharide food reserves which produce intracellular seed gums usually obtained by extraction from the seeds. Guar gum is obtained from the ground endosperms or seeds of the plant *Cyamopsis tetragonolobus* (Fam. Leguminosae). Locust bean gum is obtained from the endosperms of the hard seeds of the locust bean tree (Carob tree), *Ceratonia siliqua* (Fam. Caesalpiniaceae) (Parija *et al.*, 2001). Other sources of seed gums include mucuna gum from the seeds of *Mucuna flagillepes* (Udeala and Chukwu, 1985), psillium gum from the dried ripe seeds of *Plantago afra* (Fam. Plantaginaceae) (Tyler *et al.*, 1981), tamarind gum obtained from the endosperm seeds of the tamarind tree, *Tamarindus indica* (Fam. Fabaceae) (Parija *et al.*, 2001), durian seed gum obtained from the seeds of the seasonal fruit *Durio zibethinus* (Fam. Malvaceae) grown in South East Asia (Amin *et al.*, 2007), okra mucilage from *Hibiscus esculentus* (Fam. Malvaceae) (Agarwal *et al.*, 2001), yellow mustard (from *Sinapis alba* Fam. Brasiccaceae), flax mucilage (from *Linum usitatissimum* Fam. Linaceae) and quince seed gum obtained from the seeds of *Cydonia oblonga* (Fam. Rosaceae) (Bhardwaj *et al.*, 2000; Parija *et al.*, 2001). Yellow mustard mucilage is extracted from whole mustard seeds or from the bran (Izydorczyk *et al.*, 2005).

Plant gums have also been extracted from roots and tubers of certain plants. Konjak manneri gum is extracted from the corms and tubers of *Amorphophallus konjak* (Fam. Ulmaceae), and marshmallow gum from the roots of the perennial herb, *Althaea officinalis* (Fam. Malvaceae).

1.2.3 Seaweed polysaccharides

Seaweed gums are typified by the carrageenans, agar and the alginates. The carrageenans are marine hydrocolloids obtained by extraction from seaweeds of the class Rhodophyceae, represented by *Chondrus crispus, Euchema spinosum, Gigartina skottsbergi,Gigartina stellata, Iradaea laminariodes*. These are red seaweeds growing abundantly along the Atlantic coasts of North America, Europe and the western Pacific coast of Korea and Japan (Izydorczyk *et al.*, 2005).

Gum agar is extracted from the red-purple marine algae of the Rhodophyceae class. The species include *Gelidium cartilagineum* and *Gracilaria confervoides* which grow abundantly in the waters along the coast of Japan, New Zealand, South Africa, Southern California, Mexico, Chile, Morocco, and Portugal (Bhardwaj *et al.*, 2000; Parija *et al.*, 2001; Izydorczyk *et al.*, 2005).

The alginates are the primary structural polysaccharides isolated from the brown seaweeds of the family Phaeophyceae. The species include *Macrocystis pyrifera, Laminaria hyperborean, Laminaria digitata* and *Laminaria japonica. Microcystis pyrifera* grows primarily along the coasts of Australia, New Zealand and the California coast of the USA (Izydorczyk *et al.*, 2005). The alginates have also been synthesised by bacteria such as *Pseudomonas aeruginosa* and *Azobacter vinelandii.*

1.2.4 Microbial polysaccharides

Natural polysaccharide gums have also been obtained as carbohydrate fermentation products including Xanthan gum, produced in pure culture fermentation by the bacteria *Xanthomonas campestris*. It was originally obtained from the rutabaga plant (Parija *et al.*, 2001). Gellan gum is a microbial polysaccharide obtained by fermentation by *Pseudomonas elodea* (Whistler and Smart, 1953; Parija *et al.*, 2001). Pullulan is an extracellular homopolysaccharide of glucose produced by many species of the fungus *Aureobasidium*, specifically *A. pullulans.*

1.2.5 Animal polysaccharides

Natural gums have also been obtained from animal sources. Examples include chitin and chitosan. Chitin is a structural polysaccharide which takes the place of cellulose in any species of lower plants and animals. It therefore occurs in fungi, yeast, green, brown and red algae and form the main component of the exoskeleton of insects and shells of crustaceans (Izydorczyk *et al.*, 2005). Chitin is insoluble in water but when

treated with strong alkali, it forms the water-soluble polysaccharide chitosan which is the only polysaccharide carrying a positive charge (Izydorczyk *et al.*, 2005).

1.2.6 Less known sources of polysaccharide gums

Other sources of polysaccharide gums have been reported (Smith and Montgomery, 1959; Bhardwaj *et al.*, 2000; Parija *et al.*, 2001; Femi-Oyewo *et al.*, 2004). Kendu fruit gum is obtained from *Dyspyros cardifolia* (Fam. Ebenceae), a tree grown in tropical countries like India (Parija *et al.*, 2001), pectins are obtained from plant cell walls, and scleroglucan, a natural exocellular polysaccharide, is secreted by fungi of the genus *Sclerotium rolfsii* (Bhardwaj *et al.*, 2000). Mesona blumes gum is extracted from the leaves of Liangfen Cao, also called Hsian tsao. Liangfen cao is a herb found growing in south China, Indonesia, Vietnam and Burma (Feng *et al.*, 2007). Mesquite gum from *Prosopis julifora* (Fam. Leguminosae) and gums from *Cissus populnea* of the family ampelidaceae are also intracellular gums obtained from the stem bark of the plants.

1.3 CLASSIFICATION OF NATURAL GUMS

Natural gums have been classified in various ways based on source, chemical composition and structure, physical properties or whether they are acidic or neutral (Whistler and Smart, 1953; Smith and Montgomery, 1959; Izydorczyk, 2005).

The classification of natural gums based on source has been discussed in the previous section (see section 1.2).

Classification of plant gums according to their chemical composition and structure is based on the hydrolytic nature of the product. Those gums hydrolysing to only a single monosaccharide type are classified as homoglycans while those hydrolysing into two monosaccharide species are classified as di-heteroglycans. The antigenic polysaccharide produced by *Eubacterium saburreum*, strain T17, is a homoglycan composed of d-*glycero*-d-*galacto*-heptose residues (Nazakawa, 1985). Plant gums containing three, four and five different monosaccharide residues are classified as tri-, tetra-, and penta-heteroglycans respectively (Smith and Montgomery, 1959; Izydorczyk, 2005).

Polysaccharides have also been classified based on the arrangement of the different monomers present. They may be linear, branched or cyclic. This classification is related to the physical properties of the gum. Linear polysaccharides do not dissolve in

water but swell to form thick gels and give translucent viscous solutions. The linear polysaccharides are packed closely and form many intermolecular secondary attachments which make the structure strong, rigid and insoluble. They are therefore associated with structural material in those plants in which they occur (Berger, 1962; Mithal and Khasgiwal, 1972). Examples include cellulose, xylan and chitin. Branched polysaccharides on the other hand, generally dissolve in water to give clear solutions with adhesive properties (Smith and Montgomery, 1959). The commonly branched polysaccharides are those which serve as food reserves such as glycogen and dextran. Pullulan is a branched polysaccharide obtained from the fungus *Aureobasidium pullulans*.They are easily soluble in water and have immense thickening properties (Whistler and Smart, 1953). The cyclic polysaccharides are also known as cycloamyloses.

Polysaccharides have been classified based on the number of the different monomers present as either homopolysaccharides (examples include pullulan, starch, cellulose and glycogen) or heteropolysaccharides (examples include xanthan gum which consists of trisaccharide chains, guar gum made of mannose and galactose and acacia gum) (Apinall, 1972). While the homopolysaccharides consist of only one type of monosaccharide, the heteropolysaccharides consist of two or more types of monosaccharide units joined by O-glycosidic linkages (Izydorczyk, 2005). The homopolysaccharides are further classified based on the types of glycosidic linkages which join the monosaccharide units.

A classification of gums which accommodates all the natural gums is that based upon whether the gum is acidic, neutral or basic. Acidic polysaccharide gums contain sugar acids in their structure and carry negative charges. Examples include xanthan gum, gum acacia, and tragacanth gum. They are said to be anionic polysaccharides and carry a negative charge (Izydorczyk, 2005). Polysaccharide gums which consist of sugar units only are known as neutral polysaccharides. Most seed gums containing galactose and mannose (galactomannan) are neutral gums (Cunha *et al.*, 2009). Chitosan obtained by modification of the naturally occurring animal polysaccharide chitin is the only example of a cationic polysaccharide (Izydorczyk, 2005) and is an example of a polysaccharide with basic groups (Whistler and Smart, 1953; Smith and Montgomery, 1959).

1.4 COMPOSITION OF NATURAL GUMS

Natural gums are chain macromolecules which are highly complex polymers usually composed of different monosaccharide residues joined together by glycosidic bonds. The monosaccharide residues may be hexose, pentose, and/or sugar acid units. The most common hexoses found in natural gums are glucose, galactose, rhamnose and mannose. The pentoses include arabinose and xylose, while glucuronic, galacturonic and mannuronic acids constitute the sugar acids frequently isolated from plant gums (Smith and Montgomery, 1959; Berger, 1962; Kharbade and Joshi, 1995; Bhardwaj *et al.*, 2000; Parija *et al.*, 2001). Figure 1.1 shows the molecular structures of some monosaccharides and sugar acids present in polysaccharides.

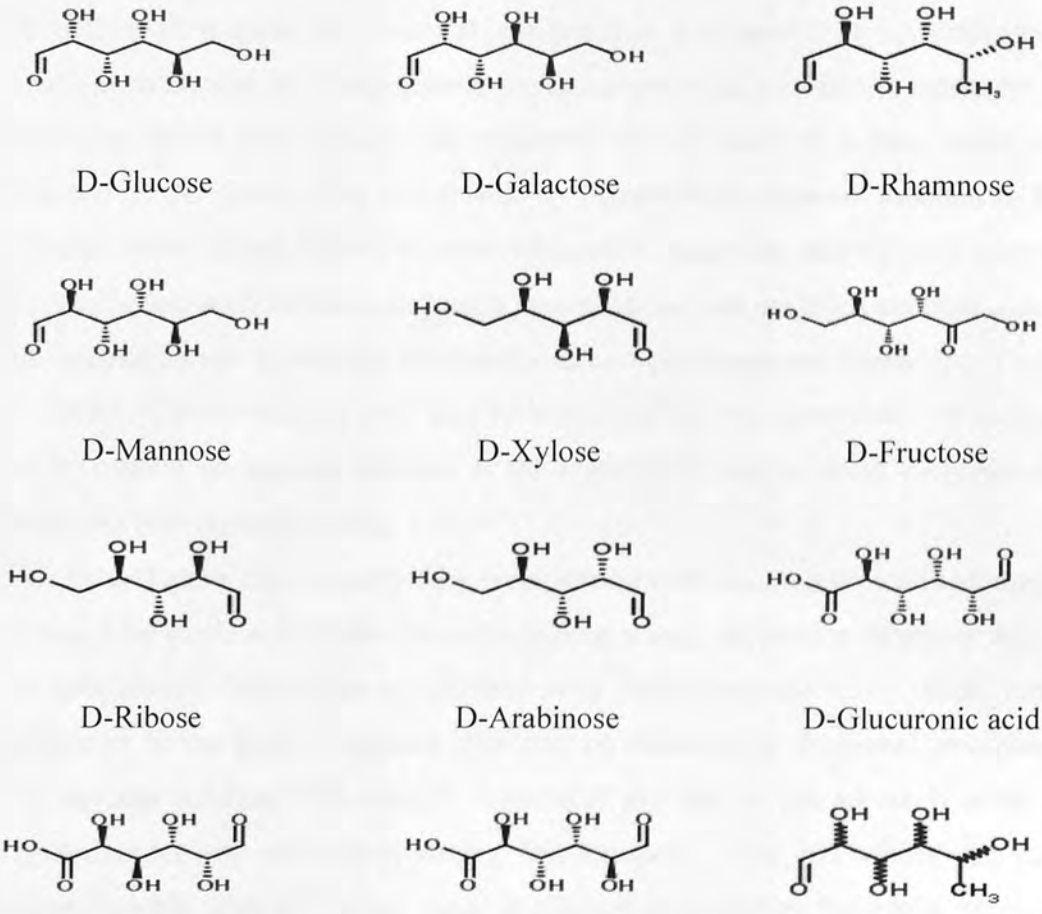


Figure 1.1: Molecular structure of some monosaccharides and sugar acids

Lipids, lignin, proteins, salts, colouring matter and other substances may be associated with natural gums. The composition of natural gums may be achieved by degradation

of the polysaccharide to the constituent monosaccharide units before analysis. This degradation process has been achieved in a number of ways: controlled acid hydrolysis of the gum in mineral acid (such as HCl or H_2SO_4) or in organic acid such as trifluoroacetic acid (TFA), methanolysis, and also by acetolysis of the polysaccharide under the influence of heat (Cui, 2005). Usually polysaccharides with sugar acids present some difficulty in hydrolysis because the glycosidic linkages of the uronic acids are highly resistant to hydrolysis. In such instances the stronger mineral acids are the choice for hydrolysis. The components of the hydrolysate can be separated and detected by chromatographic techniques.

1.5 EXTRACTION AND PURIFICATION OF NATURAL GUMS

The source of a gum, its chemical composition and nature of contaminants or impurities determine, to a large extent, the technique employed for the extraction and purification of the gum. Usually, the extraction process involves milling of the crude dried part of the plant. This is followed by dispersion in aqueous medium to form mucilage which is then filtered to remove insoluble impurities and the gum recovered by precipitation with alcohol. Repeated precipitation from acidified aqueous solution with ethanol serves to remove inorganic ions and proteinaceous impurities (Yang *et al.*, 2006). Electro-dialysis may also be employed for the elimination of inorganic ions by passing an aqueous solution of the material through a cation exchange resin (Smith and Montgomery, 1959).

Some natural gums can be purified by precipitation from aqueous or acidified aqueous solution with acetic acid while others containing a large proportion of uronic acid are best precipitated from aqueous solution with dilute mineral acid. When further purification of the gum is required, this may be achieved by fractional precipitation from aqueous solution with ethanol. Levels of pH that do not adversely affect the polysaccharides are maintained during fractionation. Low pH values can cause hydrolysis while high pH values result in alkaline degradation. Separation of natural gums may also be partially achieved by successive or repeated extraction with solvents of increasing efficacy. Successive extraction with cold water, hot water, cold dilute alkali or hot dilute alkali can give fractions of different compositions and properties (Smith and Montgomery, 1959).

Natural gums that have undergone purification are usually dried by solvent exchange or by freeze-drying. Such procedures produce light amorphous powders that are reactive. When gums containing moisture are dried by methods other than this, they form hard, horny masses that are difficult to pulverise and study (Smith and Montgomery, 1959). Other methods for purification of plant polysaccharides include ultra filtration, gel filtration, column chromatography and electrophoresis (Smith and Montgomery, 1959; Flindt *et al.*, 2005; Mortlagh *et al.*, 2006).

1.6 PHYSICAL PROPERTIES OF NATURAL GUMS

The physical properties of natural gums including solubility, flow behaviour, viscosity or gelling potential, and /or surface and interfacial properties are dictated by the diversity of structural features of polysaccharides. This great diversity originates from differences in the monosaccharide composition, linkage types and patterns, chain shapes and the degree of polymerization (Cui, 2005). It is this structural diversity that dictates the functional properties exhibited by the different natural polysaccharide gums.

1.6.1. Viscosity and flow behaviour of polymer solutions or dispersions

One of the most important physical characteristics of polysaccharide gums that has been extensively studied is viscosity. Viscosity is the measure of the resistance of a fluid to flow or the measure of the internal friction of the fluid. Polysaccharide gums dramatically increase solution viscosity at relatively low substance weight or concentration. The viscosity of polysaccharide solutions or dispersions is dependent upon the molecular weight, temperature, shear rate and the concentration of the polysaccharide (Cui and Wang, 2005). Viscosity is integral to the understanding of the molecular structure of these plant gums and also in their utilisation.

Most natural gums form very viscous solutions at low or high substance weight. High viscosity is important in the production and stabilization of emulsions and suspensions. Factors which affect the viscosity of plant gums include-concentration of the polymer, temperature, presence of electrolytes, pH, organic solvents, ageing and mechanical treatment (Whistler and Smart, 1953). The viscosity of most natural gum solutions increases as the concentration of the polymer increases. As the temperature of a polymer solution increases, both viscosity and density of the solutions decreases (Whistler and Smart, 1953; Stephens, 1990; Cui and Wang, 2005). The addition of

electrolytes to dilute solutions of plant gums generally decreases viscosity (Whistler and Smart, 1953; Carter, 1972). This decrease in viscosity is proportional to the increase in the valency of the cation or the increase in the concentration of electrolyte. The addition of more than one electrolyte gives an additive effect. The lowering of viscosity is more pronounced in concentrated solutions than in dilute solutions of the polymer. In the preparation of emulsions for example, the lowering of viscosity and interfacial tension produces favourable emulsifying conditions (Stephens, 1990).

The viscosity of natural gums is also affected by pH (Whistler and Smart, 1953). Acacia gum, for example, is most viscous between pH of 4 and 6 whereas tragacanth gum is most viscous at pH 8. Increases in pH result in a fall in viscosity until a certain maximum value. With the addition of organic solvents such as acetone or ethanol, the relative viscosity of plant gums decreases slowly, becoming more significant as the concentration of the solvent increases. At a certain concentration of the solvent, the gum will precipitate (Cui and Wang, 2005). Solvents such as glycerol, propylene glycol and polyethylene glycol are usually miscible with most plant gums and act essentially as diluents and/or plasticizer.

Mathematically, viscosity (η) is defined as the ratio of applied shearing stress (τ) to rate of shear strain ($\frac{d\gamma}{dt}$) or simply shear rate ($\dot{\gamma}$) and is given by the equation:

$$\eta = \frac{\tau}{d\gamma/dt} = \frac{\tau}{\dot{\gamma}} \qquad \text{---------- } \textit{(Equation 1.1)}$$

The amount of force called shear, required to overcome the internal friction and cause the fluid to move in relation to another body is dependent on the magnitude of the internal friction. Highly viscous fluids therefore require more shear to move than less viscous materials (www.brookfieldengineering.com/education/what-is-viscosity-asp).

1.6.1.1 Newtonian fluids

When the viscosity of a material does not change irrespective of the shear rate at a constant temperature, in accordance with Newton's law, such a material is said to exhibit Newtonian flow and obey equation 1.1. Examples of fluids that exhibit Newtonian flow are water and thin motor oils. The viscosity of a Newtonian fluid will remain the same at constant temperature irrespective of the model of viscometer (www.brookfieldengineering.com/education/what-is-viscosity-asp)

1.6.1.2 Non-Newtonian Fluids

The relationship $\frac{d\gamma}{dt}$ is not a constant in non-Newtonian fluids. Such polymer solutions or dispersions do not obey equation 1.1. In these fluids the viscosity changes as the shear rate is varied and the model of viscometer, spindle number and speed (in the case of the Brookfield viscometer) all affect the measured viscosity. The measured viscosity is called the apparent viscosity of the fluid and is accurate only when the explicit experimental parameters are furnished and adhered to (Cui and Wang, 2005; www.brookfieldengineering.com/education/what-is-viscosity-asp). In these types of fluid when an increase in shear rate results in a decrease in apparent viscosity, the polymer solution or dispersion is said to exhibit a shear thinning flow behaviour. This phenomenon is also called pseudoplastic flow behaviour. Examples of pseudoplastic fluids are paints, emulsions and different types of dispersed systems. Conversely, when a polymer solution or dispersion shows an increase in viscosity with an increase in shear rate, it is said to exhibit a shear thickening behaviour also called dilatant flow behaviour. Dilatant flow is very often displayed by highly deflocculated systems such as clay slurries, candy compounds, corn starch in water, and sand/water mixtures. However, under static conditions, certain fluids behave as solids and force must be applied in order to induce flow. This force that must be applied in order to induce flow is called the yield value. In such systems the polymer solution or dispersion only starts to flow when the yield value is exceeded usually by shaking vigorously. Such fluids are said to exhibit plastic flow behaviour. Once the yield value is exceeded and flow begins, plastic fluids will exhibit Newtonian, pseudo plastic or dilatant flow characteristics (www.brookfieldengineering.com/education/what-is-viscosity-asp).

Under conditions of constant shearing, some fluids show a change in viscosity over time. Two categories are identifiable. If when shearing is constant over a period of time a fluid shows a decrease in viscosity, it is said to exhibit thixotropy. When a fluid's viscosity increases as a function of time under continuous shear at a constant rate, it is said to exhibit rheopexy.

The knowledge of a material's rheological characteristics is valuable in predicting pumpability and pourability performance in a dipping or coating operation, or the ease with which it may be handled, processed or used. It is also of value in predicting redispersibility of dispersed systems during storage.

1.6.2 Solubility characteristics and swelling behaviour

A wide range of solubility is displayed by plant polysaccharides. Some dissolve readily in cold water while some are only soluble in hot water, and some insoluble ones will not dissolve even in boiling water. For example xanthan gum dissolves in cold water, *Khaya grandifolia* (Fam. Meliaceae) gum dissolves only in hot water (Aslam *et al.*, 2006) while karaya gum will not dissolve even in boiling water. This behaviour is determined primarily by two factors: molecular structure and molecular weight of the plant gum or polysaccharide (Wang and Cui, 2005). Neutral polysaccharides are not as soluble as the charged polysaccharides while linear polysaccharides with high regularity in structure are essentially insoluble in aqueous media. Polysaccharides with regular conformation that can form crystalline or partial crystalline structures are usually insoluble in water. The irregularity of molecular structure prevents the formation of a closely packed structure and thus allows many polysaccharides to readily hydrate and dissolve when water is present. Generally, solubility increases with reduced regularity in molecular structure. Polysaccharides with high levels of branching or substitution of polysaccharide chains tend to show an increase in solubility due to the reduced possibility of intermolecular association. According to Wang and Cui (2005), any structures that contain especially flexible units such as (1-6) glycosidic linkages are more soluble because of large favourable entropy of the solution.

The dissolution rate for polysaccharide samples of similar particle sizes usually decreases with increasing molecular weight. This is because disentanglement from the particle surface and subsequent diffusion to bulk solution of large molecules takes a longer time compared to small molecules (Wang and Cui, 2005).

The process of dissolution of plant gums is sometimes complex and occurs in stages. When the polymer is introduced into the solvent, no obvious interaction may take place initially. Later, the polymer becomes swollen as the solvent molecules diffuse into it. Swelling is usually rapid for finely divided polymers and when the solvent is a small, compact molecule. This is usually the rate determining step in the dissolution process. The volume of the polymer phase increases as the solvent is imbibed, but few polymer molecules enter the solvent phase because of their low diffusion rates (Collins *et al.*, 1973). Finally, the swollen polymer particles disintegrate, and the individual polymer molecules diffuse apart until a true solution is formed. Agitation is

helpful in speeding up this final process. The higher the molecular weight of the polymer, the slower is this final stage in the dissolution process.

Up to 37% w/v of acacia gum dissolves in water at 25°C to form a clear solution whereas tragacanth gum forms a thick, viscous and mucilaginous semi-gel in water (Smith and Montgomery, 1959; Whistler and Smart, 1953). Some plant gums swell in water to form viscous dispersions while others are either completely water-soluble or water-insoluble. Upon addition of monovalent, divalent or trivalent metals, solutions of most plant gums form or produce precipitates. The stability of these polymer solutions is also affected by pH changes. The polymers are precipitated out of solution by high concentrations of organic solvents such as ethanol or acetone.

Swelling is an integral property requirement for efficient and effective disintegrant action and water penetration or swelling is now the most widely accepted mechanism for tablet disintegration (Hahm, 2000; Zhao and Augsburger, 2005). The ability of plant gums or polysaccharides to absorb water and swell is suggestive of good disintegrant action which is dependent upon the concentration of the gum used. Swelling has been described as either intrinsic or bulk swelling, both of which have been shown to be dependent on the pH and temperature of the environment and on the concentration of the polymer in question (Shangraw *et al.*, 1980; Chen *et al.*, 1997).

1.6.3 Molecular weight

Natural gums or polysaccharides are polymers of high molecular weight and they owe their unique properties to their size and asymmetry. Their physical properties are largely influenced by their molecular weight. Molecular weight therefore, represents a fundamental characteristic of the natural gums or polysaccharides (Wang and Cui, 2005).

Monosaccharide composition and the arrangement of these units in the polymer chain differ for each polysaccharide (see section 1.4). The chains of different numbers of monosaccharide units that make up the polysaccharides give each a distribution in molecular weight. As a result the molecular weights of polysaccharides are said to be polydispersed in nature. The molecular weight distribution for a given polysaccharide will vary depending on a number of factors such as the pathway and environment of synthesis, and the prevailing conditions under which the polysaccharide was extracted (Wang and Cui, 2005).

In order to be able to give a comprehensive and precise description of the molecular weight of a polysaccharide, information on both the average molecular weight and the molecular weight distribution of the polysaccharide must be supplied (Hosier *et al.*, 2004; Wang and Cui, 2005). Average molecular weight is described by different molecular weight averages. Molecular weight distribution is described by the mode of distribution and polydispersity. Statistically described molecular weight averages include (Cui, 2005): Number average molecular weight *(M_n)*, Weight average molecular weight *(M_w)*, Z-average molecular weight *(M_z)*, and Viscosity average molecular weight *(M_v)*.

Number average molecular weight *(M_n)*, is a measure of chain length of a polysaccharide. It is the summation of the mole fraction of each species multiplied by its molecular weight. Weight average molecular weight *(M_w)* corresponds to a measure of the size of the polymer chain. It is the sum of the weight fraction of each species multiplied by its molecular weight. Z-average molecular weight is determined by sedimentation techniques, and represents a third power average. Viscosity average molecular weight *(M_v)* is obtained from measurement of intrinsic viscosity and bears a relationship with the molecular weight through the Mark-Houwink equation (see section 1.6.3.2). For heterogeneous polysaccharides: $M_z > M_w > M_v > M_n$ (Wang and Cui 2005). The polydispersity index (M_w / M_n) is used as a convenient measure of the range of molecular weight present in a distribution. For natural polysaccharides this is usually in the range 1.5 -2.0.

Several techniques have been used in the determination of molecular weights of polymers. They include colligative property-based techniques, end-group analysis, light scattering, ultracentrifugation, agarose electrophoresis, dilute solution viscometry and gel permeation/filtration chromatography or size exclusion chromatography (Stephens, 1990; Testereci and Usanmaz, 1995; Pavlov *et al.*, 1995; Mutaudo *et al.*, 1997; Idris *et al.*, 1998; Al-Assaaf *et al.*, 2003; Moyses *et al.*, 2004; Mortlagh *et al.*, 2006; Al-Assaaf *et al.*, 2007; Feng *et al.*, 2007) among others. According to Hosier *et al.*, (2004), the accurate determination of the molecular weight characteristics of a polymer is very important and will depend to a large extent on the technique used.

The different techniques that have been used for molecular weight determination provide different information on molecular weight. These techniques can be categorised into three: absolute, relative and combined techniques. Absolute

techniques include membrane osmometry, static light scattering and sedimentation equilibrium. These techniques require no assumptions about molecular weight and no calibration using standards of known molecular weight is required. Relative techniques on the other hand require knowledge or assumptions concerning molecular conformation and calibration using standards of known molecular weight. Examples of relative techniques include viscometry and size exclusion chromatography. The combined techniques are those that combine information from two or more techniques e.g., sedimentation velocity combined with dynamic light scattering.

1.6.3.1 Size exclusion chromatography

Size exclusion chromatography (SEC) is also known as gel permeation chromatography or gel filtration chromatography. Separation is by molecular size rather than by chemical properties. The polymer solution is pushed through the SEC column causing polymer chains to separate according to differences in hydrodynamic volume. Column construction allows access of smaller molecules and excludes the larger ones, thus the retention volume (or time) of a fraction provides a measure of the molecular size. The resulting chromatogram represents a molecular size distribution. SEC does not yield absolute molecular weight rather it requires calibration using standards of known molecular weight by converting the retention volume to a molecular weight for a given column set. Several approaches have been used for calibration. The peak position method is that approach in which narrow fraction standards (e.g. pullulan and dextran) are used for calibration. The peak retention volume of several standards are recorded, and used to make a calibration curve of log molecular weight (*MW*) against retention volume. The calibration curve can then be used for determination of unknown molecular weight.

Under or over-estimation of the molecular weight of test polymer can occur if there is a difference in structure between the calibration standard and the test polymer. Values are therefore reported as pullulan or dextran equivalent molecular weight as appropriate (Wang and Cui, 2005).

Inconsistency caused by use of improper standards as already described, can be overcome by using the universal calibration approach which is based on the observation that, $[\eta] \times MW$ is directly proportional to hydrodynamic volume of a polymer (Benoit *et al.*, 1966). In this case, a plot of $\log[\eta]\ MW$ against retention volume emerges to a common line (the so-called universal calibration curve). Narrow

fraction standards are also used. Provided the intrinsic viscosity is known, the peak *MW* is read from the calibration curve.

1.6.3.2 Intrinsic viscosity

When a polymer is in solution, its viscosity is always directly related to the relative size and shape of the polymer molecules (Wang and Cui, 2005). Dilute solution viscometry is one of the methods most widely used for the characterisation of the molecular weight of polysaccharides. The instrumentation is minimal and data interpretation simple and straight forward.

In dilute solution viscometry, relative viscosity $[\eta_r]$ is measured for a series of dilute solutions of the polymer or polysaccharide, which allow for calculation of the intrinsic viscosity $[\eta]$. Molecular weight can then be calculated using the Mark-Houwink equation (Idris *et al.*, 1998):

$$[\eta] = K M^{\alpha} \quad \text{---------- (Equation 1.2)}$$

Where, M is the viscosity average molecular weight, α and K are constants. This requires that the constants K and α be known. These are determined by use of absolute methods such as sedimentation and osmometry. Dilute solution viscometry does not give absolute molecular weight values but only a relative measure of the polymer's molecular weight (Wang and Cui, 2005).

The determination of intrinsic viscosity $[\eta]$, is theoretically based on the Huggins (Huggins, 1942) and Kraemer (Amalvy, 1997) equations:

$$\eta_{sp} = [\eta]c + K'[\eta]^2 c^2 \quad \text{---------- (Equation 1.3)}$$

$$In\,(\eta_{rel}) = [\eta]c + (K'-0.5)[\eta]^2 c^2 \quad \text{---------- (Equation 1.4)}$$

Where K' is called the Huggins coefficient and c is the concentration of the polymer.

Determination of $[\eta]$ requires that the viscosity of several dilute solutions are determined and plotted in the forms of η_{sp}/c or $In(\eta_{rel})/c$ vs. c. Ideally, both lines usually extrapolate to the same point at zero concentration to give the intrinsic viscosity. The average of the two intercepts gives the intrinsic viscosity where the two intercepts are not identical (Vinod *et al.*, 2007).

The intrinsic viscosity of the polymer solution is an index of the inherent ability of a polymer to increase solution viscosity (Xiaodong and Marek, 2007) and is directly related to its molecular weight. Intrinsic viscosity is not a viscosity but a measure of the dynamic volume occupied by the isolated polymer chains in a given solvent. Unlike reduced or specific viscosity, intrinsic viscosity does not depend on polymer

concentration. It depends on three factors: molecular structure, molecular weight and solvent quality.

The intrinsic viscosity $[\eta]$, of a polymer can be related to its molecular weight by the Mark-Houwink equation (*Equation 1.2*).

1.6.3.3 Ultracentrifugation (Sedimentation)

This is an absolute technique for the determination of molecular weight of polysaccharides. The technique is based on the sedimentation property of materials in which macromolecules in solution are transported from the surface to the bottom under gravitational force. Two sedimentation methods are in use: sedimentation equilibrium and sedimentation velocity. In the sedimentation equilibrium technique, both diffusion and sedimentation are critical in determining the molecular weight. The technique allows the average molecular weight (M_w) to be calculated from the equation (Wang and Cui, 2005):

$$M_w = \frac{\mathcal{J}(b)-\mathcal{J}(a)}{\mathcal{J}_0(b^2-a^2)}\frac{2RT}{\omega^2(1-\upsilon\rho)} \qquad \text{-------- } \textit{(Equation 1.5)}$$

Where a and b are the distance from the centre of the rotor to the cell meniscus and cell bottom respectively, $\mathcal{J}_0$ is the initial loading concentration, ω is the rotor speed, υ is the partial specific volume, ρ is the solution density, R is the gas constant, T is the absolute temperature and $\mathcal{J}$ is the polymer concentration.

Sedimentation equilibrium technique is time consuming and data interpretation often too complex although it can be used for a wider range of molecular weight compared to techniques like light scattering and osmotic pressure methods.

Conversely in the sedimentation velocity method, diffusion is negligible because of the extremely high speed of the ultracentrifuge. The molecular weight M can be calculated from the Svedberg's equation:

$$S = \frac{M(1-\upsilon\rho)}{f} \qquad \text{---------- } \textit{(Equation 1.6)}$$

Where S is the sedimentation coefficient, ρ is the solution density, υ is the specific volume of the polymer and f the frictional coefficient. The sedimentation velocity method is very useful for monodisperse systems and offers an advantage over the sedimentation equilibrium method in the fact that it can be completed within a relatively short time (Wang and Cui, 2005).

1.6.3.4 Membrane osmometry

In membrane osmometry, the osmotic pressure (a colligative property of the material) of the polysaccharide is measured. For thermodynamically ideal solutions (very dilute solutions), the molecular weight can be derived from the relationship expressed in the Van't Hoff's equation (Tanford, 1961):

$$\frac{\pi}{c} = \frac{RT}{M_n} \qquad \text{--------- } \textit{(Equation 1.7)}$$

Where, $\frac{\pi}{c}$ is the reduced osmotic pressure, R is the gas constant, T is the absolute temperature and M_n is the number-average molecular weight. For polymer solutions however, the relationship is expressed in terms of the virial equation:

$$\frac{\pi}{c} = RT(\frac{1}{M_n} + A_2c + A_3c^2 + \ ...) \qquad \text{-------- } \textit{(Equation 1.8)}$$

Where, A_2 and A_3 are second and third virial coefficients, respectively. Determination of M_n requires that a plot of π/c verses c be extrapolated to zero concentration. The intercept gives a RT/M_n, and the slope of the plot gives RTA_2.

1.6.3.5 Static light scattering

The measurement of molecular weight by static light scattering is based on the relationship between the amount of light scattered by a polymer solution and the mass of the solute molecules. The measurement of the intensity of scattered light by a dilute polymer solution therefore enables the determination of the molecular weight of the polymer. The static light scattering technique is a convenient method for determining the weight average molecular weight, virial coefficients and the radius of gyration R_g for a particular polymer simultaneously (Wang and Cui, 2005).

1.6.4 Polysaccharide gels

Polysaccharides have the ability to form gels and are often used as gelling agents in both the food and pharmaceutical industries. Gellation by a polysaccharide is induced in the first instance by dissolution or dispersion of the polysaccharide in a solution. This forces the disruption of the hydrogen bonds in the solid state resulting in the formation of sols. In order for the sols to transform to gels, subsequent treatment by addition of cations or co-solutes, temperature and/or pH change is required (Wang and Cui, 2005). These treatments can decrease intramolecular interactions and increase intermolecular interactions. Intermolecular attractions contributing to the gelation process include hydrogen bonding, ionic or ion dipole bonding, *Van der waals* attractions, and hydrophobic interactions.

The gelation of polysaccharides can be affected by several factors. These factors include structural features of the polysaccharide, polymer concentration, molecular weight, ionic strength, pH, and the presence of co-solutes. The formation of polysaccharide gels requires that the polysaccharide chains or chain segments must assume ordered structures which cross-link with each other to form a stable three-dimensional network. Gels with different properties and gelation mechanisms can result for the same polysaccharide if there are variations in the fine structure of the polymer chains.

The formation of gels only occurs when the polymer concentration exceeds a critical concentration and there is a minimum critical chain length necessary for the cooperative nature of the interaction. This is in the range of 15 to 20 residues (Whistler, 1973). Co-solutes such as sugars can enhance the gelation of polysaccharides. Altering the pH or the type and amount of counter-ions can also considerably affect the properties of polysaccharide gels (Wang and Cui, 2005).

1.6.5 Surface activity

The property of some polysaccharides that has made them useful as emulsifiers and emulsion stabilizers is the ability to reduce the surface tension of water. They form protective layers through adsorption at oil-water interfaces (Dickinson, 2003). The general trend by which the surface tension is reduced has been documented (Wang and Cui, 2005): The initial addition of polysaccharides dramatically reduces the surface tension; further increase in polysaccharide concentration results in continued decrease of the surface tension of water until a saturated concentration is reached; above which, no further reduction of surface tension should be observed forming protective layers.

A polymer must possess a number of characteristics in order to be effective in stabilizing dispersed particles or emulsion droplets (Dickinson, 2003):

1. The polymer must be able to adsorb strongly at the oil-water interface. This means that the polysaccharide should possess a good degree of hydrophobic as well as hydrophilic character (amphiphilic). This property will ensure that the polysaccharide remains permanently anchored at the interface.
2. The polysaccharide must have complete surface coverage of both oil and water phases. This requires good solubility of the polysaccharide in water so that sufficient polymer chain is present in the continuous phase that will allow for full coverage of the surface of the oil droplets.

3. The ability to form a thick steric protecting layer and/or charged protecting layer that will help to stabilize the emulsion systems.

Polysaccharides such as methylcellulose and hydroxypropyl methylcellulose have been converted to amphiphilic polymers by the introduction of hydrophobic groups to the sugar units (Gaonkar, 1991).

1.7 DEGRADATION OF POLYSACCHARIDES

1.7.1 Degradation of polysaccharides

The determination of the monosaccharide composition of polysaccharide polymers requires first of all that the polysaccharide be broken down into its constituent monosaccharide units. The hydrolysis and specific or partial degradation of the polysaccharide prior to analysis of the monosaccharide or oligosaccharide components can be achieved in a number of ways.

1.7.1.1 Acid hydrolysis

The monosaccharide components of polysaccharides are linked by glycosidic bonds. In order to free the constituent monosaccharides, the glycosidic bonds or linkages must be cleaved. This can be achieved by the use of a mineral or organic acid in the presence of heat. However the glycosidic bonds do not all cleave at the same rate. Notably, the glycosidic bonds linking uronic acid units are difficult to cleave. This indicates that the hydrolysis of acidic polysaccharides presents greater difficulty as compared to neutral polysaccharides (Brummer and Cui, 2005). For this reason sufficient time must be allowed for the hydrolysis of all the glycosidic linkages in a sample. It must however be borne in mind that while sufficient time is required to allow for complete hydrolysis of the sample, the length of time must not be too lengthy as to lead to degradation of the sample.

Sulphuric acid has been reported to be more efficient than trifluoroacetic acid (TFA) in the hydrolysis of fibrous substrates such as microcrystalline cellulose, wheat bran, apple and straw (Garleb *et al.*, 1989). The draw-back with sulphuric acid however, is the difficulty to remove it from sample after hydrolysis. This is not the case with TFA which is volatile and relatively easily removed prior to analysis.

Several hydrolysis procedures have been reported for neutral polysaccharide gums using TFA (Brummer and Cui, 2005). When working with a new polysaccharide sample, it is worthwhile to monitor the hydrolysis protocol. The quantity of each sugar

present in the solution is monitored at time intervals to ensure that the protocol is not resulting in excessive decomposition and bonds are cleaved quantitatively. The progress of hydrolysis is indicated by an initial increase in the quantity of each sugar to an optimum. Thereafter when further hydrolysis results in degradation, this will be evident as a decrease in monosaccharide concentration as hydrolysis time increases (Brummer and Cui, 2005).

1.7.1.2 Methanolysis

Methyl glycosides are formed when the glycosidic bonds of polysaccharides are broken down and a methyl group introduced. Methanolysis is usually done on permethylated polysaccharides. Acid catalysed methanolysis of permethylated polysaccharides results in the formation of methyl glycosides (Cui, 2005).

1.7.1.3 Acetolysis

When polysaccharides are heated in a mixture of acetic anhydride, acetic acid, and sulphuric acid in the ratio 10:10:1 (Lindberg *et al.*, 1975), there is peracetylation and cleavage of selected glycosidic bonds. This process is termed acetolysis. It has been reported that glycosidic linkages in α-configuration are more susceptible to acetolysis than those in β-configuration (Pazur, 1986; Harvey, 2001).

1.7.1.4 Smith degradation

Smith degradation is a combination of three procedures: periodate oxidation, reduction, and mild acid hydrolysis. The procedure results in monosaccharide units and/ or oligosaccharides (Cui, 2005).

1.7.1.5 Enzyme hydrolysis

Enzymes have been used to cleave acid resistant polysaccharide structures to give high yields of oligosaccharides. β-mannase is an example of a purified and specific enzyme that has been used in the enzymatic analysis of galactomannans (McCleary, 1994). This enzyme hydrolyses the mannan backbone chain.

1.7.2 Monosaccharide analysis using GC

Following the hydrolysis of polysaccharides to the monosaccharide components, the composition can be determined by a suitable method for identifying the released monosaccharides, using chromatographic techniques such as HPLC or GC. GC techniques will however require treatment of the monosaccharides (derivatisation) to some volatile derivatives.

1.7.2.1 Derivatisation

Following hydrolysis, neutral monosaccharides are often derivatised into alditol acetates before GC analysis. The procedure essentially involves reduction of the neutral sugars to alditols using sodium borohydride. The alditols are then acetylated using a mixture of acetic anhydride and pyridine with heating at about 100°C. The resultant alditol acetates are dissolved in suitable solvent such as anhydrous chloroform and injected into the GC column. Acidic sugars or uronic acid units are treated to yield trimethylsilyl (TMS) derivatives (Brummer and Cui, 2005).

1.7.2.2 Gas chromatography (GC)

Gas chromatography is a technique in which the sample is dissolved in a gas phase and moved through a small bore column with its interior coated with the stationary phase under high temperature and pressure. This high temperature and pressure volatilises the sample as separation occurs through the column. The components in the sample mixture with high affinity for the stationary phase remain in the column while those with less affinity elute faster. The structure, properties and chemistry of the stationary phase determine the degree of interaction with sample components. The procedure can be used for both qualitative and quantitative analysis of the sample components. Quantification is based on determination of the response factor (RF) for each monosaccharide (Brummer and Cui, 2005).

HPLC, a separation technique similar to GC, has also been used for monosaccharide analysis of polysaccharides. Here sample components are also separated on a stationery phase but, both mobile phase (eluant) containing the sample and the stationary phase are liquid. Separation is a function of the differing compatibility of sample components for the eluent.

Polysaccharides may also have some proportion of fibre and fibre analysis of a polysaccharide may be carried to determine the fibre content.

1.8 STRUCTURAL ANALYSIS OF POLYSACCHARIDES

1.8.1 Determination of linkage pattern

The determination of the linkage pattern of polysaccharides requires derivatisation of the monosaccharide or oligosaccharides units following hydrolysis. This has been achieved through a number of means, some of which have already been discussed. These include specific degradation of the polysaccharides as well as methylation

analysis, reduction cleavage analysis, and peroxidation. The derivatised monomers or oligomers are then analysed using the techniques discussed below.

1.8.1.1 Mass spectroscopic techniques

Techniques that have been used in carbohydrate structural analysis are represented by the mass spectroscopic (MS) techniques (a key technique in carbohydrate structural analysis) which include fast atom bombardment mass spectroscopy (FAB-MS), matrix-assisted laser desorption ionisation mass spectroscopy (MALDI-MS) and matrix-assisted laser desorption ionisation time of flight (MALDI-TOF) techniques. These are proven techniques that are suitable for low volatile to non-volatile samples, and have been used to solve structural problems of complex polysaccharides.

1.8.1.2 NMR spectroscopy

Nuclear magnetic resonance spectroscopy has become a very useful tool in the determination of polysaccharide structures (Cui, 2005). Detailed structural information including monosaccharide composition, linkage patterns, α- or β- anomeric configurations, and the sequences of the sugar units in the polysaccharides can all be obtained using this technique.

The technique is based on the magnetic properties of some nuclei. Structural information about an individual nucleus and its surroundings can be obtained from the energy absorbed from a pulse radio frequency which is subsequently released. The signals from this released energy (which represent the structural information about the individual nucleus and its surroundings) is detected, analysed, and expressed as chemical shift and spin coupling (Cui, 2005). In carbohydrate analysis the nuclei of importance are ^{1}H and ^{13}C.

The spectrum generated for each polysaccharide is unique in both ^{1}H and ^{13}C NMR spectroscopy. Owing to the fact that the structural information signals from a polysaccharides are usually crowded within 3 to 5 ppm, the interpretation of the ^{1}H NMR spectra for polysaccharides that contain many sugar residues becomes difficult. This problem has been overcome by the use of 2-dimensional (2D) and multi-dimensional (MD) NMR techniques, which has brought a lot of improvement to the resolution and sensitivity of NMR spectroscopy. COSY (Homonuclear Correlated Spectroscopy) is a ^{1}H homonuclear shift correlation spectrum. It provides information on spin coupling networks within a constituent residue through the observation of cross peaks off the diagonal (Cui, 2005).

Other spectroscopic techniques that have been used in carbohydrate analysis include infra-red spectroscopy and solid state ^{13}C NMR. Solid state ^{13}C NMR provides a method for analysis of materials in the solid state. It provides information on structure and conformation, molecular motion and resonances, and inter-nuclear distances (Tishmack *et al.*, 2002). In infra-red (IR) spectroscopy the amount of infrared frequency that passes through the sample without being absorbed is measured as percentage transmittance. The spectrum has a finger print region and absorption bands by which bond bending or stretching vibrations and functional groups have been assigned. The finger print region usually contains complicated series of absorptions and lie from 1500 to 500 cm^{-1}. This is unique for the polysaccharides.

1.8.1.3 Other techniques in polysaccharide analysis

X-ray photoelectron spectroscopy (XPS)

The last two decades has witnessed a considerable increase in the interest of scientists in the surface properties of particles (Duke and Plumier, 2002). XPS, also known as electron spectroscopy for chemical analysis (ESCA), a technique that is very sensitive and provides useful information on the surface properties of a material or particle (Briggs and Seah, 1996).The technique involves irradiating the surface of a sample with monochromatic X-rays of a characteristic energy which causes photo electrons to be ejected with a range of kinetic energy depending on the element from which they are emitted and the chemical state of that element. The emitted electrons are then collected by an energy analyzer and sorted by their energies (Leinen *et al.*, 1999). The energy difference between incident X-rays and the kinetic energy of the emitted photoelectrons is used to determine the binding energy and oxidation states of different atoms in the system. This electron energy identification provides information on the elements present at a sample surface and their chemical state, giving an indication of the types of compounds present in the surface (Morales *et al.*, 2007). Typically XPS examines the surface to a depth of 7 nm. Greater depths can be analysed by removing surface layers by argon ion etching. XPS gives quantitative information on all elements excluding H and He and the detection limit is normally 0.1 %.

XPS has been used to determine the efficacy of encapsulation process in morphine loaded microparticles by the chemical analysis of the particles in the polymer surface, and to also verify that no drug molecules are adsorbed to the polymer surface (Morales

et al., 2007). Although other techniques such as thermal analysis or infrared analysis have been used for the same purpose, XPS is highly useful in assays to determine the extent of encapsulation, in spite of its relatively uncommon use (Oliva *et al.*, 2002, 2003).

Differential scanning calorimetry

Physical and chemical changes that occur in a polysaccharide during thermal processing can be monitored by means of a differential scanning calorimeter (DSC). This method yields thermograms that are unique for a given polysaccharide (Vinod *et al.*, 2007). It has been reported that structural and functional group differences in polysaccharide gums influence the thermal behaviour and affect the transition temperature, T_g (Zohuriaan and Shokrolahi, 2004). The temperature at which a material becomes glassy and brittle on cooling or soft on heating is referred to as the glass transition temperature, T_g.

Thermogravimetric analysis

Thermogravimetric analysis (TGA) is a simple and accurate method which has been used for studying the decomposition pattern and the thermal stability of polymers (Zohuriaan and Shokrolahi, 2004).

1.9 PHARMACEUTICAL APPLICATIONS OF NATURAL GUMS

Natural polymers are often preferred to synthetic materials because of their non-toxicity, low cost and relative availability. They can however exhibit uncontrolled rates of hydration and thickening, decreases in viscosity on storage and microbial contamination which may make modification necessary (Durso, 1980). Irrespective of these, natural or plant gums have several applications in pharmacy and medicine. In pharmaceutical formulations, they have been used as thickeners, binders and disintegrants. Others have been used for film coating of tablets and some in drug delivery systems to control the release of medicaments. As medicines, some have been used as plasma expanders, bulk laxatives or demulcents.

1.9.1 Binders and disintegrants in tablets

Naturally occurring polymers such as tragacanth, acacia, starch and alginic acid are used as diluents, binders and disintegrants in some tablet formulations. Binders, when added to tablet formulations, impart cohesiveness to powders and provide the necessary binding to form granules that under compaction form a mass or compact

(Lieberman *et al.*, 1989; Guo *et al.*, 1998). Pectin, carrageenan and guar gum have been used as binders for solid dosage formulations using either wet or dry granulation or direct compression procedures (Guo *et al.*, 1998). More recently, synthetic and semi-synthetic polymers have achieved wide use in tablet formulation. Microcrystalline cellulose, sodium carboxymethylcellulose and polyvinylpyrolidone are among polymers widely used in tablet formulations. Studies have shown that binder levels affect the hardness and dissolution of tablets (Guo *et al.*, 1998).

1.9.2 Bulk laxatives

Naturally occurring and semi-synthetic polymers have been used as bulk laxatives or demulcents. Agar and chondrons are used as demulcents. They become heavily hydrated in the intestine thereby producing a stool which is readily evacuated (Rhodes and Banker, 1970). Methylcellulose has been extensively used as a bulk laxative because of its unique gelling and solubility characteristics which result in more effective bulking action in the large intestine (Windover, 1963).

1.9.3 Plasma expanders

Naturally occurring polymers such as dextran and okra mucilage derived from the immature fruits of *Abelmoschus esculentus* (Fam. Malvaceae), have been used in medicine as plasma expanders (Rhodes and Banker, 1970; Tamoda *et al.*, 1980). Polyvinylpyrolidone, a synthetic polymer is also used for this purpose (Azorlosa and Martinelli, 1962; Rhodes and Banker, 1970).

1.9.4 Thickeners and stabilizers

The ability of plant gums to increase the apparent viscosity of a solution when dissolved in water has been exploited in some pharmaceuticals and cosmetics to thicken preparations such as suspensions, creams and lotions. In this capacity the polymers function as viscosity imparting agents based on the large spatial requirements which the polymers have in their hydrated and maximally extended configuration (Guo *et al.*, 1998). The extended polymer chains may be partially inter-twined. Polar groups on the polymer molecules are hydrated in aqueous solution and the long chain molecules may also immobilize the water molecules by simple entrapment (Bean *et al.*, 1964). Synthetic and semi-synthetic polymers such as carboxyvinyl polymer (Carbopol®), hydroxypropyl methylcellulose and sodium carboxymethylcellulose are amongst agents that have largely replaced the natural gums as thickening agents based on their greater chemical purity, microbiological

resistance, wide stable pH range, greater chemical stability and their greater range of compatibility with mainly common pharmaceutical materials.

It is worthy of note to state that in areas in which development and formulation are still in progress, a broad understanding of the behaviour of a natural polymer in solution is a requisite criterion. Viscosity and flow behaviour of the polymer become important areas of study.

1.9.5 Sustained-release polymer matrices

When compressed tablets of plant gums are placed in water, they absorb the water and swell to form a gel. Release of drug contained in the tablet is achieved through gradual diffusion across the gel layer and thus achieving a sustained release of the drug (Nakano *et al.*, 1984). This entire process is affected by the hydration time and hydration rate of the polymer (Guo *et al.*, 1998). The time required for a polymer to reach maximum viscosity is called the hydration time, and the rate at which this occurs is termed the hydration rate. It has been proposed that faster hydrating polymers are more suitable for sustained-release formulations because rapid gel development limits the amount of drug initially released from a matrix and further extends the duration of release (Guo *et al.*, 1998). Since viscosity is the measure of the internal friction of a polymer solution, the more viscous a polymer upon hydration the slower will be the rate of drug release from the polymer matrix.

To achieve rate controlled release of drugs, natural and synthetic polymers are used frequently where they function as diffusion barriers for drug movement. These polymers have been used in coating, microencapsulation, as films in laminated structures, as slabs in monolithic systems, and as flakes in many erodible devices as well as matrix formers in general to regulate the release of drugs (Folkman, 1978). The controlled drug release devices made of polymeric matrices and the mechanism of release of the core are discussed in detail in literature (Wood, 1980).

1.9.6 Mucoadhesive delivery systems

Oral controlled-release drug delivery systems are designed to provide a continuous delivery of drugs at predictable and reproducible kinetics for a predetermined period during the course of gastrointestinal transit. A major problem with oral drug delivery formulations is the speed with which they pass through the gastrointestinal tract. With a short intestinal transit time of less than 3 hours, the contact time within the small intestine is often too short to allow complete absorption of drugs. Thus, a drug

delivery system may pass rapidly through the small intestine, the area of maximum absorption and arrive at the colon and release most of its drug there at a non-optimal site (Naisbett and Woodley, 1995). The potential advantages of these oral controlled release systems are re-patenting of successful drugs; reduced dosage frequency and total dose, decreased occurrence and intensity of toxicity and a constant therapeutic effect (Chien, 1983). These systems can be modified to modulate the gastrointestinal transit time so that the system reaches the site of absorption and resides there for an extended period so as to maximize absorption.

One approach to delaying intestinal transit has been the use of mucoadhesives. The delay in transit is really an effect caused by a delay in gastric emptying (Fell *et al.*, 1987; Read and Sugden., 1987; Harris *et al.*, 1988). Mucoadhesives have been used to extend dosage form residence time as well as improving intimacy of contact with various absorptive surfaces of biological systems. They act as platforms for controlled-release dosage forms and may exert some control over the rate and amount of drug released, and contribute to the therapeutic advantage of such systems (Gupta *et al.*, 1990).

A number of terminologies have been used to describe the adhesion of two surfaces to one another. Bioadhesion has been defined as the ability of a material (synthetic or biological) to adhere to a biological tissue for an extended period of time (Peppas and Buri, 1985; Duchene *et al.*, 1988; Park, 1989). When applied to mucosal epithelia, a bioadhesive polymer may adhere primarily to the mucus layer in a phenomenon known as mucoadhesion (Dondetti *et al.*, 1996). Mucoadhesive or bioadhesive delivery systems have been demonstrated as effective dosage forms for controlled delivery of various drugs using buccal, ocular, rectal, vaginal, nasal, sublingual and oral routes (Duchene *et al.*, 1988; Dondetti *et al.*, 1996).

The potential usefulness of polymers as matrices for oral controlled drug delivery where they find application in eye, nose, vaginal, buccal and rectal formulations have also been reported (Park and Robinson, 1984). Tragacanth a plant gum, has been used as bioadhesive polymer to promote dosage form residence time as well as in improving intimacy of contact with various absorptive surfaces of biological systems (Park and Robinson, 1984).

1.9.7 Film coating

The application of film coating on the surfaces of pharmaceutical tablets is a practise that has been in place for over a hundred years. Major drivers for film coating include: masking of unpleasant taste and odour, improvement of product stability, facilitation of handling and modification of drug-release characteristics (Guo *et al.*, 1998). In film coated formulations, polymeric materials have been shown to produce a relatively thin coat compared to conventional sugar coat. This improves product stability and appearance much better than the conventional coatings (Lappas and Mckechan, 1967; Pickard and Rees, 1974; Rowe, 1982; Porter, 1982). Polymeric coatings are also widely used in pharmaceutical technology for the production of tablets and suppositories. These coatings are applied for protective reasons or to assist pharmacokinetic control.

Formulations designed to release drug in the colon must provide protection for the drug during transit through the stomach and small intestine before allowing rapid release on entry into the colon. Pectin and guar gum are bacterially degradable and have been shown to have potential value for such systems (Guo *et al.*, 1998).

1.10 ORAL DRUG DELIVERY

Oral dosage forms offer the advantage of convenience of administration, patient compliance and cost effective manufacturing. Consequently, the oral route has become the preferred route of administration for most therapeutic agents. This has remained so in spite of the fact that a substantial number of therapeutic agents are not well absorbed when administered by this route.

1.11 TABLETS AS PLATFORM FOR ORAL DRUG DELIVERY

1.11.1 Tablets

A tablet consists of one or more active pharmaceutical ingredient(s) (API) as well as a number of other substances used in the formulation to make administration of the drug convenient and to influence absorption and/or delivery of the drug to the site of action or absorption. The European Pharmacopoeia (1997), defined tablets as "solid preparations each containing a single dose of one or more active ingredients and obtained by compressing uniform volumes of particles".

Tablets vary in their shapes from flat or convex, round, oval, cylindrical or triangular and are intended for oral administration. Some are swallowed whole, or chewed, while others are either dissolved in water before administration or are retained in the mouth, where the active ingredient is liberated. They are widely prescribed today for a number of reasons which include - safe and convenience of administration, physical stability, portability, low price, elegance and accurate dosing of the drug amongst others. Tablets like other dosage forms should fulfil a number of specifications regarding their chemical, physical and biological properties. The quality attributes of a tablet include: the tablet should include the correct dose of the drug, the drug should be released from the tablet in a controlled and reproducible way, the tablet should be of sufficient mechanical strength to withstand fracture and erosion during handling, it should be chemically, physically and microbiologically stable during the lifetime of the product, it should be biocompatible, i.e. not include excipients, contaminants and microorganisms that could cause harm to patients, and the appearance of the tablet should be elegant and its weight, size and appearance should be consistent.

The British Pharmacopoeiea (B.P 2010) classifies tablets into coated tablets and uncoated tablets. Coated tablets have coating materials included in the formulation to reduce irritation on the gut wall, prevent loss of active ingredients through degradation, delay release of the drug or conceal unpleasant taste. Uncoated tablets contain no special coating materials. Other types of tablets include buccal, sublingual, chewable, vaginal, effervescent, soluble and sustained release tablets etc.

Tablets are modified with additional substances necessary for their manufacture, disintegration and appearance called tablet excipients.

1.11.2 Tablet excipients

The compressed tablet is a dosage form resulting from a physical process of powder mixing and use of extreme pressure, forcing particles to deform and rearrange with formation of a robust tablet mass. This is achieved by calculated inclusion of certain pharmacologically inert excipients that are incorporated to the active ingredient(s) and serve different functions. Tablet excipients enhance the manufacturability of the tablet and appearance of the finished product. They ensure that the tableting operation is run satisfactorily and tablets of specified quantity are prepared. Tablet excipients have been classified according to their functions in tablet formulation and they include

diluents/fillers, disintegrants, binders, glidants, lubricants, anti-adherents, sweeteners, colourants, flavourants and absorbents.

Diluents are inert substances added to tablet formulations to make a reasonably sized tablet and also referred to as bulking agents or fillers. Diluents are not necessary if the dose of the drug per tablet is high, i.e. above 500 mg weight of tablet. The ideal diluents should be chemically inert, non-hygroscopic, cheap, biocompatible, and possess good biopharmaceutical properties (e.g. water-soluble), good technical properties (such as compatibility and dilution capacity) and acceptable taste. These requirements cannot be fulfilled by a single substance (Stainiforth, 2002). The most commonly used diluent is lactose. Other diluents include sucrose, mannitol, dextrose and starches of wheat, rice and potatoes. These are used frequently in concentrations ranging between 5-75% w/w of the formulation.

Disintegrants ensure that the tablet breaks up into small fragments when in contact with a solvent to facilitate rapid drug dissolution. Ideally, the tablet should break up into individual drug particles in order to obtain the largest possible effective surface area during dissolution (Stainiforth, 2002). The disintegrants function mainly to oppose the efficiency of the tablet binder and the physical forces that act under compression to form the mechanical body of the tablets. Examples of disintegrant used in conventional tablets are starch, among which potato, maize and corn starches are also the most common types used. Superdisintegrants such as croscarmellose sodium and cross-povidone are now very popular. Other examples include gums (natural and synthetic), cellulose derivatives (methylcellulose) and alginates (sodium alginate).

Binders (also referred to as adhesives) are used in tablet formulation to improve compactibility and flowability of powders during the granulation and compaction stages of manufacture (Symeko and Rhodes, 1995). The quantity used must be carefully regulated since the tablet must hold together until swallowed and then disintegrate and dissolve to release the medicaments. Inadequate binding results in tablets with poor mechanical strength, which are easily prone to lamination and capping while excessive binding result in very hard tablets which are difficult to disintegrate (Gunsel and Kanig, 1976). Binding capacity and concentration affects tablet properties such as hardness, dissolution rate, friability and disintegration time. Examples of binders include natural gums such as acacia (1-5%), tragacanth (1-3 %.),

gelatin (1-3%), alginic acid (1-5%), guar gum (1-10%), starch mucilage, mucilage of acacia (20%); Semi-synthetic binders such as cellulose derivatives (hydroxypropyl methylcellulose, microcrystalline cellulose) and cross-linked polyvinylpyrrolidone (Stainiforth, 2002).

Lubricants function to ensure that tablet formation and ejection can occur with low friction between the solid and the die wall. They prevent adherence of tablets to punch surfaces and dies. High friction during tableting can cause problems including inadequate tablet quality (capping or even fragmentation of tablets during ejection, vertical scratches on the tablet edges) and may even stop production. Lubrication is achieved by mainly two mechanisms, fluid lubrication and bounding lubrication. The most effective of the boundary lubricants are stearic acid or its salts, primarily magnesium stearate. Magnesium stearate has become the most widely used lubricant because of its superior lubrication properties. The hydrocarbon chain in magnesium stearate confers deforming properties on it. The hydrophobic property of stearates may cause retardation of tablet disintegration *in vivo* necessitating the addition of wetting agents (e.g. surface active agents and polyethylene glycol) and limiting the amount of lubricants incorporated (Stainiforth, 2002).

Glidants are materials that improve the flowability of the powder of the granules prior to tableting. This is especially important during tablet production at high speeds and during direct compaction. The most commonly used glidant today is probably colloidal silica, added in low (very) proportions i.e. about 0.2%, although talc was traditionally used as a glidant in tablet formulation in concentrations of about 1-2% by weight.

Anti-adherents function to reduce adhesion between the powder and the punches faces and thus prevent particles sticking to the punches. Such adherence is especially prone to happen if the tablet punches are engraved or engrossed. Many lubricants, such as magnesium stearate have also anti-adherent properties; others are talc and starch (Stainiforth, 2002). Adsorbents are included in a formulation capable of adsorbing some quantities of fluid in an apparently dry state. Oils and/or oil-drug solutions can be incorporated into a powder mixture, which is granulated and compacted into tablets. Examples of adsorbents include microcrystalline cellulose and silica, which are used in tablets.

Other excipients used in tablet formulation include colourants, flavours and sweeteners. The use of colourants is optional and is regulated by the food and drug administration (FDA). Colorants like dyes and lakes are used to aid identification, improve aesthetic elegance or to provide control during manufacturing. Examples include caramel, titanium dioxide, and riboflavin. Flavours are incorporated to impart a pleasant taste or to mask unpleasant taste. Flavours are thermolabile and are not incorporated to formulations prior to procedures involving heat. Sweeteners impart sweetness or pleasant taste to a preparation. They are often used together with flavouring agents in chewable tablets (Gunsel and Kanig, 1976). Examples include sucrose and aspartame.

1.11.3 Tablet technology

1.11.3.1 Direct compression

Powders possessing free flow as well as compressible properties like potassium chloride and ammonium chloride can be compressed directly without granulation. Here, powdered materials are mixed and compressed into tablets without modifying the physical nature of the materials. Direct compression is used to define process of tablet compression directly into tablets from powdered blends of active medicaments and suitable excipients, which flow uniformly and can form firm compacts. Direct compression has no pre-treatment like granulation (wet) and has three steps, which are mixing, lubrication and compression.

The method is cheap and simple due to the few stages and machinery involved. Also, drugs sensitive to heat and moisture can be formulated using this method. However, the few number of directly compressible medicinal compounds are limited by segregation, which may occur at the compression stage due to differences in particle size and bulk density between the drug and diluents, limits applicability. This may lead to poor weight and content uniformity within the compressed tablet.

1.11.3.2 Granulation methods

Granulation is a pharmaceutical process in which primary powder particles are made to adhere to form larger, multi-particle entities called granules or aggregates with the aim of

1. Improving flowability of the powder in order to ensure that tablets with a low and acceptable tablet weight variation can be prepared.

2. Increasing the bulk density of the powder mixture and thus ensure that the required volume of powder can be filled into the die.
3. Improving the compactibility of the powder by adding a solution binder, this is effectively distributed on the particle surface.
4. Improving the mixing homogeneity and reduce segregation by mixing small particles which subsequently adhere to each other. (Stainiforth 2002):

A good tablet granulation must also possess a uniform distribution of all ingredients. Granulation is achieved by the following methods;

Dry granulation

The primary powder particles are aggregated under high pressures. There are two main processes, either a large tablet (known as a slug) is produced in a heavy-duty tableting press (a process known as 'slugging') or the powder is squeezed between two rollers to produce a sheet of material (roller compaction). In both cases, the intermediate products are broken into granular material (using a suitable milling technique), which is usually sieved to separate the desired size fraction. The unused fine material is reworked to avoid waste. Dry granulation is used for drugs that do not compress well after wet granulation, or those sensitive to moisture such as aspirin.

Wet granulation

This is the most common and popular method of tablet production. The method involves the massing of a mix of dry primary powder particles using a granulating liquid/fluid. The fluid contains a solvent, which must be non-toxic and volatile so that it can be removed by drying. Typical liquids include water and ethanol, either alone or in combination. The granulating liquid may be used alone, or more usually as a solvent containing a dissolved adhesive (also referred to as a binder or binding agent), which is used to ensure particle adhesion once the granule is dry.

In the traditional wet granulation method, the wet mass is forced through a sieve to produce wet granules, which are then dried. A subsequent screening stage breaks agglomerates of granules and removes the fine material, which can then be recycled. Variations of this traditional method depend on the equipment used, but the general principle of initial particle segregation using a liquid remains in all of the processes. Wet granulation is the method of choice for high dose drugs with poor flow. It also prevents segregation of components giving a homogenous powder mix during processing, transferring and handling, reduces the level of dust during manufacture

thereby reducing cross-contamination and risk to workers. This is especially important for hormonal drug products. However, this method is limited by the high cost due to labour, time and equipment. Also moisture and heat sensitive drugs are degraded when they are wet granulated and dried using heat.

Other methods of granulation are high shear granulation, fluidised bed granulation and melt granulation.

1.11.4 Powder and granule evaluation

1.11.4.1 Flow rate of powders and granules

This parameter determines the rate of flow of granules and powders in the hopper, which is essential for die filling accuracy. Flow rate is affected by particle size; shape, porosity, density and surface texture (Cooper and Gunn, 1986*a*).

Studies have shown that flow rate improves with particle size and increased diameter up to a certain limit after which flow rate begins to decrease (Marshall, 1985).

Flow of powders through the orifices depends on the diameter of the orifice and efflux tube length, and is governed by the equation,

$$W = (DCL)^{2.5} \quad \text{---------- } \textit{(Equation 1.9)}$$

Where, W= flow rate, L= efflux tube length, C= constant for powder, D= Diameter of the tube

The flow rate through an orifice will therefore be given by the equation,

$$Flow\ rate = \frac{weight\ of\ powder\ (g)}{time\ (seconds)} \quad \text{---------- } \textit{(Equation 1.10)}$$

1.11.4.2 Angle of repose

The angle of repose is the maximum angle possible between the surface of a heap of powder and the horizontal plane, the tangent of which is the coefficient of friction (Cooper and Gunn, 1986*a*). Angle of repose is an indirect method of quantifying powder flowability because of the relationship with inter-particulate cohesion. As granule and powder cohesion increases, the angle of repose also increases. When the particle shape of materials increasingly departs from the spherical, the angle of repose increases. Free flowing powders give an angle of repose of 25°, while powders with angle of repose of 45° or more will not flow satisfactorily (Martin *et al.*, 1983). Very non-cohesive powders do not form heaps at all. Angle of repose provides a reliable index of powder flow.

1.11.4.3 Granule density

Density is the ratio of mass to volume of a substance. Generally, as the density of a substance increases, its flow rate also increases (Sheth *et al.*, 1980). Bulk density of a granule sample is the mass or weight of the sample divided by the bulk volume it occupies prior to tapping.

$$Bulk\ density(\rho_b) = \frac{weight\ of\ sample}{bulk\ volume} \quad \text{---------- (Equation 1.11)}$$

Bulk density of granules or powder depends primarily on particle size distribution, particle shape and the tendency of the particles to adhere to one another. A powder can have only a single true density but several bulk densities, depending on the packing of the particles and the bed porosity. Granules may pack in such a way that leaves large gaps between their surfaces, which results in the granules having a low bulk density. On the other hand, granules with smaller particle size may sift between the larger ones to form a high bulk density leading to increase in cohesion (Martin *et al.*, 1983).

Tapped density is the ratio of the weight of granules or powder to volume occupied after tapping for a given period of time. Tapping leading to consolidation reduces voids.

$$Tapped\ density(\rho_t) = \frac{weight\ of\ sample}{tapped\ volume} \quad \text{--------- (Equation 1.12)}$$

1.11.4.4 Hausner's quotient

This is a measure of inter-particulate friction in the powder. It is useful in predicting the flowability properties of powder.

Hausner's quotient is expressed as:

$$Hausner's\ quotient\ (ratio) = \frac{tapped\ density}{bulk\ density} \quad \text{--------- (Equation 1.13)}$$

Hausner showed that powders with low inter-particulate friction and good flow had a ratio of approximately 1.2 while more cohesive and less free flowing powers have the ratio greater than 1.6. Between 1.2 and 1.6, added glidant normally improves flow (Stainiforth, 2002)

1.11.4.5 Carr's compressibility index

The percentage compressibility of a powder is an indirect method of measuring powder flow from bulk densities. It is a direct measure of the potential powder/granule bridge strength and stability. It is related to apparent powder densities and is given by:

$$Carr's\ compresibility\ \% = \frac{tapped\ density - bulk\ density}{tapped\ density} X\ 100 \quad \text{- (Equation 1.14)}$$

This is a simple index that can be determined on small quantities of powder or granules. Values of 23-35% indicates poor flow, 18-21% indicates fair flow, 12-16% indicates good flow and 5-15% indicates excellent flow (Stainiforth, 2002).

1.11.4.6 Granule and powder moisture content

Granules for compression must have minimum moisture content, which varies with the material in order to compress satisfactorily.

1.11.5 Effects of particle size distribution on tablet properties

A particle population, which consists of spheres or equivalent spheres with uniform dimensions, is mono-sized and its characteristics can be described by a single diameter. In order to be able to define a size distribution or compare the characteristics of two or more powders consisting of particles with many different diameters, the size distribution can be broken down into different size ranges (Stainiforth, 2002).

Particle size is important in determining tablet properties. It is important in the formulation of pharmaceutical dispersions, flow of granules during tableting, capsule filling, extraction and in mixing (Marshall, 1986). The presence of an inadequate proportion of fines leads to non-uniform weight and inadequate die cavity filling due to existing inter-particulate spaces. Excess fines leads to structural defects with production of soft tablets. Spherical particles minimize inter-particulate friction and static changes, which is the primary objective in granule production.

The dimensions of particulate solids are important in achieving optimum production of efficacious medicines. The particle size of a drug and other powders determines and influences the subsequent physical performance of the formulation and the pharmacological performance of the drug. Particle size influences the production of formulated medicines as solid dosage forms.

Powders with different particle size have different flow and packing properties, which alter the volumes of powder during each tablet compression event. In order to avoid such problems, the particle size of drug and other powder may be defined during formulation so that problems during production are avoided (Stainiforth, 2002).

Common methods of particle size determination or analysis include: sedimentation rate, gas absorption (surface methods), sieving, permeability, conductivity, elutriation, microscopical methods and centrifugal methods (Cooper and Gunn, 1986*a*). Particle size distribution involves determination of weight of granules lying in a particular size

range obtained by sieve retention plotted against a size range to give a frequency distribution range (Marshall, 1986).

1.11.6 Tablet compression

The compressibility of a powder is defined as its propensity when held within a confined space, to reduce in volume while loaded (Stainiforth, 2002). Compression is the process of pressing a material together to make it more firm and solid. This process applied to a particulate or granular system results in the formation of a compact, known as a compressed tablet.

Compression of material into tablet involves application of a compression force unto the powder or granules within the die cavity between the opposing faces of the two punches. The punches consist of the lower punch, the tip of which moves, up and down within the die but never actually leaving it, and the upper punch which descends to compress the granules in the die cavity and then withdraws to permit the ejection of the formed tablet. During the compression phase, the lower punch can be stationary or can move upward in the die. After maximum applied force is reached, the upper punch leaves the granules (i.e. the decompression phase).

1.11.7 Standardisation and evaluation of tablet properties

Evaluation of tablets ensures that the patient receives a product containing the required amount of drug substance in a form that enables the active drug to exert its maximum therapeutic effects. The tests carried out are classified as in-process quality control tests and non- in- process quality control tests. They may also be classified as pharmacopoeial and non- pharmacopoeial tests.

1.11.7.1 In-process control tests/ pharmacopoeia tests

Uniformity of diameter (Tablet dimension)

The dimensions of particulate solids are important in achieving optimum production of efficacious medicines. The dimension of a tablet can be characterized by determining the diameter of the tablet (Stainiforth, 2002). Tablets of the same diameter are reasonably assumed to be of the same weight range if compressed under uniform pressure.

Although the pharmacopoeia does not insist on a standard weight for official tablets, it nevertheless specifies their diameter. The thickness is not directly controlled, but in practice, most manufacturers make uncoated tablets of thickness equal to `half the diameter since this result in an elegant and pleasing shape. If the diameter and

thickness are both defined, the volume and weight of the tablets are obviously fixed and there is very little variation in the dimensions of official tablets produced by different manufacturers. The pharmacopoeia permits some slight deviation, usually + 5% from the nominal diameter (Cooper and Gunn, 1986*b*). The diameter can be determined using a vernier caliper while the thickness is measured using a micrometer screw gauge.

Uniformity of weight

This test is designed to ensure uniformity of dosage and is preferred to content of active ingredients where drug substance forms a greater part of tablet weight. Small variations in the weight of individual tablets are inevitable and admissible, and accordingly, accepted limits are officially specified for uncoated tablets (Cooper and Gunn, 1986*b*).

To pass the test, not more than two tablets are permitted to deviate from the mean by greater than a stated percentage and no tablet by more than double that percentage (Gunsel and Kaanig, 1976).

Uniformity of content of active ingredients

This test gives the average content of active ingredients in a tablet batch utilizing 20 tablets powdered and assayed together according to the monograph method. It should be realized that variations in the percentage of medicaments might occur for various reasons such as variations in the weight of the tablets, permitted variation in the purity of the drug, errors of 'random sampling', and limits of accuracy in the analysis.

The official limits are wider if fewer than 20 tablets are used for the test. It will be noted that these variation are allowed when the stated limits are between 90 and 110 percent.

Dissolution rate profiles (release profiles)

Dissolution testing is the most important way to study the release of a drug from a solid dosage form, and represents an important tool to assess factors that affect the bioavailability of a drug from a solid preparation. During a dissolution rate test, the cumulative amount of drug that passes into solution is studied as a function of time. The test thus describes the overall rate of all the processes involved in the release of the drug into a bioavailable form.

Dissolution rate studies are carried out for several reasons, (Stainiforth, 2002):

1. To evaluate the potential effect of formulation and process variables on the bioavailability of a drug.
2. To ensure that preparation comply with product specifications.
3. To indicate the performance of the preparation under *in vitro* condition.

Dissolution rate testing is essential in tablets where the rate-limiting step affecting bioavailability is the rate of dissolution in gastrointestinal fluids and not necessarily the disintegration rate. The apparatus and media for dissolution simulate the composition of gastrointestinal tract fluids and a method of forced convection by gradual fluid turbulence and peristaltic activity obtained *in vivo*.

Spectrophotometry (Beer's plot)

It provides a relatively sensitive and convenient assay method for a wide range of tablets. This technique measures the maximum absorbance of ultraviolet radiation at particular wavelength specific to that compound governed by the Beer-Lambert's law according to the equation:

$$A = \mathcal{E}\, l\, c \qquad \text{---------- } \textit{(Equation 1.15)}$$

Where, $\boldsymbol{\varepsilon}$ is the molar absorptivity, l is the path length of the solution that the light passes through, c is the concentration of the solution, and A is the absorbance. It gives an accurate method of concentration determination since concentration is proportional to optical density.

Disintegration time test

Tablets containing insoluble or slightly insoluble ingredients may be slow to disintegrate when swallowed, and the inclusion of a disintegrant such as starch to hasten the process has been described in section 1.11.2. Tablets may remain in the stomach for 4 hours or more, but the British Pharmacopoeia (BP) normally requires them to disintegrate within 15 minutes. This applies to conventional tablets. Tablets intended to delay the release of medicaments will take longer to disintegrate.

An essential disintegration apparatus consists normally of six chambers i.e. tubes open at the upper end and closed by a screen (or rust-less wire gauze) at the lower end. This test is carried out by agitating a given number of tablets in an aqueous medium at a defined temperature and constant frequency. Disintegration is considered to be achieved when no tablet fragment remains on the screen. Disintegration tests are useful as a means to assess the potential importance of formulation and process variables on the biopharmaceutical properties of the tablet.

1.11.7.2 Non- pharmacopoeal tests

Hardness/ Crushing test for tablets

The crushing or mechanical strength of a tablet is associated with the resistance of the solid specimen towards fracturing and attrition. This test is carried out for several reasons (Stainiforth, 2002):

1. To assess the importance of formulation and production variables for the resistance of a tablet towards fracturing and attrition during formulation, process design and scaling up.
2. To control the quality of tablets during production (in-process and batch control).
3. To characterize the fundamental mechanical properties of materials used in tablet formulation.

The test consists of breaking or crushing the tablet by application of a compressive load. Since crushing strength is governed by the rate of application of pressure and tablet dimensions, which may be variable, tensile strength may be utilized.

Friability test

The friability of a tablet is a measure of its resistance to abrasion. It measures the abrasive effect of tumbling motion encountered by tablets during coating, packing or transportation. After a period, the abraided material is sifted from the tablets and the percentage estimated. Ten tablets are selected from a batch and the total weight determined after subjecting to abrasion / agitation, the final weight is determined and the loss from the tablet is expressed as a percentage applying the equation:

$$Friability\ (\%) = \frac{original\ weight - final\ weight}{original\ weight} X100 \qquad \text{------- } \textit{(Equation 1.16)}$$

To pass this test, a batch must possess friability of not more than 1%. A friabilator is used to carry out this test.

1.11.8 Problems of tableting

Many problems influenced by physiochemical properties of excipients, drug substance or production processes are encountered at one stage or the other of tablet manufacture, among which the most important are detailed in the following sections (Stainiforth, 2002):

1.11.8.1 High weight and dose variation of tablets

The factors responsible for weight variation in a batch of tablets include;

1. Poor granule flow: This will lead to variable filling of the dies and tablets with variable weights will be produced.
2. Improper mixing of the granules with lubricants: Granule size and size distribution, if not done well could lead to inconsistent flow of granules.
3. Variation in the size of punches or multiple stationary tableting machines.

1.11.8.2 Low mechanical strength of tablets

When changes occur in the compression speed of the tablet press, tablets of low mechanical strength are likely to result. This means that tablets lack sufficient hardness or mechanical strength necessary to withstand the stress and rigours of shipment.

1.11.8.3 Capping and lamination of tablets

Capping is the partial or complete separation of the top or bottom while laminating is the incomplete separation of a tablet into two or more distinct layers.

1.11.8.4 Chipping of tablets

Chipping is a situation where some parts of the edges of the tablet break off. Factors responsible for capping, lamination and chipping include insufficiency in binder cohesiveness, presence of entrapped air, presence of excess fines of powders in the granules, pressure and speed of compression, moist and soft granulation, worn and imperfect dies and punches, and deep marks on tablet punches.

1.11.8.5 Picking and adhesion or sticking of powder material to punch tips

Picking is the removal of a localized portion or materials from tablet surface unto punch surfaces, while sticking is the adherence of the granules or tablet particles on the die wall and punch surfaces. These problems occur if the granules are too wet or under -dried.

1.11.8. 6 High friction during tablet ejection

Such problems are related to the properties of the powder intended to be formed into tablets, and also to the design and condition of the tablet press.

1.11.8.7 Mottling

This is a problem which predominantly affects coloured tablets. It is the unequal distribution of colour on the surface of the tablet with light or dark areas standing out in an otherwise uniform surface. This could be due to migration of a dye during drying of a granulation, or the drug having a different colour than its excipients or whose degradation products are highly coloured.

1.12 PHARMACEUTICAL ORAL SUSPENSIONS

When finely divided, insoluble solid (drug) particles are dispersed uniformly throughout a fluid, they are known as suspensions (Billany, 2007). The solid particles are also called the dispersed phase while the liquid or fluid in which they are dispersed is known as the dispersion medium or continuous phase. Pharmaceutical oral suspensions are coarse dispersions (usually aqueous) having a dispersed phase with mean particle diameter of greater than 1.0 μm while colloidal suspensions have a dispersed phase of mean particle diameter that is less than 1.0 μm.

Aqueous suspensions are useful for administration of insoluble or poorly water soluble drugs. The dispersed drug provides a large surface area that ensures and enhances dissolution and hence absorption of the drug. They however come with the challenge of physical stability.

1.12.1 Physical properties of well formulated pharmaceutical oral suspensions

There are a number of features desirable in pharmaceutical oral suspensions. These include: the suspended material should not settle too rapidly; the particles which do settle to the bottom of the container must not form a hard mass but should be readily dispersed into a uniform mixture when the container is shaken; and the suspension must not be too viscous to pour freely from the orifice of the bottle (Billany 2007).

1.12.2 Physical stability of pharmaceutical oral suspensions

That condition in which the dispersed phase does not aggregate and in which the particles remain uniformly distributed throughout the dispersion defines the physical stability of an oral pharmaceutical suspension. However, since this ideal condition is seldom realized, the dispersed phase when settled should be easily resuspended by moderate amount of agitation or shaking. A suspension is therefore said to be stable when the dispersed phase remain discrete.

In pharmaceutical oral suspensions which are usually coarse dispersions, the system sediments over time because of the size of the particles and the effect of gravity. The dispersed particles slip past one another forming closely packed arrangement at the bottom of the container with smaller particles filling up the void between larger ones and gradually particles below are pressed together by the weight of particles above. The repulsive barrier surrounding the particles is overcome and a physical bonding occur leading to "cake"formation due to formation of bridges between the particles as a consequence of crystal growth and hydration effects requiring forces greater than

agitation to disperse the sediments (Billany, 2007). A reduction in the zeta potential to a point where attractive forces pre-dominate produces primarily coarse masses or coagulation which is difficult to readily redisperse.

Conversely, the particles may form a loosely bonded structure or aggregate called a floc or flocculate and the process of its formation flocculation. These flocs separate out and sediment fairly rapidly but because they are loosely packed, high-volume sediments which retain their structure, they are easily redispersible. The supernatant liquid is clear because the colloidal particles are trapped within the flocs and sediment with them. This later state is desirable for a pharmaceutical suspension. Figure 1.2 shows a schematic representation of stability dynamics in a coarse or colloidal system.

One parameter for assessing a pharmaceutical suspension is the sedimentation volume ratio, F, defined as the ratio of the final volume V_u to the original volume V_o:

$$F = V_u/V_o \qquad \text{-------- } \textit{Equation 1.17}$$

The ratio F is a measure of the aggregated-deflocculated state of a suspension. The ratio F may be plotted together with the measured zeta potential, against concentration of additive to enable assessment of the state of the dispersion to be made. Also the appearance of the supernatant liquid should be noted and the redispersibility of the suspension evaluated (Billany, 2007).

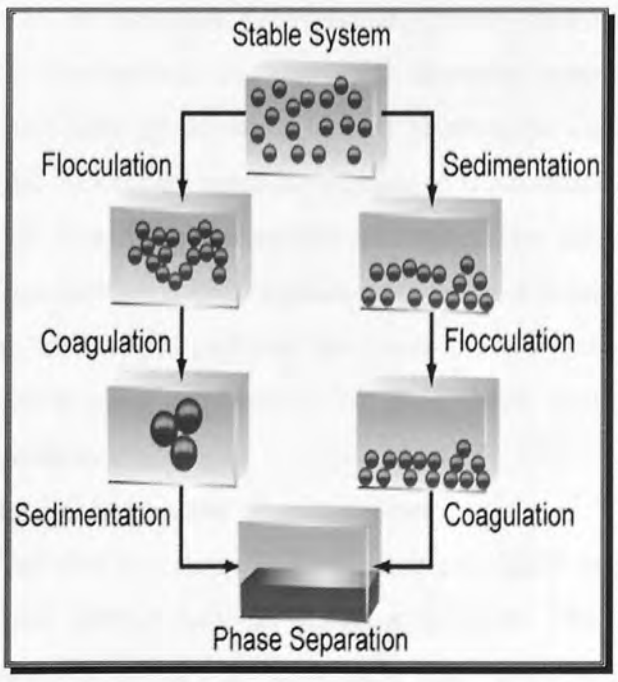


Figure 1.2: Stability dynamics of a coarse or colloidal dispersed system. Adapted from www.malvern.com/LabEng/industry/colloids/colloids_stability.htm

1.12.3 Stabilization of suspensions

Non-ionic polymeric materials have been shown to stabilize colloidal particles against coagulation in the absence of a charge on the particles. This has been applied to pharmaceutical suspensions. Naturally occurring gums such as tragacanth and semi-synthetic or synthetic materials like non-ionic surfactants and cellulose polymers have been used to produce satisfactory suspensions. These materials stabilize suspensions by increasing the viscosity of the aqueous vehicle thereby slowing the sedimentation rate of the particles. They also form adsorbed layers around the particles thereby hindering the approach of their surfaces and aggregation that leads to coagulation (Billany, 2007).

In the formulation of pharmaceutical suspensions one of the problems encountered in dispersing the drug particles in water is that of wettability. The powder may not be readily wetted when the drug particles have hydrophobic surfaces or due to entrapped air. Suspension particles may also adhere to the walls of the container just above the liquid line. For such powders to be completely wetted there must be a decrease in the surface free energy as a result of the immersion process. Surfactants have been used to overcome such problems.

1.12.4 Rheological properties of suspensions

Depending on concentration, flocculated systems can exhibit plastic or pseudoplastic flow behaviour as the structure progressively breaks down under shear (also see section 1.6.1.2). Flocculated systems will therefore show the time-dependent reversibility of this loss of structure termed thixotropy. Conversely, deflocculated dispersions exhibit Newtonian behaviour owing to the absence of such structures and may even at high concentrations exhibit dilatancy. The dilatant flow exhibited by deflocculated dispersions has been explained to be as a result of electrical repulsion that occurs when the charged particles are forced close together causing the particles to rebound, creating voids into which the fluid flows, leaving other parts of the dispersion dry (Billany, 2007).

1.12.5 Electrokinetic properties of suspensions

The surfaces of particles in a suspension or emulsion usually acquire an electric charge when brought into contact with an aqueous medium. The charge is more often negative than positive and it may arise in a number of ways: the surface of the particles may contain chemical groups which can ionize to produce charged surfaces;

the particle surface may adsorb ions of one sign of charge in preference to charges of the opposite sign; deliberate addition of chemical compounds that preferentially adsorb on the particle surface to generate the charge. The amount of charge on the particle surface is an important particle characteristic and it determines many of the properties of the suspension (Billany, 2007).

Although particles are referred to as being electrically charged, the suspension is neutral overall because the charge on the surface of each particle is counterbalanced by charges (ions) of opposite sign in the surrounding solution. This distribution of ions is in turn affected by thermal agitation which tends to redisperse the ions in solution and results in the formation of an electric double layer made up of charged surface and a neutralizing excess of counter-ions over co-ions distributed in a diffused manner in the aqueous medium (www.colloidal-dynamics.com/docs/CDElTut1.pdf)

Figure 1.3 is a schematic representation of the electric double layer. The liquid layer surrounding the particle exists as two parts; an inner region (Stern layer) where the ions are strongly bound and an outer (diffuse) region where they are less firmly associated. Within this diffuse layer is a notional boundary known as the slipping plane, within which the particle acts as a single entity. This surface separating the bound charge from the diffuse charge around the particle, marks where the solution and the particle move in opposite directions when an external field is applied. It is called the surface of shear or the slip surface. The electrostatic potential on slip surface or slipping plane is called the zeta potential and it is that potential which is measured, when the velocity of the particles in a direct current electric field is determined.

The potential stability of a dispersed system may be indicated by the magnitude of the zeta potential. Dispersed systems with high negative or positive zeta potential show no tendency to coagulate because the particles tend to repel one another. However, low zeta potential means that the particles can easily come together and coagulate. The general dividing line between stable and unstable suspensions is generally taken at either +30mV or -30mV. Particles with zeta potentials more positive than +30mV or more negative than -30mV are considered as stable (www.nbtc.cornell.edu/facilities/downloads/Zetasizer%20chapter%2016.pdf

Figure 1.3: The electric double layer. Adapted from: www.silver-colloids.com/Tutorials/Intro/pcs17A.html

1.13 ORAL CONTROLLED RELEASE OF MEDICAMENTS

In the design of sustained-release dosage forms, a single dose unit is expected to provide a prompt or immediate release of an amount of drug that would produce the desired therapeutic plasma concentration followed by a gradual and continuous release of smaller amounts of the drug to maintain the attained therapeutic plasma level. Several formulation methods have been used to achieve sustained-release of drug substances.

1.13.1 Coated granules/tablets and similar multilayered systems

Multi-layered systems are designed in such a way that the drug has to cross barrier(s) on its way from the device to the physiological environment. The release process is controlled by the nature and number of barriers (Zahirul, 1995). To design appropriate barriers, formulation scientists have relied on polymers from the very beginning of the emergence of controlled-release technology. This is perhaps attributable to the fact that these substances can be fabricated according to the needs of the system and most of them are inert and biocompatible.

In their simplest form, coated tablets comprise a core containing the drug and a coating layer which surrounds the core. The core is usually the drug either alone or

loaded into an inert material which may be hydrophilic or hydrophobic. The coating layer is a hydrophobic or slightly water permeable polymeric film or a mixture of both hydrophilic and hydrophobic films of which the ratio is optimized according to the needs of the system (Zahirul, 1995). Several differently coated groups of granules may have to be combined to produce the final desired availability rate. This may produce more uniform absorption from the gastrointestinal tract. The coated granules may be encapsulated or tableted or even formulated into suspension (Rawlins, 1980; Zahirul, 1995).

In order to extend the release profile of drugs from coated tablets, more complex systems have been introduced. The common approach is to use multiple coatings with polymers of different physicochemical properties or polymers in combination with other hydrophobic materials. Other attempts to extend the release of the drug from coated tablets include modification of the core by using hydrophobic matrices or by complex formation of the drug with a carrier in the matrix which results in prolongation of the drug release. Multi-layered systems having the drug in both outer layers and within the core have also been described in the literature (Zahirul, 1995). The rationale behind this type of formulation is that a rapid release of the drug followed by a controlled-release as required for prolonged action can be achieved using these devices.

1.13.2 Ion-exchange products

Ion-exchange resins are water-insoluble cross-linked polymers containing salt-forming groups in repeating positions on the polymer chain (Rawlins, 1980). The design of ion-exchange preparations involves an insoluble resin capable of reacting with either an anionic or cationic drug to form an insoluble Resin-Drug complex. Upon exposure to the ions in the gut (K^+, Na^+, Cl^-), the drug is displaced from the resin releasing the drug which is then absorbed freely.

The charging of drug ions to an ion-exchange resin may be accomplished in two ways: highly concentrated drug solution is percolated through a column or a bed of resin particles until equilibrium is reached. Alternatively, resin particles are stirred with a large volume of concentrated drug solution. The charged resin particles are then washed with deionised water to remove unassociated drug and other ions, and the resin particles subsequently dried (Rawlins, 1980; Zahirul, 1995). The particles may then be tableted or encapsulated.

1.13.3 Micro-encapsulation

Micro-encapsulation is a means of applying relatively thin coatings to small particles of solid, liquid and dispersions. It involves the coating of particles of 0.1- 5000μm in size. The uniqueness of the method lies in the size range of the coated particles and their application to a wide range of dosage forms. The technique improves drug absorption and reduces side-effects related to dose - dumping or localized building- up of irritating drugs against the gastrointestinal tract mucosa (Rawlins, 1980).

1.13.4 Complex formation

Chemical complexes are formed from reaction of certain substances with some chemical agents. The complex formed is slowly soluble in body fluids depending on the pH of the environment or dissolution medium. This slow dissolution is effective in providing sustained-release action (Zahirul, 1995).

1.13.5 Osmotic pump devices

In osmotic systems usually an osmotic agent (often with an osmotic adjuvant) is contained within a rigid compartment at least one wall of which is a semi-permeable membrane. The drug is dispersed in another compartment by a partition. In the physiological environment the aqueous fluid penetrates across this membrane and the increased volume within the osmotic compartment pushes the drug out of the device through a delivery orifice (Zahirul, 1995). In simpler devices, the drug itself serves as the osmotic or is dispersed in an osmotically active carrier and is surrounded by a semi- permeable membrane. Penetration of water causes the drug to be driven out through the orifice or the perforated membrane.

1.13.6 Drug particle-size increase

This is usually aimed at increasing the surface to volume ratio which allows the slow rate of drug release. The particle-size increase is limited to poorly soluble drugs. Good grinding techniques and/or the addition of filler with larger or smaller particles provide means of getting desired particle-size range (Zahirul, 1995).

1.13.7 Hydrocolloid systems (Floating tablets/capsules)

The system has been exploited in controlled-release formulation of diazepam (Valrelease®) by Roche (Zahirul, 1995). The drug delivery system is said to be hydrodynamically balanced and is made of a matrix designed such that on contact with gastric fluid, the dosage form shows a density of less than 1 g/cm^3 and thus, actually remains buoyant. Such dosage forms are also called 'hydrodynamically balanced drug

delivery systems' (HBS^{TM}). The system is based on the principle that devices with specific gravity less than that of gastric juice will float in the gastric juice at the stomach and retain the drug in the stomach for an extended period of time thereby increasing the total residence time or residence time in the proximal region of the gastrointestinal tract (Zahirul, 1995).

1.13.8 Matrix embedded products

A matrix may be defined as a uniform dispersion of a drug in a solid which is less soluble than the drug in the depot fluid and which as the continuous phase of the dispersion effectively impedes passage of the drug from the matrix to the depot fluid (Rawlins, 1980). This is one of the least complicated approaches to the manufacture of sustained-release dosage forms. It involves the direct compression of blends of drug, retardant material and additives to form a tablet in which drug is embedded in a matrix core of the retardant. Alternatively, retardant/drug blends may be granulated before compression. As the drug leaches from the matrix, the path over which it must diffuse increases so that with time the drug-containing portion is surrounded by an empty matrix through which the remaining drug must diffuse (Rawlins, 1980).

1.13.9 Mucoadhesion

Mucoadhesive or bioadhesive delivery systems have been demonstrated as effective dosage forms for controlled delivery of various drugs using buccal, occular, rectal, vaginal, nasal, sublingual and oral routes (Duchene *et al.*, 1988; Dondetti *et al.*, 1996). Also refer to section 1.9.6. Several other methods of achieving sustained-release of medicaments have been described to include lantiated drug or prodrug, use of lysosomes, presentation of drugs in resealed erythrocytes (Zahirul, 1995).

1.14 HYDROPHILIC MATRIX MATERIALS

Hydrophilic matrix materials include methylcellulose, hydroxymethylcellulose, sodium carboxymethylcellulose, carboxypolymethylene, galactomannose, sodium alginate, tragacanth and carboxyvinyl polymer. These are non-digestible materials that absorb water and swell to form gels. The drug release is controlled by hydration of the polymer and diffusion of drug through the swollen, hydrated matrix, in addition to the erosion of the gel layer. The extent to which diffusion or erosion control the release of the drug from polymer matrix depends on the polymer type and also on the drug/polymer ratio. Hydrophilic matrix materials have been largely used in the

formulation of sustained-release formulations (James *et al.*, 1985; Peppas and Khare, 1993; Risk *et al.*, 1994). Hydrogels are a special class of hydrophilic water-insoluble or slowly dissolving materials available in a variety of molecular weights and compositions that absorb water and swell to form a gel-like structure (Peppas and Khare, 1993). Bioadhesion or mucoadhesion is also a property observed with some of these hydrogels and therefore can serve dual purpose of release control and mucoadhesion (Guru *et al.*, 2001). Most mucoadhesive polymers described in literature are hydrophilic materials (Park and Robinson, 1984; Harris *et al.*, 1988; Duchene *et al.*, 1988; Gupta *et al.*, 1990; Zahirul, 1995; Dondetti *et al.*, 1996).

1.15 HYDROPHOBIC MATRIX MATERIALS

These include polyethylene, polyvinylchoride, polymethylacrylate-methacrylate copolymer and ethyl cellulose. They have been used as the basis for many marketed sustained-release formulations. Tablets produced from these materials are designed to be ingested intact and not to break apart in the gastrointestinal tract. The tablets may be directly compressed from mixtures of drug and ground polymer. The rate limiting step in controlling release from these formulations is liquid penetration into the matrix, unless wetting agents are included to promote permeation of the polymer matrix by water which allows drug dissolution and diffusion from the channels created (Efentakis *et al.*, 1997).

1.16 KINETICS AND MECHANISMS OF DRUG RELEASE FROM MATRIX TABLETS

The mechanism of drug release from tablet matrices can be obtained by fitting the release data into the zero-order, first-order and Higuchi kinetic equations (Higuchi, 1963):

$$Zero\ order: \quad Q_t = Q_o + K_o t \qquad \text{---------- (Equation 1.18)}$$

$$First\ order: \quad lnQ_t = lnQ_o + K_1 t \qquad \text{---------- (Equation 1.19)}$$

$$Higuchi: \quad Q_t = K_H t^{1/2} \qquad \text{---------- (Equation 1.20)}$$

Where, Q_t is the amount of drug released at time t, Q_o is the initial amount of drug released, and K is the kinetic constant.

The release of drug from polymer matrix tablets is a diffusion controlled mechanism (Higuchi, 1963). It involves the uptake of aqueous fluids or water by the tablet with

swelling of the polymer to form a gel-layer or barrier from which soluble drug diffuses out of the swollen matrix. The operating principle controlling drug release from matrix tablets is that on exposure to aqueous fluids the tablet surface becomes wet and the polymer starts to partially hydrate to form a gel layer. An initial burst of soluble drug from the external layer may be released. An expansion of the gel layer as water penetrates the tablet further increases the thickness of the gel layer and soluble drug diffuses through the gel barrier. Concomitantly, the outer layer becomes fully hydrated and dissolves, a process generally referred to as erosion. Water continues to penetrate towards the core until it dissolves. Thus, drug release from a matrix could involve diffusion and erosion of the gel layer. This drug release through a matrix tablet can be characterised mathematically according to the exponential relationship derived by Korsmeyer *et al.*, (1983) for drug release from a polymeric system:

$$\frac{M_t}{M_f} = Kt^n \qquad \text{-------- } \textit{(Equation 1.21)}$$

Where, $\frac{M_t}{M_f}$ is the fraction of drug release, t is the released time, K is the kinetic constant incorporating the properties of the macromolecular polymeric system and the drug (or the structural and geometric characteristics of the release device), and n is the release exponent indicative of the mechanism of release. A value of $n = 0.45$ indicates square root of time kinetics (case I or Fickian diffusion), $n = 0.89$ indicates case II transport, $0.45 < n < 0.89$ indicates anomalous behaviour or non fickian diffusion, and $n > 0.89$ for super case II transport (Korsmeyer *et al.*, 1983; Peppas, 1985; Ritger and Peppas, 1987; Talukdar and Kinget, 1995; Sujja-Areavath *et al.*, 1996).

Converting *equation 1.21* to the logarithmic form:

$$Log\ (M_t/M_f) = Log\ k + n\ Log\ t \qquad \text{-------- } \textit{(Equation 1.22)}$$

A plot of log of fractional drug release or percent release ($Log\ (M_t/M_f)$) against the log of time ($Log\ t$) will yield a straight line. The slope of the line gives n, and the intercept gives $Log\ k$ (Peppas, 1985; Ritger and Peppas, 1987).

1.17 ADVANTAGES OF ORAL CONTROLLED DRUG DELIVERY

Oral controlled-release drug delivery systems provide a continuous delivery of drugs at predictable and reproducible kinetics for a predetermined period during the course of gastro-intestinal transit. There is a reduced frequency of drug administration and total dose. There is also an increased safety margin of high potency drugs and reduced

adverse effects. Other advantages include convenience of drug administration and improved patient compliance as well as maintenance of more even blood level and increased reliability of therapy.

The conventional controlled-release formulations can simply delay the release process to some extent but are unable to restrain and localize the system in required areas of the gastro intestinal tract for the required time. The bioadhesive system can delay the gastric emptying of the device thereby increasing the residence time. This means solution of bioavailability problems resulting from a short duration of dosage form at absorption level of the active ingredient (Mikos and Peppas, 1986). This approaches the goal of a once daily dosing. Various dosage forms have been formulated in this way (Mikos and Peppas, 1986).

1.18 MUCOADHESIVE DRUG DELIVERY SYSTEMS

1.18.1 Mucoadhesion

Orabase®, one of the earliest developed mucoadhesive formulation was formulated from natural gums. Natural or hydrophilic polymers have since gained a considerable growing interest in the development of mucoadhesive delivery systems. The adhesive properties of these delivery platforms account for the intimate contact between the delivery system and the absorbing surface and the consequent advantages offered by such systems.

1.18.2 Mechanism of mucoadhesion

The mucoadhesion process involves three regions (Murtazavi *et al.*, 1993): the surface of the mucoadhesive polymer, the mucosal surface, and the interfacial layer between the 2 surfaces which primarily consists of mucus (Duchene *et al.*, 1988). For mucoadhesion to occur therefore, a succession of phenomena is required, whose role depends on the mucoadhesive nature.

1.18.2.1 Intimate contact

The mucoadhesive material has to penetrate the crevices of the tissue on which it is applied. The tissue surface roughness is an important factor for mucoadhesion. This is defined by the aspect ratio of maximum depth, d to maximum width, h (Mikos and Peppas, 1986). Insignificant roughness for adhesive purposes occurs when aspect ratio takes values of $d/h < {}^{1}/_{20}$. For higher values of this ratio, only highly fluid materials

can penetrate the tissue anomalies, and therefore their viscosity and melting power are of the greatest importance for satisfactory mucoadhesion (Duchene *et al.*, 1988).

1.18.2.2 Interpenetration

Intimate contact of mucoadhesive polymer with tissue is followed by interpenetration of chains from the polymer and mucus to a depth sufficient to create semi-permanent bonds. During chain interpenetration, the molecules of the mucoadhesive and glycoproteinic network are brought together into contact and due to the concentration gradient, the adhesive polymer chains penetrate at rates which depend on the diffusion coefficient of a macromolecule through a cross-linked network and the potential gradient (Mikos and Peppas, 1986; Duchene *et al.*, 1988; Murtazavi *et al.*, 1993). This process is shown in figure 1.4.

With cross-linked polymers, interpenetration of large chains occurs with greater difficulty. However, smaller chains and chain ends may still contribute to inter-diffusion (Murtazavi *et al.*, 1993).

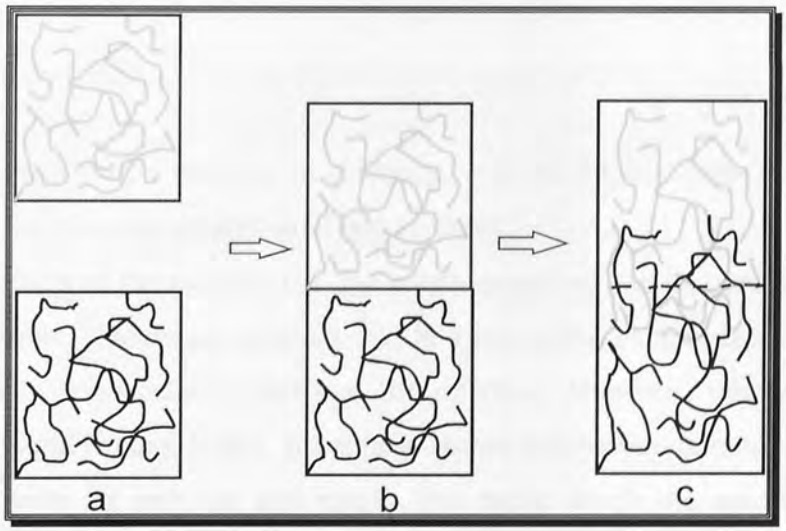


Figure 1.4: Diffusion theory of adhesion (a) Top (polymer) layer and bottom (mucus) layer before contact; (b) top layer and bottom layer immediately after contact; (c) top layer and bottom layer after contact for a period of time. Adapted from Andrews *et al.*, (2009).

1.18.2.3 Chemical interactions

Adhesive chemical bonds are of the primary or secondary type (Murtazavi *et al.*, 1993). Primary chemical bonds are covalent in nature. Their high strength results in permanent bonds undesirable in mucoadhesion. Secondary chemical bonds comprise a group of many different forces of attraction including electrostatic forces, *Van der*

waals forces, and hydrogen and hydrophobic bonds. *Van der waals* forces are all the interactions between uncharged molecules. They can be attributed to three types of effect: Polar (or Kessom) forces resulting from the orientation of permanent dipoles in two molecules, induction forces arising from a permanent dipole in another molecule, and dispersion (or London) forces resulting from instantaneous changes in the charge distribution around non-polar molecules. Hydrogen bonding occurs when a specific hydrogen atom from one molecule is associated with another atom from a second molecule. Hydrophobic bonding occurs when non-polar groups associate with each other in aqueous solution, due to the tendency of water molecules to exclude non-polar molecules. This type of force is so far the most important in mucoadhesion (Duchene *et al.*, 1988).

1.18.2.4 Surface separation

The fracture theory of mucoadhesion attempts to relate the difficulty of separation of two surfaces after adhesion due to the adhesive bond strength. The fracture strength, δ, equivalent to the mucoadhesive bond strength, may be calculated by the following equation:

$$\frac{E\in}{L} \qquad \text{------- } \textit{(Equation 1.23)}$$

Where, E is the Young's modulus of elasticity, $\in$ is the fracture energy, and L is the critical crack length upon separation of two surfaces.

Thus, the stiffness of the material (i.e. the elastic modulus) can be used as a measure of mucoadhesion. This theory assumes that in a separation experiment the failure of the bioadhesive bond occurs exactly at the interface. However, this almost never occurs (Mikos and Peppas, 1986). It has been shown that rupture does not occur at the interface between the polymer and mucus, but rather inside the mucus (Park and Robinson, 1985; Mikos and Peppas, 1986).

1.18.3 Factors affecting mucoadhesion

The likelihood and degree of polymer/mucus interaction can be affected by a number of factors made obvious by the mechanism of mucoadhesion already outlined (see section 1.18.2). This leaves a lot of room for designing and tailoring of mucoadhesive delivery systems by modification or control of such polymer properties. Although most of the mucoadhesive polymers described in literature are hydrophilic in nature, not all hydrophilic polymers possess good mucoadhesive properties (Duchene *et al.*,

1988). There are several comprehensive reviews available in the literature that describes the properties a polymer should have in order to possess good mucoadhesive properties (Park and Robinson, 1985; Gu *et al.*, 1988; Duchene and Ponchel, 1992; Jimenez - Castellanos *et al.*, 1993; Dondetti *et al.*, 1996).

1.18.3.1 Functional group contribution

Mucoadhesive polymers attach and bond to the biological substrate primarily by interpenetration (also see 1.18.2.2). Interpenetration is quickly followed by secondary non-covalent bonding between the mucoadhesive polymer and the biological substrate. This secondary bonding is a consequence of hydrogen bond formation and as a result hydrophilic polymers which possess functional groups such as carboxyl (*COOH*), hydroxyl (*OH*), amide (NH_2) and sulphate (SO_4H) groups may be better candidates for formulation of mucoadhesive drug delivery platforms (Andrews *et al.*, 2009). Polymers with high density of available hydrogen bonding groups interact strongly with mucin glycoproteins because physical entanglements and secondary interactions (hydrogen bonds) contribute to the formation of a strengthened network (Madsen *et al.*, 1998). Because mucoadhesives are hydrophilic polymers that contain numerous polar functional groups, they are able to interact with mucus through both physical entanglements and secondary chemical bonds which result in the formation of weakly cross-linked networks (Capra *et al.*, 2007).

1.18.3.2 Degree of hydration and swelling

This characteristic is related to the polymer itself, and also to its environment. As mentioned earlier (1.18.2.2), interpenetration of chains is easier as polymer chains are disentangled and free of interactions. Hydration and swelling is essential for interpenetration of the polymer chains and depends on polymer concentration and on water presence. Mucoadhesive polymers have hydrophilic functional groups which form hydrogen bonds such as carboxyl, hydroxyl, amide and sulphate groups. Hydrogen bonding seems to play a dominant role and hence, the amount of water present at the interphase between the adhesive polymer and biological membrane and/or mucus is critical for bioadhesion. When mucoadhesives hydrate in aqueous media, they swell and form a gel. The rate and extent of water uptake by the mucoadhesive depends on the type and number of hydrophilic functional groups present in the polymer structure as well as the pH and ionic strength of the aqueous medium. It has been found that as the percent composition of charged groups on a

polymer decreases, the degree of hydration decreases (Leung and Robinson, 1990). Swelling time is an important parameter for assessing mucoadhesiveness. The faster a polymer is hydrated, the faster will be initiation of diffusion, formation of bonds and an entangled interface and thus the faster the initiation of the mucoadhesion process. The degree of swelling of a polymer is another parameter that contributes to the mucoadhesive behaviour.

It has been reported that when hydration and swelling is excessive, a decrease in mucoadhesion occurs due to the formation of slippery mucilage (Gurny *et al.*, 1984; Mortazavi and Smart, 1993). Such a phenomenon must not occur too early, in order to lead to a sufficient action of the mucoadhesive system. Nevertheless, its appearance allows easy detachment of the mucoadhesive system after the discharge of the active ingredient. A prolonged mucoadhesive effect may be better provided by polymers that only permit a certain degree of hydration (Andrews *et al.*, 2009).

1.18.3.3 Polymer molecular weight, chain length, conformation and degree of cross-linking

As described by Gurny *et al.*, (1984), it seems that the mucoadhesive force increases with the molecular weight of the polymer up to one hundred thousand, and beyond this level there is not much effect. To allow chain interpenetration the polymer molecule must have an adequate length. It is also necessary to consider the size and configuration of the polymer molecule. For example, with polyethylene oxide, adhesive strength increases even up to molecular weights of four million (Yang and Robinson, 1998). These polymers are well known to contain molecules of highly linear configuration, which contribute to interpenetration. On the other hand with dextran, molecules with molecular weight as high as 19.5 million do not exhibit better mucoadhesion than molecules with a molecular weight of two hundred thousand (Gu *et al.*, 1988).

Chain mobility and resistance to dissolution can be significantly influenced by the degree of cross-linking within a polymer system. While the cross-linked mucoadhesive polymers hydrate and swell in aqueous medium to retain their structure, the high molecular weight linear hydrophilic polymers are swellable and readily dispersible. Apart from allowing for greater control of drug release, the swelling process also increases the surface area for polymer/mucus interpenetration. There is a decrease in chain mobility and the effective chain length which can penetrate into the

mucus layer with increase in cross-linking density, resulting in a reduction in mucoadhesive strength (Sudhakar *et al.*, 2006). Critical for interpenetration and entanglement with mucus gel is the chain flexibility. An increase in chain mobility can lead to increased inter-diffusion and interpenetration of the polymer within the mucus network (Imam *et al.*, 2003).

1.18.3.4 Polymer pH and charge

The absorption of water by a polymer, and hence its swelling, depends to a great extent on the pH up to an optimum. Mucoadhesivity is also dependent on pH. There is an optimal pH for polymer adhesion (Ching *et al.*, 1985).

1.18.3.5 Polymer concentration

Bremecker (1983) stated that there is an optimum concentration of polymer corresponding to the best mucoadhesion. In highly concentrated systems, the adhesive strength drops significantly. In fact, in concentrated solutions, the coiled molecules become solvent - poor and the chains available for interpenetration are not numerous. This is of interest only for more or less liquid mucoadhesive forms (Gurny *et al.*, 1984). Ponchel *et al.*, (1987) showed that the higher the polymer concentration, the stronger the mucoadhesion for solid dosage forms such as tablets.

1.18.3.6 The applied strength

Another factor that affects mucoadhesive strength is the pressure applied to the interacting adhesive-mucin interface. It is obvious that, to place a solid mucoadhesive system, it is necessary to apply a defined strength. The adhesive strength increases with the applied strength or with the duration of its application to an optimum (Ritger and Peppas, 1987; Duchene and Ponchel, 1992).

1.18.4 Advantages of mucoadhesive delivery systems

Mucoadhesion has been advocated as a means of achieving site-specific drug delivery. Mucoadhesive hydrophilic polymers when incorporated into pharmaceutical formulations along with the active pharmaceutical ingredient (API) are capable of attaching to mucosal surface for localised drug delivery as the API gets released close to the site of action with consequent enhanced bioavailability. However, when systemic absorption occur the use of mucoadhesive polymers will not prevent wide distribution of the API.

Mucoadhesive drug delivery systems offer a number of advantages (Andrews *et al.*, 2009):

(1) Adhesion, intimate contact and increase residence time of the formulation at the site of action means improve bioavailability using lower concentrations of API.

(2) Increased residence time of dosage form in conjunction with controlled release of the API can lead to lower administration frequency and as a consequence improve patient compliance.

(3) There is possibility of target specific delivery of API by the use of specific mucoadhesive molecules. Therefore site specific delivery to the gastro-intestinal tract and other sites have become a possibility.

(4) First-pass metabolism of API can be avoided.

(5) Reduced cost of medicaments can be achieved because of the possibility of reduced dosage, and dose related side effects can be reduced by API localisation at site of action.

1.19 GREWIA POLYSACCHARIDE GUM/BACKGROUND TO STUDY

Grewia gum is a polysaccharide derived from the inner stem bark of the edible plant *Grewia mollis* Juss (Fam. Tiliaceae). The plant is a savannah shrub which grows widely in Nigeria but is also cultivated (Keay *et al.*, 1964). The leaves and bark of the plant contain mucilage (Gill, 1992). In Nigeria, the dried and pulverised inner stem is used as a thickening agent in some local dishes. The mucilage obtained from this plant has been previously studied (Okafor *et al.*, 2001). The physicochemical properties of the gum studied so far show that the gum consists of glucose, rhamnose and galacturonic acid with traces of metals (Ca, K, Na, Mg, Zn, Fe) and a viscosity–average-molecular weight of 316,000 (Okafor *et al.*, 2001). Metals constitute about 6.1 % of the gum, while proteins and lipids account for 1.24 and 0.021 %, respectively. It has a high intrinsic viscosity with a swelling capacity greater than tragacanth and methylcellulose. The gum is claimed to be compatible with many drugs and excipients and is also non-toxic. It has been reported that aqueous dispersions of the gum in moderate concentrations exhibit pseudoplastic flow behaviour (Okafor *et al.*, 2001). The gum has been shown to be as effective as gelatin when employed as a binder in concentrations of 2-6 % w/w. At the same concentration, the gum is more effective than maize starch and acacia as a binder (Okafor *et al.*, 2001).

The foregoing accounts for the interest in grewia polysaccharide gum as a potential excipient for the pharmaceutical industry.

1.20 AIMS AND OBJECTIVES OF STUDY

Natural gums have a wide range of applications in both food and pharmaceutical industry (see section 1.9). Their exploitation provides the industry with a range of options to choose from. Their relatively free availability and renewable nature has made them a more affordable source of industrial raw material. Grewia gum may provide a suitable alternative to its natural, semi-synthetic or synthetic counterparts such as tragacanth, acacia gum, guar gum, carboxymethylcellulose, sodium carboxymethylcellulose and hydroxypropyl methylcellulose. In Nigeria where the plant is cultivated and also found growing wild, it may provide a more affordable choice of excipient for industry, save foreign exchange, and provide a source of income for the local people.

The aims of this research include, but are not limited to the evaluation of the potential applications of grewia gum as a pharmaceutical excipient. The characterization of the material with particular focus on the effects of drying techniques on its physicochemical properties will be investigated. To achieve these aims, the following objectives will be pursued:

1. Procurement, authentication and processing of the plant
2. Extraction and purification of the gum from the crude pulverized inner stem bark of the plant and drying by three methods- air-drying, freeze-drying and spray-drying.
3. Physicochemical characterization of the processed gum. Properties such as pH, moisture, solubility, ash content, viscosity and flow of dispersions, parameters of powder flow (angle of repose, Carr's compressibility index, Hausner ratio, bulk and tapped densities, true density), elemental composition, intrinsic viscosity and molecular weight, compressibility, and thermal, film and swelling properties.
4. Comparative evaluation of the suspending ability of grewia gum in ibuprofen suspension. The suspending ability of grewia gum will be compared with standard suspending agents such as acacia gum, sodium carboxymethylcellulose and xanthan gum at three levels of concentration (0.5%, 0.75% or 1.0% w/v). The evaluation parameters will include viscosity and rheology, redispersibility,

sedimentation volume, degree of flocculation, electrokinetic properties (zeta potential), and microbiological evaluation.

5. Comparative evaluation of the release retardant property of grewia gum in cimetidine and ibuprofen matrix tablets. Standard hydrophilic matrix materials such as guar, acacia, Metolose®, Methocel® and carboxymethylcellulose will be used as reference polymers including ethyl cellulose, a hydrophobic release retardant.
6. Evaluation of mucoadhesive performance of grewia gum. Mucoadhesive systems of grewia gum will be compared with similar systems of hydroxypropyl methylcellulose, carboxymethylcellulose, guar gum and Carbopol 971P®.

CHAPTER TWO

MATERIALS AND METHODS

2.1 SUMMARY

An overview of the experimental techniques used in this thesis is presented in this chapter. Any methods specifically relevant to individual chapters are detailed in the appropriate chapters and sections. Unless otherwise stated, all materials were of analytical grade and procured from Sigma/Aldrich (Poole, U.K.) The results from all studies are expressed as the mean ± the standard deviation of at least three values, unless otherwise indicated.

2.2 STATISCAL ANALYSIS

The main statistical test employed for analysis of results was the one-way analysis of variance (ANOVA), carried out using the GraphPad software (GraphPad Instat) at 95% confidence interval.

2.2.1 Dissolution profile comparison using similarity factor, f_2

The equation of similarity factor (f_2) as proposed by Moore and Flanner (1996) was employed to compare dissolution profiles of drug in formulations containing the test polymer and the dissolution profiles of drug in formulations containing the reference polymers. This is a model independent approach for comparing dissolution profiles.

$$Similarity\ factor, f_2 = 50\ log\left\{\left[1 + \frac{1}{n}\sum_{n-1}^{n}(R_t - T_t)^2\right]^{0.5} \times 100\right\} \quad -Equation\ 2.1$$

Where, n is the number of observations made, R_t is the average percentage drug dissolved from reference formulation and T_t is the average percentage drug dissolved from test formulation. When two profiles are identical, $f_2 = 100$. A similarity factor, f_2 of 50-100 has been set by the FDA to indicate similarity between two dissolution profiles.

The similarity factor (f_2) which represents a pair-wise model independent procedure (Costa and Manuel, 2001) was used to assess the similarity between any 2 dissolution profiles. This was done for both ibuprofen and cimetidine matrices. An f_2 of 100 suggests that the test and reference profiles are identical. As the value becomes smaller, the dissimilarity between the dissolution or release profiles increases. The test profiles for ibuprofen matrices were grewia 16%, 32% and 48% for the monolithic matrices as appropriate while grewia 16% was the test profile for the binary composite matrices. For the cimetidine matrices grewia 40% was the test profile for both monolithic and binary composite matrices.

2.2.2 Coefficient of determination (r^2)

The coefficient of determination obtained by fitting the release data into the zero-order, first-order, Korsmeyer-Peppas and Higuchi kinetic equations (see section 1.16) was used to predict correlation of the release profiles with the various kinetic equations. As the strength of the relationship between the release profiles and the kinetic equations increases so does the correlation coefficient. A perfect fit gives a coefficient of 1.0. Thus the highest correlation coefficient gives the best correlation with the kinetic equation in question.

2.3 EXTRACTION, PURIFICATION AND DRYING OF GREWIA GUM

The method reported by Tamoda *et al.*, (1977), was adopted with slight modification. Briefly, 2000 grams of the dried and pulverized inner stem bark of *Grewia mollis* was dispersed in 10 litres of 0.1 % sodium metabisulphite and allowed to hydrate for 48 hours. After this time the mixture was stirred continuously for 2 hours and filtered through a muslin bag. The filtrate was treated with 0.1N NaOH and centrifuged (Mst, Miww centrifuge, UK) at 3,000 rpm for 10 minutes to remove alkali-soluble impurities. The supernatant was then treated with acidified ethanol containing 0.1N HCl to dissolve and isolate acid soluble impurities, and centrifuged again as described. Absolute ethanol was then used to precipitate the solid material in the supernatant. This precipitate was washed several times with absolute ethanol and then wet-milled using a blender. Excess ethanol was removed by expressing through a muslin bag before air-drying. The air-dried product was first dry-milled before further drying in the oven at 50°C for 24 hours. The dried product was passed through a 1.0 mm sieve, weighed and stored in air-tight containers.

In order to freeze dry or spray dry, the pure gum precipitate obtained following the procedure outlined above was re-dispersed in distilled water. Freeze drying was then achieved in a freeze dryer (Modulyo, Germany) at - 40°C for 72 hours after freezing for 24 hours at -70°C. A mini spray dryer, B290 (Buchi, Switzerland) was used at an inlet temperature of 160°C, while the aspirator and pump were set to 85 % and 5 % respectively to spray-dry the product.

2.4 TOTAL AND SOLUBLE ASH

The AOAC (1990) method was adopted. A 1.0 gram portion of the gum sample was weighed into a pre-ignited and pre-weighed crucible, and transferred into a furnace (Carbolite, Sheffield-England). Ignition was maintained at 550°C for 24 hours. After ignition the ash in the crucible was transferred into a desiccator to equilibrate to room temperature before weighing.

To determine soluble ash, the resultant ash, after ignition of the gum sample for 24 hours, was mixed with distilled water (25 ml), boiled and filtered through an ash-less filter paper (Whatman Ashless 542). The filter paper was rinsed with distilled water until the filtrate volume reached 60 ml. Both filter paper and residue were then transferred into the crucible and ignited for 24 hours until constant weight before cooling in a desiccator and weighing. The ash content was determined for air-dried, freeze-dried or spray-dried grewia polysaccharide gum samples.

Percent total ash was calculated using the formula:

$$\%\, Total\ ash = \frac{ash\ weight}{original\ sample\ weight}\ X\ 100 \qquad \text{------- } \textit{(Equation 2.2)}$$

Percent soluble ash was calculated from the formula:

$$\%\ soluble\ ash = \%total\ ash - \%\ insoluble\ ash \qquad \text{------- } \textit{(Equation 2.3)}$$

2.5 VISCOSITY AND FLOW BEHAVIOUR OF AQUEOUS DISPERSION OF GREWIA GUM

A 1.0 % w/v dispersion of the polysaccharide gum sample was hydrated and homogenised using a Silverson L4R homogenizer ((Silverson, England). Viscosity (in centipoises), was read using a Brookfield DV – 1+ viscometer version 5 (Brookfield Engineering Labs, Stoughton-USA). Viscosity was read at varying shear rates between 5 to 100 rpm and at 23°C using a sample volume of 200 ml in a 250 ml beaker (Schott Duran, Germany). Spindle number 2 was used and 3 min was allowed for stability of readings before viscosity was read.

2.6 SOLID-STATE ^{1}H NMR ANALYSIS

A Varian VNMRS spectrometer (Varian, USA) operating at 102.56 MHz for ^{13}C and with a cross-polarization methodology was used for the analysis of air-dried, spray-dried or freeze-dried grewia polysaccharide gum samples. Acquisition time was 20.0

ms. The samples were run without modification and the spectra were referenced with respect to tetramethylsilane.

2.7 DILUTE SOLUTION VISCOMETRY

Grewia polysaccharide gum sample was hydrated in water (0.1% w/v) for 24 hours at room temperature. The hydrated dispersion was then filtered and serially diluted to obtain 0.00625, 0.0125, 0.025 and 0.05% w/v solutions of the polysaccharide. The apparent viscosity of these solutions of the gum was determined using automated Anton Paar micro-viscometer (AmVn, Graz-Germany) calibrated using water at 20°C, and at an angle of 50°. The intrinsic viscosity [η] was determined according to the Huggins (Huggins, 1942) and Kraemer (Amalvy, 1997) equations:

$$\eta_{sp} = [\eta]c + K'[\eta]^2c^2 \quad \text{------ (Equation 2.4)}$$

$$ln(\eta_{rel}) = [\eta]c + (K' - 0.5)[\eta]^2c^2 \quad \text{------ (Equation 2.5)}$$

Where, η_{sp} = specific vicosity,

η_{rel} = relative viscosity,

c = concetration of polymer and,

K' = Huggins coefficient.

The results were plotted in the forms of $\frac{\eta_{sp}}{c}$ or $\frac{\ln(\eta_{rel})}{c}$ Vs c. The average of the two intercepts was used as the intrinsic viscosity of the gum (Amalvy, 1997).

2.8 GEL FILTRATION CHROMATOGRAPHY

Gel filtration chromatography was carried out to estimate the molecular weight of grewia polysaccharide gum samples relative to pullulan polysaccharide calibrants. The eluent used was 0.2M $NaNO_3$ and 0.01M NaH_2PO_4 (pH of 7.0) at a nominal flow rate of 1.0 ml/min and a temperature of 35°C. The columns used were Plaquagel Guard Plus 2 x mixed-OH, 30 cm 8 μm columns and a refractive index detector (with differential pressure and light scattering) was used. The data were collated and analysed using Polymer Laboratories 'Cirrus' software. The sample was prepared by adding 10 ml of eluent to 20 mg of sample, hydrated overnight and warmed to 40°C for at least 20 minutes. After cooling, the solutions were thoroughly mixed and filtered through a 0.45 μm PVDF membrane prior to injection on the column.

2.9 X-RAY PHOTOELECTRON SPECTROSCOPY

The analysis was conducted in a Thermofisher ESCALAB 250 electron spectrometer equipped with a hemispherical sector energy analyzer. Monochromatic Al K_α X-ray source was used at a source excitation energy of 15 KeV and emission current of 6 mA. Analyzer path energy of 20 eV with step size of 0.1 eV and dwell time of 50 ms was used throughout the experiments. XPS survey scans were first recorded for the gum sample. Narrow region energy scans were then collected for all the elements identified on the surface. To improve statistics, multiple scans were always used for all the constituents in the surface. The base pressure within the spectrometer during examinations was always higher than 5×10^{-10} mbar and this ensured that all signals recorded were from the sample surface. Each area of analysis was chosen to be 500 microns diameter, but a number of representative areas were analysed.

2.10 FT-IR SPECTROPHOTOMETRY

KBr discs of the polysaccharide gum sample were prepared by dispersion of a small amount of the polysaccharide in KBr (1:10) and blending same in a mortar using a pestle. A small amount of the powder blend was then introduced into a 13 mm mould and compressed in a KBr press to 3 tonnes for 30 minutes and then to 8 tonnes for 5 minutes. The KBr discs so obtained were placed in the oven at 50°C for 10 minutes to remove moisture before FT-IR spectroscopy. FT-IR spectra were obtained at a frequency range of 500 – 4000 cm^{-1}.

2.11 THERMAL ANALYSIS

A Thermo-gravimetric analyzer (Pyris 1 TGA Perkin Elmer, U.S.A) was used to study the thermal degradation of the polysaccharide gum sample under a nitrogen atmosphere. Approximately 1 mg of sample was introduced into the sample pan and heated at 10°C per minute up to 500°C. Results were obtained in triplicate and the representative plots and derivatives collated. Differential Scanning Calorimetry (Diamond DSC Perkin Elmer, USA) was also used to study the thermal properties of the grewia polysaccharide gum. About 4 mg of sample was accurately weighed into the sample pan and the temperature was held at 30°C for 1 minute before heating up to 200°C at a rate of 10°C per minute under nitrogen atmosphere.

2.12 ATOMIC ABSORPTION SPECTROSCOPY

Elemental composition of air-dried grewia polysaccharide gum sample was determined on an atomic absorption spectrometer (AAnalyst 100, Perkin Elmer UK). Samples were prepared by hydrating 100 mg of the gum in 100 ml of distilled water for 24 hours. The dispersions were filtered with Whatman no.1 paper and filtrates separately diluted to give 2.0, 1.0, and 0.3 mg/ml solutions. The standards used were Fe, Zn, Ca, Mg, Cu, K and Na (1 mg/ml). These were diluted according to specification and used to obtain the calibration curves.

The procedure involved selection of the appropriate lamp for each element to be determined, turning on the acetylene and compressed air to 12 and 60 Psig respectively ensuring the extractor fan was on to extract acetylene from the air. The appropriate lamp current, slit width and wavelength was entered into the program and slit height set to default.

Atomic absorption spectroscopy (AAS) determines the presence of metals in liquid samples. Metals include Fe, Cu, Al, Pb, Ca, Zn, Cd and many more. It also measures the concentrations of metals in the samples. Typical concentrations range in the low mg/L range. Figure 2.1 -2.3 shows the calibration plots for magnesium, zinc and calcium.

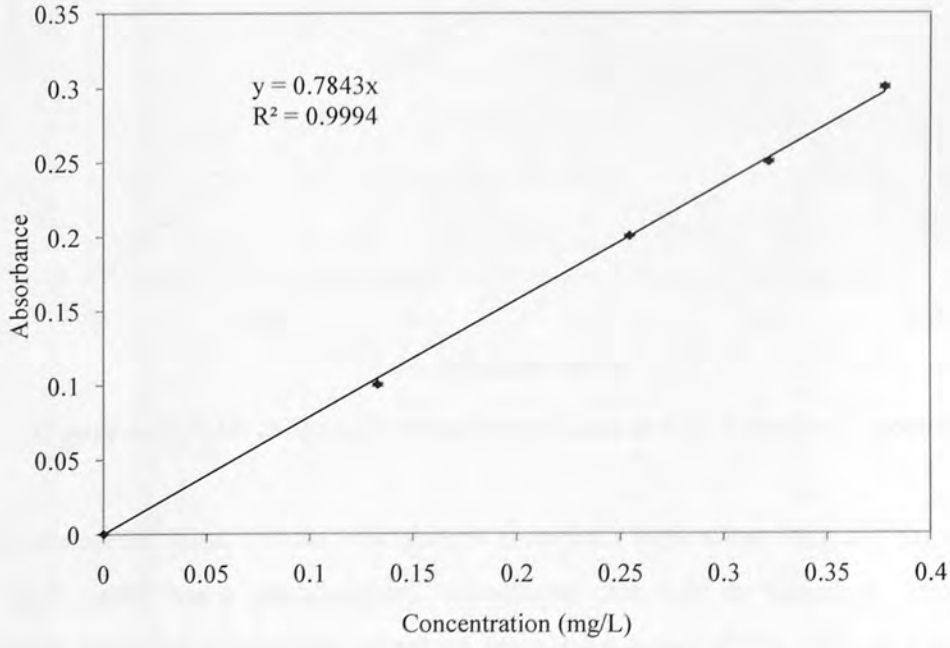


Figure 2.1: AAS calibration curve for magnesium at 285.2 nm (n=3, mean ± s.d.)

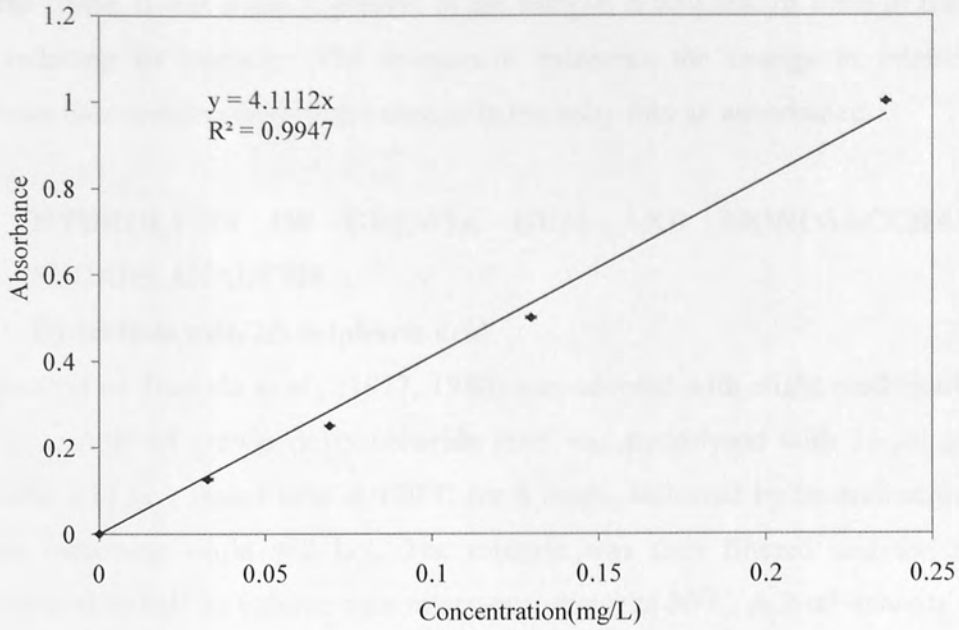


Figure 2.2: AAS calibration curve for zinc at 213.9 nm (n=3, mean ± s.d.)

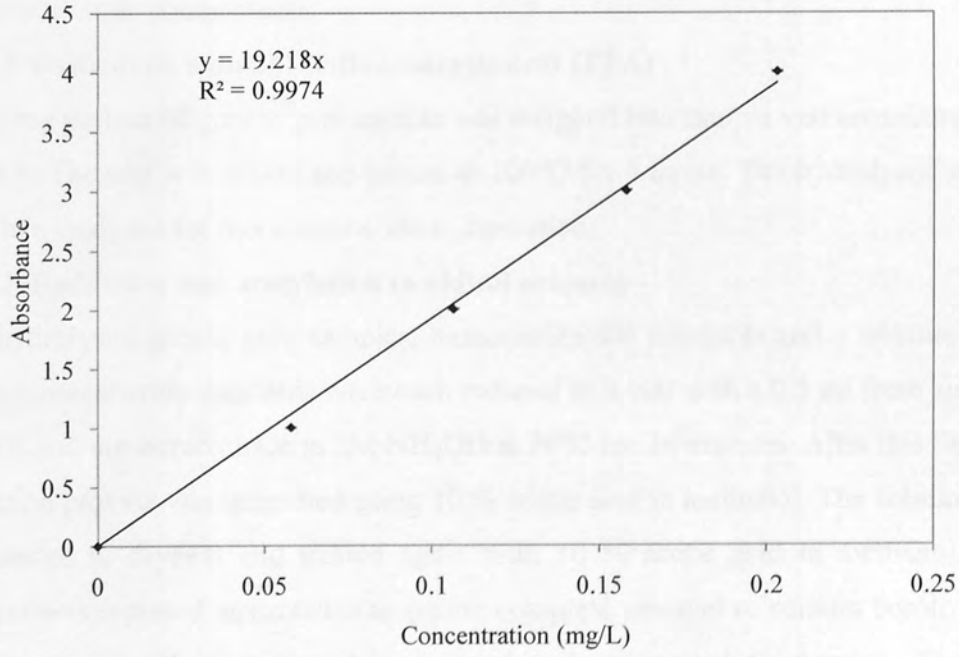


Figure 2.3: AAS calibration curve for calcium at 422.7 nm (n=3, mean ± s.d.)

In their elemental form, metals will absorb ultraviolet light when they are excited by heat. Each metal has a characteristic wavelength that will be absorbed. The AAS instrument looks for a particular metal by focusing a beam of UV light at a specific wavelength through a flame and into a detector. The sample of interest is aspirated

into the flame. If that metal is present in the sample, it will absorb some of the light, thus reducing its intensity. The instrument measures the change in intensity. A computer data system converts the change in intensity into an absorbance.

2.13 HYDROLYSIS OF GREWIA GUM AND MONOSACCHARIDE SUGARS ANALYSIS

2.13.1 Hydrolysis with 2N sulphuric acid

The method of Tomoda *et al.*, (1977, 1980) was adopted with slight modification. A 0.75 g quantity of grewia polysaccharide gum was hydrolysed with 15 ml of 2 N sulphuric acid in a sealed tube at 100°C for 6 hours, followed by neutralisation with barium carbonate while still hot. The mixture was then filtered and the filtrate concentrated to half its volume on a rotary evaporator at 50°C. A 2 ml quantity of the filtrate was mixed with 18 ml of methanol and the resultant cloudy precipitate was centrifuged at 1000 rpm for 40 minutes. The supernatant was then analysed for monosaccharide composition.

2.13.2 Hydrolysis with 2M trifluoroacetic acid (TFA)

A 1.0 mg portion of grewia gum sample was weighed into sample vial containing 2 ml of TFA. The vial was sealed and heated at 100°C for 4 hours. The hydrolysed sample was then analysed for monosaccharide composition.

2.13.3 Reduction and acetylation to alditol acetates

The hydrolysed grewia gum samples, monosaccharide standards and a mixture of all the monosaccharide standards were each reduced in a vial with a 0.3 ml fresh solution of 10% sodium borohydride in 2M NH_4OH at 20°C for 30 minutes. After this time the reduction process was quenched using 10 % acetic acid in methanol. The solution was evaporated to dryness and treated again with 10 % acetic acid in methanol. This process was repeated again twice to ensure complete removal of sodium borohydride. Thereupon 0.5 ml of methanol was added and evaporated to dryness. This was repeated twice. Acetylation was then achieved by adding 0.1 ml of acetic anhydride and 0.1 ml of pyridine and heating at 100°C for 20 minutes. The acetylated product was then evaporated to dryness. 0.5 ml of toluene was added and then evaporated to dryness. This was done twice. Finally, partitioning was done with 0.5 ml distilled water and 0.5 ml anhydrous chloroform with shaking. The alditol derivatives were recovered with the chloroform layer. Further partitioning was done using 0.5 ml of

chloroform a second and a third time. The recovered chloroform was then concentrated to dryness and made up to about 0.2 ml with chloroform prior to monosaccharide analysis by gas chromatography.

2.13.4 Monosaccharide analysis

Gas chromatography (GC) was used to detect the neutral sugars of the grewia polysaccharide gum. An ATI – Unicam 610 GC (Unicam, UK) with a flame ionization detector (FID) was used under the following conditions: Column- Supelco SP-2380 (30 m, 0.25 mm id and 0.2 μm film thickness), detector temperature- 280°C, injector temperature - 260°C, column temperature - 250°C, split injection volume - 1 μL, split ratio - 50:1, Helium flow - 1 ml/minute, and 15 minute isothermal run. The standard sugars used were L-rhamnose monohydrate, D-(+)-galactose, DL-arabinose, D-xylose and D-(+)-glucose. The sample was first hydrolysed (see sections 2.13.1 and 2.13.2) and subsequently both the sample and sugar standards were converted to the alditol acetates (see section 2.13.3) before GC analysis.

2.14 ^{1}H AND ^{13}C NMR SPECTROSCOPY

Grewia polysaccharide gum hydrolysed according to the method outlined in section 2.13.1 was dissolve in D_2O and analysed using ^{13}C, and ^{1}H NMR spectroscopy on an AC 250 NMR spectrometer (Bruker, UK). The program was Iconnmr and the software was Topspin (Bruker). Before running the analysis, the polysaccharide solution containing about 20 mg in D_2O was placed in a 5 mm NMR tube and the tube with the top affixed placed in the spinner of the NMR spectrometer.

2.15 PREPARATION OF PHOSPHATE BUFFER

Exactly 100 ml of 0.1M KH_2PO_4 was added to 69.4 ml of 0.1M NaOH to produce a 0.1 Molar solution of phosphate buffer (pH 7.2). A pH meter was used to confirm the pH.

2.16 IBUPROFEN ASSAY

2.16.1 Ibuprofen

Ibuprofen is RS-2,4-(isobutyl-phenyl) propionic acid. The structure is shown below in figure 2.4. Ibuprofen is available as two enantiomers: S(+) enantiomer and the R(-)

enantiomer. It is mostly available in racemic form but some coutries use the S(+) isomer alone (Sweetman, 2004).

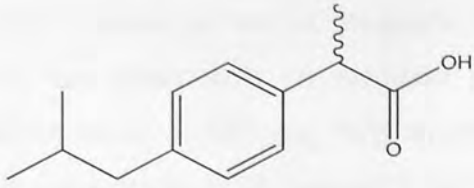


Figure 2.4: Molecular structure of ibuprofen

Ibuprofen is characterised by its safety and high therapeutic efficiency. Among the NSAIDS it is well tolerated with low incidences of gastrointestinal tract irritation (Fries *et al.*, 1991). It is the drug of first choice in the treatment of musculoskeletal disorders and different pain conditions (Valle-James *et al.*, 1984).

Ibuprofen acts by inhibition of the cyclooxygenase enzyme (COX), an enzyme responsible for conversion of arachidonic acid to prostaglandin H_2 (PGH_2). As many other NSAIDS, the analgesic and antipyretic action of ibuprofen is due to the inhibition of prostaglandin synthesis. This inhibition of COX is non-selective but mainly through COX-2. The R (-) ibuprofen show less inhibition of COX-1.

Ibuprofen is white crystalline powder that is practically insoluble in water and with a melting point of 75-78°C. Table 2.1 gives some of the physicochemical properties of ibuprofen.

Table 2.1: Physicochemical properties of Ibuprofen

Molecular formula	$C_{13}H_{18}O_2$
Solubility (mg/ml)	0.37
pKa	4.5
Molecular weight	206.3

Ibuprofen has high permeability across the cell membrane and as a result it is fully absorbed from the gastro-intestinal tract when given orally. The rate limiting step to bioavailability is the dissolution of the drug from the dosage form. Different formulations have been developed to increase ibuprofen bioavailability such as microcapsules and pro-drugs (Murtha and Ando *et al.*, 1994).

Immediate release tablets of 200 and 400 mg doses are used for pain relief in acute cases of headache, toothache, menstrual cramps, muscular aches and common cold where rapid relief is essential and achieved by rapid release of ibuprofen. These conventional immediate release tablets of ibuprofen require that the drug be administered three to four times daily. A modified release ibuprofen has been developed and is administered as 800 mg daily in chronic cases of rheumatoid arthritis, osteoarthritis and recently as an adjunct to morphine to reduce side effects (Plummer *et al.*, 1996). The advantages of the modified release tablets are providing pain relief and anti-inflammatory action over a long period of time (24 hours), and improving patient compliance by decreasing the frequency of administration (Avgerinos and Malamataris, 1990). Also, the modified release tablets decreases the early morning stiffness in rheumatoid patients which cannot be avoided when using the immediate release tablets, and local irritation to the gastro-intestinal membrane that occurs with the immediate release tablets can be avoided (Galeone *et al.*, 1981).

2.16.2 Assay of ibuprofen.

Exactly 100 mg of ibuprofen (API) was accurately weighed and dissolved in 100 ml of phosphate buffer (pH 7.2) to give a 1 mg/ml stock solution of ibuprofen. Dilutions of the stock were made using the phosphate buffer (pH 7.2) to give 0.8, 0.6, 0.4, and 0.2 mg/ml dilutions. The absorbance of the solutions was read on a Jenway 6300 UV spectrophotometer (Bibby scientific, UK) at 265 nm. The absorbances of the solutions were then plotted against the concentrations (mg/ml) to give the calibration curve for ibuprofen. Figure 2.5 shows the calibration curve for ibuprofen constructed from UV absorbance at 265 nm against concentration.

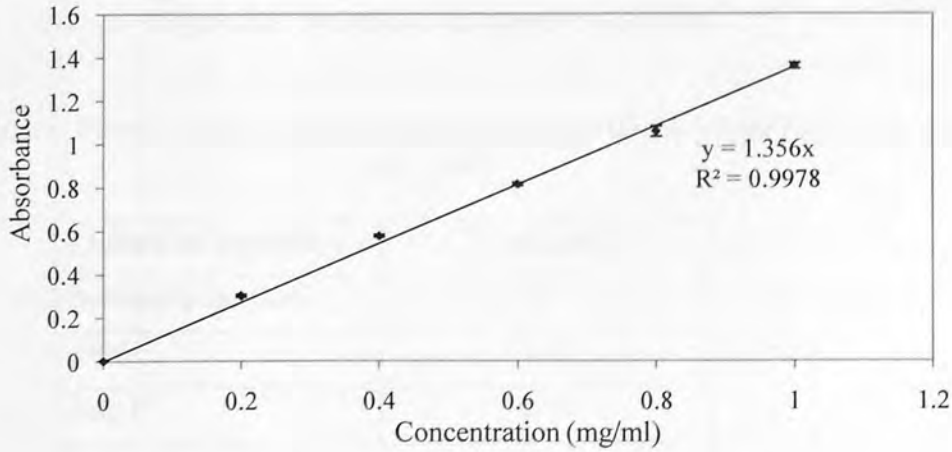


Figure 2.5: Typical calibration graph for ibuprofen assay in phosphate buffer (pH 7.2) by UV determined at 265 nm (n=3, mean ± s.d.)

2.17 CIMETIDINE ASSAY

2.17.1 Cimetidine

Cimetidine is approved by the FDA for the reduction of gastric acid secretion stimulated by food, histamine, pentagastrine, caffeine and insulin. Cimetidine is therefore administered for the treatment or management of conditions such as gastric and duodenal ulcer disease, both now known as peptic ulcer disease. Others include erosive gastro-oesophageal reflux disease or heartburn, and hyper-secretory conditions like Zollinger- Ellison syndrome (gastrinoma) and multiple endocrine adenomas and scleroderma oesophagus.

Cimetidine is marketed under the brand name Tagamet® by GlaxoSmithkline. It is also marketed as generic formulation, and is available over the counter and by prescription. It is a histamine H_2-receptor antagonist and does this by competitive inhibition of the action of histamine at the histamine H_2-receptor site of the parietal cells. It does this without significant interaction at catecholamine, β-receptors, histamine H_1-receptors or muscarinic receptors (www.gsk.com.au/resources.ashx/). By so doing the secretion of basal and gastric hydrochloric acid is inhibited along with a reduction in pepsin output. The molecular structure of cimetidine is shown in figure 2.6 and the physicochemical properties are summarised in table 2.2.

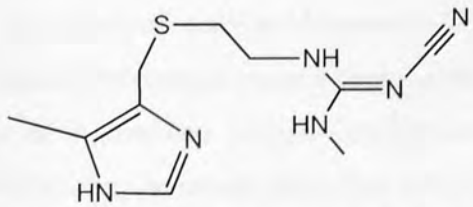


Figure 2.6: Molecular structure of cimetidine

Table 2.2: Physicochemical properties of cimetidine (Glaxosmithkline, 1984; Bavin *et al.*, 1984)

Molecular formula	$C_{10}H_{16}N_6S$
Solubility (mg/ml)	11.0
pKa	6.8
Log P	0.40
Molecular weight	252.3

Cimetidine is a white to off-white powder that is only slightly soluble in water, very slightly soluble in chloroform, insoluble in ether, but soluble in ethanol. The hydrochloride salt is however soluble in water. It has a bitter taste with a characteristic odour. In the formulation of cimetidine (Tagamet) tablets several ingredients or excipients have been used. These include microcrystalline cellulose, maize starch, povidone, sodium lauryl sulfate, magnesium stearate, sodium starch glycollate, indigo carmine CI73015, iron oxide yellow CI77492, Opadry 06H51000 green and carnauba wax (www.gsk.com.au/resources.ashx/). The tablets are marketed in 200, 400 and 800 mg doses.

Cimetidine is well absorbed after oral administration attaining a bioavailability of 70% (GlaxoSmithkline, 1984). Studies carried out using a 200 mg dose of cimetidine show that blood levels of about 2.8 µmol/L (0.7 mg/L) can be attained within 45 to 75 minutes of administration (GlaxoSmithkline, 1984). About 34% of the drug is recovered in the urine 2 hours after dosing and up to 70% after 24 hours with a protein binding of 13-26%.

The side effects to cimetidine has been reported to include headache, diarrhoea, constipation, dizziness, drowsiness, tiredness, rash, musculoskeletal pain, nausea, vomiting, flatulence, fever, and depression.

Cimetidine is usually administered 8 hourly with meals. An additional dose at bedtime is advised to inhibit nocturnal and basal acid secretion. This frequent dosing regimen for cimetidine can present problems of patient compliance and this can be reduced by delivering cimetidine as a sustained release formulation. Controlled release matrix formulations of cimetidine may be beneficial to this effect.

2.17.2 Assay of cimetidine

Exactly 12.5 mg of cimetidine (API) was accurately weighed and dissolved in 1000 ml of phosphate buffer (pH 7.2) to give a 0.0125 mg/ml solution of cimetidine. Dilutions of this were made using the phosphate buffer (pH 7.2) to give serial dilutions of up to 3.9×10^{-4} mg/ml dilution. The absorbance of the solutions was read on a Jenway 6300 UV spectrophotometer (Bibby scientific, UK) at 228 nm and plotted against the concentrations (mg/ml) to give the calibration curve for cimetidine (figure 2.7).

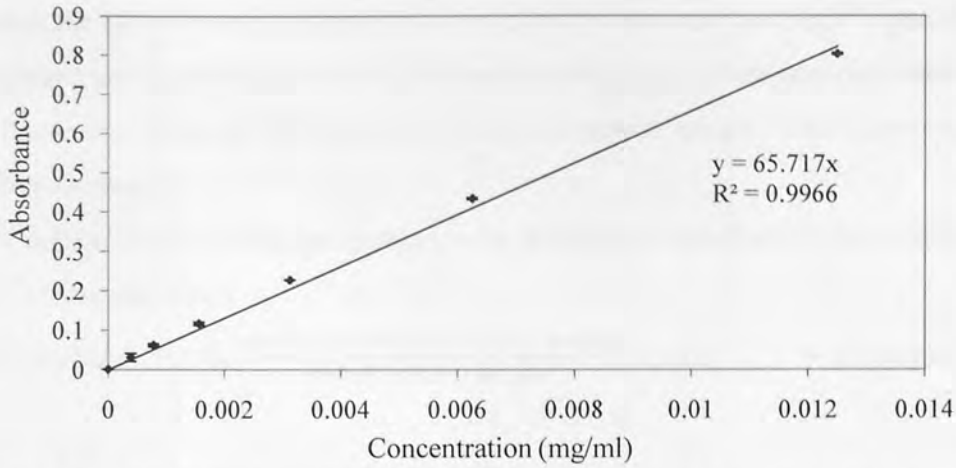


Figure 2.7: Typical calibration graph for cimetidine assay in phosphate buffer (pH 7.2) by UV determined at 228 nm (n=3, mean ± s.d.)

2.18 DRUG RELEASE STUDIES

The release of drug from the matrix tablets of ibuprofen was studied in 900 ml of phosphate buffer (pH 7.2) using the USP II (paddle method) equipped with a 40 mesh sinker. The dissolution equipment was an Erweka, DT 600 (Erweka, Germany). The dissolution test was performed at 100 rpm and the temperature was 37 ± 1°C. A 4 ml sample was withdrawn at time intervals of 15 minutes, 30 minutes, 1 hour and thereafter every 1 hour for 12 hours for both monolithic and binary systems, and further sampling was done at 24, 25 and 26 hours for the monolithic systems. The withdrawn samples were assayed spectrophotometrically at 265 nm. After each sampling, equal volume (4 ml) of fresh buffer solution with same temperature was replaced.

The *in vitro* release studies for cimetidine matrix tablet formulations were also done under same conditions. Absorbance reading was read at 228 nm and sample withdrawal was done at time intervals of 15 minutes, 30 minutes and 1 hour intervals up to a maximum of 12 hours.

2.19 WATER UPTAKE AND EROSION STUDIES

Water uptake and erosion studies were performed using the dissolution apparatus DT 600 (Erweka, Germany). The matrix tablets in a sinker were placed in 900 ml of phosphate buffer (pH 7.2) equilibrated at 37 ± 1°C and the paddle rotating at 100 rpm. The tablets were allowed to hydrate, swell and erode at different time intervals. Two tablets were used per time point. At the predetermined times (0, 30 minutes, 1, 2, 4 or

8 hours), the tablets were removed from the dissolution vessel and lightly patted with tissue paper to remove excess water. The wet weight of the tablets was determined and then they were dried at 50°C until reaching a constant weight. This remaining dry weight was recorded.

Water uptake, % remaining and erosion were determined gravimetrically according to the following equations:

$$Water\ uptake\ (\%) = \frac{wet\ weight - remaining\ dry\ weight}{remaining\ dry\ weight} \times 100 \qquad \text{--- (Equation 2.6)}$$

$$Erosion\ (\%) = \frac{original\ weight - remaining\ dry\ weight}{original\ weight} \times 100 \qquad \text{--- (Equation 2.7)}$$

2.20 DETERMINATION OF DRUG RELEASE KINETICS

Different kinetic equations (zero-order, first-order, Higuchi, and Korsmeyer-Peppas) were applied to interpret the release rate of the drug from the matrix systems. The best fit with highest correlation best describes the release kinetics of the formulation.

Zero order: $Q_t = Q_o + K_o t$ ---------- *(Equation 1.18)*

First order: $InQ_t = InQ_o + K_1 t$ ---------- *(Equation 1.19)*

Higuchi: $Q_t = K_H t^{1/2}$ ---------- *(Equation 1.20)*

Korsmeyer-Peppas: $Q_t/Q_\alpha = Kt^n$ ---------- *(Equation 1.21)*

2.21 POWDER AND GRANULE EVALUATION

2.21.1 Moisture content

The moisture content of the powders or granules was determined on a Sartorius moisture balance (Sartorius, Germany) and worked out according to the formula:

$$Moisture\ content\ (\%) = \frac{initial\ weight - final\ weight}{initial\ weight} \times 100 \qquad \text{---------- (Equation 2.8)}$$

2.21.2 Angle of repose

Apoproximately 20 grams of granules or powders was accurately weighed and carefully introduced into a funnel clamped to a stand with its tip 10 cm from a plane paper surface. The granules or powders were allowed to flow freely unto the paper surface. The height of the cone, H formed after complete flow and the radius of the cone, R were measured and used to calculate the angle of repose using the equation:

$$Angle\ of\ repose\ (tan\ \theta) = {}^{H}/_{R} \qquad \text{---------- (Equation 2.9)}$$

2.21.3 Bulk and tapped densities

Approximately 20 grams of granules or powders was accurately weighed into a 100 ml measuring cylinder and without disturbing the cylinder the volume of granules or powders was read to give the bulk volume. The measuring cylinder was then clamped to the USP I tapper of a USP tap density tester (Sotax TD2, Switzerland).The volume of the granules or powders was read after every 50 taps up to a total of 300 taps when volume of granules or powders was constant. This represents the tapped volume of the granules or powders. The bulk density and tapped density was calculated using *equation 1.11* and *1.12* respectively.

2.21.4 Hausner quotient

The Hausner ratio, quotient or factor was calculated as the ratio of tapped to bulk densities (*equation 1.13*).

2.21.5 Carr's compressibility index

The compressibility index of the granules or powders was determined according to the Carr's compressibility index percentage (see *equation 1.14*).

2.21.6 Granule size analysis

Granules (30 g) was accurately weighed and transferred into the arrangement of sieve sizes 710, 500, 355 and 250 μm respectively. The sieves were arranged such that the largest size (710 μm) is on top and the smallest (250 μm) sitting on top of the collector. The sieve analyzer (Fritsch Analysette 3 SPARTAN, Germany) was set to 12 minutes with amplitude of 1.0 mm. At the end of the run time the granules on top of each sieve was collected and weighed. The granules of < 250 μm were discarded.

2.22 EVALUATION OF TABLET PROPERTIES

All batches of tablets formulated and compressed were evaluated for the following tablet properties:

2.22.1 Appearance

The tablets formed were examined for colour, surface texture and any visible tablet defects.

2.22.2 Uniformity of diameter and thickness

The diameter and thickness of the tablets was measured using a digital micrometer. The diameter and thickness was determined for 10 tablets per batch.

2.22.3 Friability test

A soft brush was used to remove surface dust from 20 tablets randomly selected. These were then accurately weighed and placed in the drum of the friabilator (Roche) which was then rotated 100 times at 25 rpm. The tablets were removed from the drum and brushed free from adhering dust and reweighed. This procedure was followed for three sets of 20 tablets per batch. The loss in weight (friability) was calculated as a percentage of the initial weight according to *equation 1.16*.

2.22.4 Tablet crushing strength

The mechanical strength of 5 tablets randomly selected was tested with the model 6D tablet tester (Schulinger Pharmatron, Manchester, NH).

2.22.5 Uniformity of weight test

Exactly 20 tablets were randomly selected and weighed individually and collectively on a *class A* balance. The weight deviation of the individual tablets from the average weight of the 20 tablets was determined.

2.22.6 Uniformity of content

A tablet was crushed using a pestle and mortar and dissolved in 900ml of phosphate buffer (pH 7.2). The absorbance of the filtered solution was read on a 6300 Jenway UV spectrophotometer (Bibby scientific, UK) at 265 nm for ibuprofen tablet formulations, and at 228 nm for cimetidine tablet formulations. A total of 10 tablets were individually assayed and drug content calculated from the calibration curve.

2.22.7 Total drug content

Total drug content was determined from three tablets individually weighed and assayed. The tablet was first ground in a pestle and mortar and then dissolved in 900 ml of phosphate buffer (pH 7.2). The absorbance of the solution was determined on a 6300 Jenway UV spectrophotometer (Bibby Scientific, UK) at 265 nm and 228 nm for ibuprofen and cimetidine tablet formulations respectively. The drug content was calculated from the calibration curve.

2.22.8 Disintegration test

The B.P (2010) disintegration test was performed on six tablets using a disintegration tester (Erweka, Germany) set to 37°C and 30 cycles per minute. One tablet each was placed in each of the six tubes and with the guide disc in place, the time taken for the tablets to completely disintegrate was recorded. The disintegration medium was 800 ml of distilled water. This procedure was done thrice for each batch of tablets.

Although disintegration test is not a requirement for sustained-release formulations, this test was done to confirm that the tablets were non-disintegrating.

CHAPTER THREE

CHARACTERIZATION OF GREWIA POLYSACCHARIDE GUM

3.1 INTRODUCTION

Natural polysaccharide gums have found wide application in the pharmaceutical industry as polymer matrices in sustained release solid dosage forms (Talukdar and Vercamen, 1993; Huber and Christenson, 1996; Sujja-Areevath *et al.*, 1996, Bhardwaj *et al.*, 2000, Varshosaz *et al.*, 2006), binders in tablets (Udeala and Chukwu, 1985), stabilizers or suspending agents in liquid dosage forms (Bilany, 2007), and in bioadhesive drug delivery systems (Middleton *et al.*, 1990). They have the advantage of biocompatibility, low cost and relatively abundant availability (Vendruscolo *et al.*, 2009) compared to their synthetic counterparts.

The detailed literature review on grewia polysaccharide gum has been discussed in section 1.20. This study therefore aims to achieve further characterisation of grewia polysaccharide gum in order to establish its functionality as an excipient for both solid and liquid dosage forms. This aim was achieved in a number of steps. Firstly, the polysaccharide gum was extracted and purified from the crude and pulverised inner stem bark of the plant according to methods discussed in section 2.3. The drying process has been shown to have significant effect on the physico-chemical properties and formulation characteristics of materials (York, 1983). Consequently, in order to determine the effect of drying techniques on the polysaccharide gum, drying of the extracted and purified polysaccharide was achieved by three methods: air-drying, spray-drying and freeze-drying (see section 2.3). Finally, the differently dried polysaccharide gum samples were characterised using several techniques discussed in the following sections.

3.2 MATERIALS AND METHODS

All materials used in this study were procured from Sigma-Aldrich (Poole, UK) unless otherwise stated and were of analytical grade unless otherwise specified. The details of methodology not outlined in this section have already been discussed in chapter two.

3.2.1 Moisture content

Moisture content of the air-dried, freeze-dried, spray-dried and the crude gum was determined using a moisture analyzer (MA 40, Satorius, Germany). The moisture content was worked out according to *equation 2.9* (see section 2.20.1).

Also the moisture content of polysaccharide gum samples was also determined using thermogravimetric analysis. The thermogravimetric analyzer (Pyris 1 TGA, Perkin

Elmer, USA) was allowed to equilibrate for 15 minutes before analysis. The scan rate employed was 10°C per minute from 30-200°C under nitrogen atmosphere.

3.2.2 pH of 0.05%w/v dispersion of grewia polysaccharide gum

A 0.05% w/v dispersion of the gum (air-dried, spray-dried and freeze-dried) was incubated in water for 24 hours at room temperature. The hydrated gum was then stirred and filtered through a Whatman no.1 filter paper. The pH of the resultant solution was read using a pH meter (Satorius, Germany).

3.2.3 Effect of temperature on viscosity of grewia gum

The effect of temperature on the viscosity of grewia polysaccharide gum was studied using the air-dried polysaccharide gum sample only. The viscosity of a 0.5% dispersion of the gum was determined using a Brookfield viscometer (DV-1+version 5, Brookfield Engineering Labs, USA) at 10°, 20°, 30°, and 40°C by equilibrating the dispersion in a water bath at the specified temperatures. Spindle number 2 was used for all determinations at 10 rpm.

3.2.4 Effect of type and concentration of electrolyte on the viscosity of grewia polysaccharide gum

Solutions of KCl, $CaCl_2$ or $AlCl_3$ (0.125, 0.25, 0.5 and 1.0 M) were prepared and used to make 200 ml portions of 0.5% w/v dispersion of the air-dried polysaccharide gum. The viscosity of the gum dispersions was determined using the Brookfield viscometer (DV-1+version 5, Brookfield Engineering Labs., USA) at 20°C and shear rate of 5 rpm. Spindle number 2 was used for viscosity reading of all samples. A 0.5% dispersion of the polysaccharide gum with no electrolyte added served as control.

3.2.5 True density of grewia polysaccharide gum

The densities of grewia polysaccharide gum samples were determined using a multipycnometer (MVP- D160-E, Quantachrome Instruments U.S.A.). Each sample was maintained at 50°C for 12 hours before analysis to reduce moisture. Powder was inserted into the 20 cm^3 sample vial until it was approximately three quarters full and accurately weighed. The sample vial was transferred to the pycnometer and the weight was entered. Five determinations were made per sample and the pycnometer provided readings of a mean and standard deviation.

The helium pycnometer employs Archimedes principle of fluid displacement and Boyle's law to measure the volume of a given mass of a powdered material. Maximum accuracy is assured by the use of helium which can penetrate the finest

pores as the displaced fluid. The true density of solid objects can then be determined from the given mass and the determined volume of the material.

3.2.6 Bulk and tapped density, angle of repose and Carr's compressibility

Bulk density, tapped density, angle of repose and Carr's compressibility of grewia polysaccharide gum samples were determined according to the methods outlined in section 2.20.2 - 2.20.5 except that in this case only 10 grams of the polysaccharide gum was used.

3.2.7 Scanning electron microscopy

Scanning electron micrograph of the grewia gum samples (air-dried, freeze-dried and spray-dried) was carried out (StereoScan 90, Cambridge instruments) after gold coating using an Enscope SC500 gold sputter coater.

3.2.8 Aqueous solubility

The aqueous solubility of grewia gum was determined gravimetrically by accurately weighing 1g into 1000 ml of distilled water and hydrated at room temperature for 24 hours. Thereupon, the dispersion was filtered through a pre-weighed filter paper of medium porosity. The residue on the filter paper was weighed after drying in an oven at 50°C for 24 hours and the difference determined.

3.3 RESULTS AND DISCUSSION

3.3.1 Yield of grewia gum

Spray drying yielded only 3.1% w/w of the extracted gum compared to 32.4% w/w on dry weight basis for both the freeze-dried and the air-dried. The low yield of the spray dried is attributed to lose to the walls of the spray dryer due to inefficient drying at the outlet. The yield may be improved by decreasing the concentration of the dispersion. The consequent reduction in viscosity of the sample and adjustments of spray drier parameters may optimise the yield. However, due to limitations of product availability, the other drying methods were adopted. On dispersion in water the powdered gum produced by all drying techniques gave a viscous, mucilaginous gel. The air-dried powder obtained after precipitation and drying was wheat-coloured, while the freeze-dried was a light-wheat colour and the spray-dried sample was off-white. Colour variation may be attributable to the effect of atmospheric oxygen, both freeze-drying and spray drying being vacuum processes.

3.3.2 Physicochemical properties of grewia polysaccharide gum

Some of the physicochemical properties of grewia polysaccharide gum dried by the different techniques are shown in table 3.1.

Aqueous dispersions of all samples of grewia gum give an acidic pH. There is no significant difference in pH ($P>0.05$) between the samples. This suggests that grewia gum is not a neutral polysaccharide. The acidic nature of the gum indicates that the polysaccharide contains a uronic acid (sugar acid) in its structure. Polysaccharides containing sugar acids in their structure will carry negative charges, and are also described as anionic polysaccharides (Cui, 2005).

The variation in total ash between the gum samples is not significant ($P>0.05$). The spray-dried sample of the gum has a higher value of soluble ash compared to gum dried using the other methods. This may be attributable to the effect of particle size on solubility. The spray-dried gum sample has smaller particle size when compared with samples of the air-dried or freeze-dried gum (also see section 3.3.12). The BP (2010) reported a maximum upper limit of 4% total ash for acacia and tragacanth. The ash value is often indicative of the amount of inorganic metallic elements that may be present in a material. A total ash content of 6.3% has been reported previously for grewia gum (Okafor *et al.*, 2001).

Grewia polysaccharide gum possesses very low aqueous solubility. The gum disperses in water but not readily, slowly hydrating and swelling to give highly viscous dispersions.

Table 3.1: Physicochemical properties of grewia polysaccharide gum (n=3, mean ± s.d.)

	Air-dried	*Freeze-dried*	*Spray-dried*
Solubility (mg/ml)	0.15 ± 0.01	0.13 ± 0.04	0.11 ± 0.03
Soluble ash (%)	3.38 ± 0.20	3.36 ± 0.06	3.75 ± 0.14
Total ash (%)	6.13 ± 0.36	6.09 ± 0.41	6.30 ± 0.38
pH	5.74 ± 0.03	5.73 ± 0.11	5.72 ± 0.04
Moisture content (%)	10.64 ± 2.01	15.37 ± 1.15	18.84 ± 2.64
Viscosity at 5 rpm (cPoise)	319.07 ± 8.68	289.2 ± 8.62	216.97± 6.71
True density (g/cm^3)	1.98 ± 0.013	1.68 ± 0.007	2.04 ± 0.07
Angle of repose (°)	30.37 ± 0.47	32.56 ± 1.03	-
Bulk density (g/ml)	0.16 ± 0.003	0.14 ± 0.003	-
Tapped density (g/ml)	0.20 ± 0.01	0.17 ± 0.01	-
Hausner's ratio	1.25±0.02	1.27±0.01	-
% compressibility	25.2 ±1.86	27.18 ±1.36	-

3.3.3 Viscosity and flow behaviour

Figure 3.1 shows the flow behaviour of 1.0% w/v aqueous dispersions of grewia polysaccharide gum. All samples of the polysaccharide gum demonstrated typical pseudoplastic flow behaviour (shear-thinning). The apparent viscosity of the dispersions decreased with an increase in shear rate, with the air-dried demonstrating higher apparent viscosity at all shear rates than the freeze-dried and spray-dried samples. This type of flow behaviour is desirable for dispersed systems such as pharmaceutical emulsions and suspensions. The apparent viscosity of the dispersion or solution of a polysaccharide gum will increase with the amount of polysaccharide (Cui, 2005).

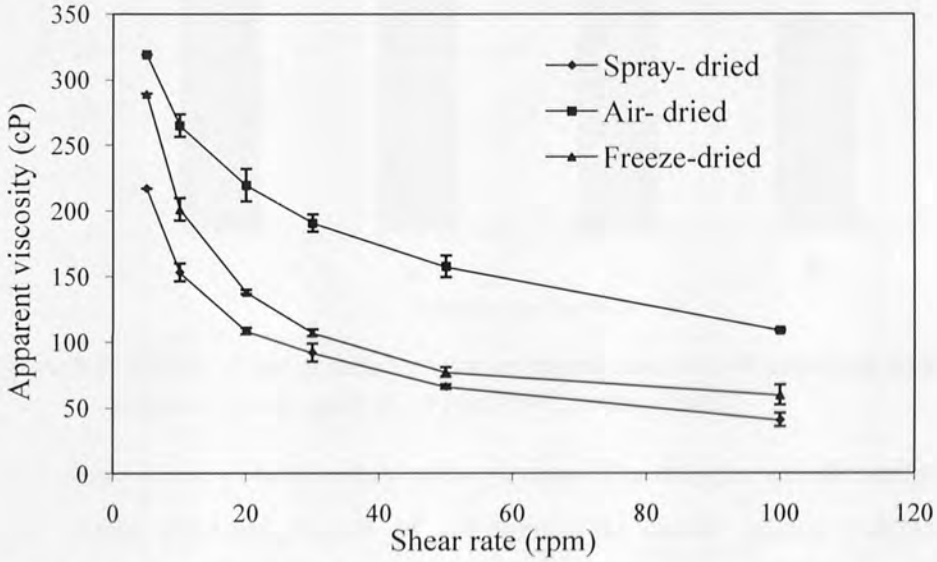


Figure 3.1: Effect of shear rate on the apparent viscosity of air-dried, freeze-dried and spray-dried grewia gum samples (n=3, mean ± s.d.)

Shear-thinning behaviour is demonstrated by several polysaccharide dispersions or solutions (Cui, 2005) and is attributed to the decreasing number of chain entanglements as the chains orient themselves with the direction of flow or shear. At high shear rates, a decrease in viscosity is observed because the extent of newly formed entanglements cannot compensate for those being disentangled. The extent of shear thinning demonstrated by a dispersion or solution of a polysaccharide is dependent on the intrinsic molecular characteristics such as conformation, molecular weight, and charges in the case of an anionic polysaccharide (Cui, 2005). This is in addition to extrinsic factors such as concentration, temperature, and pH which may also influence the flow behaviour.

3.3.3.1 Effect of temperature on the viscosity of grewia gum

The effect of temperature on the apparent viscosity of a 0.5% w/v dispersion of the air-dried grewia gum sample at 10 rpm is shown in figure 3.2. There was a gradual decrease in apparent viscosity as the temperature of the polysaccharide gum dispersion was raised from 10°C to 40°C.

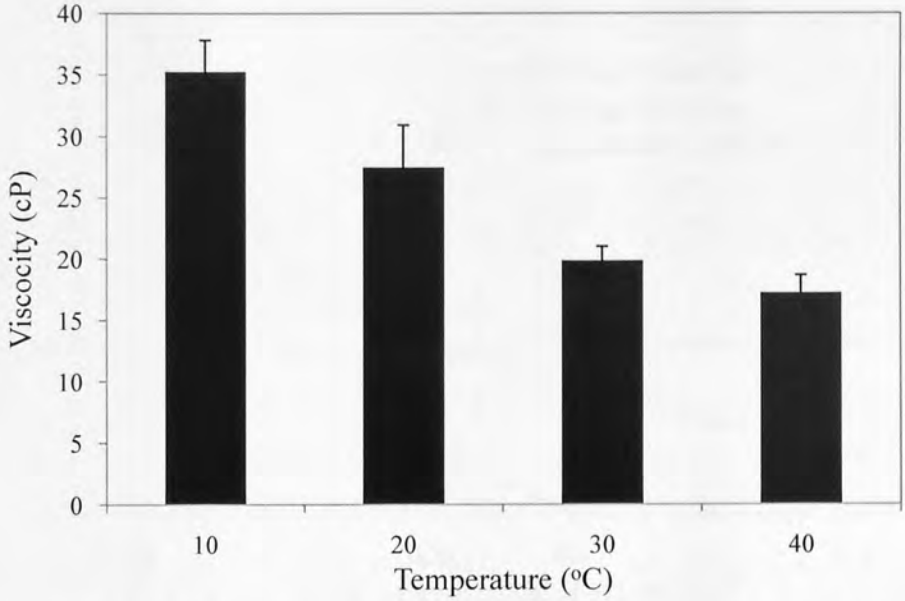


Figure 3.2: Effect of temperature on the apparent viscosity of air-dried grewia polysaccharide gum at 10 rpm (n=3, mean ± s.d.)

At a given shear stress, polymer chain entanglement /disentanglement determines flow rate (Cui, 2005). Disentanglement of polysaccharide chains causes a decrease in viscosity and this disentanglement is promoted by temperature rise.

3.3.3.2 Effect of type and concentration of electrolyte on the viscosity of grewia gum

The effect of type and concentration of the electrolytes potassium chloride (KCl), calcium chloride ($CaCl_2$) or aluminium chloride ($AlCl_3$) on the apparent viscosity of a 0.5% w/v dispersion of grewia gum (air-dried) was determined and results are shown in figure 3.3.

In the absence of electrolyte, the initial viscosity of the polymer dispersion was 28.5 cP (0.0285 Pa.s). The presence of 0.125 M potassium or calcium chloride brought about a reduction in the apparent viscosity to 15.0 and 13.25 cP (0.01325 Pa.s), respectively. Further increases in the concentration of potassium chloride to 0.25 M resulted to a further reduction in the apparent viscosity to 12.8 cP (0.0128 Pa.s). The apparent viscosity at 0.5 M concentration of KCl dropped further to 10.5 cP (0.0105

Pa.s). Beyond this point, further increases in the concentration of KCl did not result in a corresponding decrease in apparent viscosity. However in the case of $CaCl_2$, there was a continuous drop in the apparent viscosity of the gum dispersion with increase in concentration of the electrolyte. The addition of higher concentrations of $AlCl_3$ rapidly resulted in the salting-out of the gum dispersion.

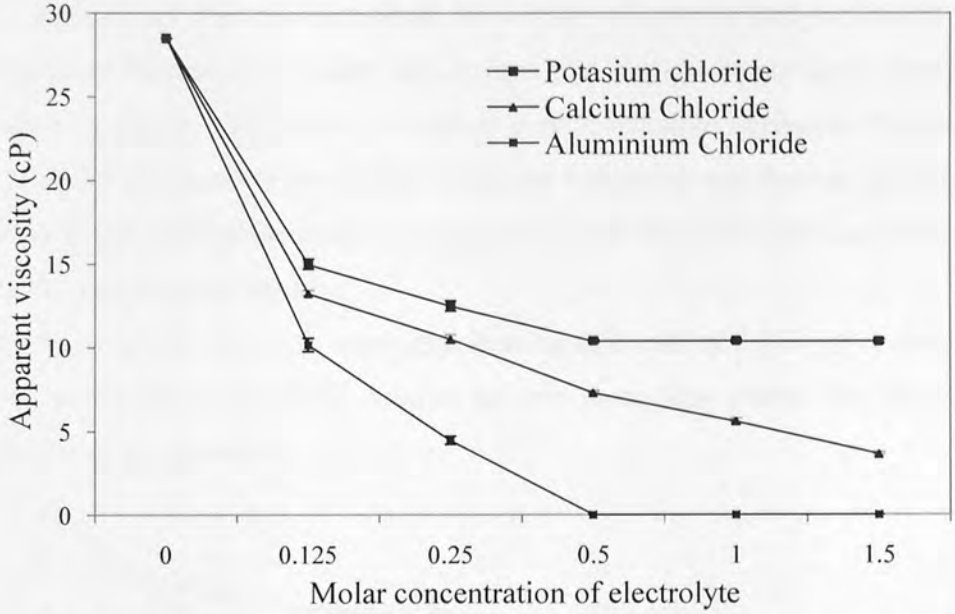


Figure 3.3: Effect of type and concentration of electrolyte on the apparent viscosity of 0.5% w/v gum dispersion (n=3, mean ± s.d.)

The effect of electrolytes on the apparent viscosity of other gum dispersions has been reported (Vernor-Carter and Sherman, 1980). The more concentrated the gum dispersion, the greater the probability of a 'polyion' encountering and attracting an ion of opposite charge around it. This mutual attraction causes the coiling up of the polymer chain which ultimately results in a drop in viscosity. It can also be seen from the figure that aluminium chloride was more effective than calcium chloride which was in turn more effective than potassium chloride in decreasing the apparent viscosity of the gum dispersion. Glicksman and Schachat (1959), explained that the decrease in the viscosity of gums brought about by electrolytes is proportional to the concentration as well as the valence of the cation. This property should be borne in mind when considering the additional use of electrolytes as flocculating agents in suspensions already containing grewia polysaccharide gum as viscosity imparting (suspending) agent.

3.3.4 Thermal analysis

3.3.4.1 Thermogravimetric analysis

Thermogravimetric analysis (TGA) is a simple and accurate method which has been used for studying the decomposition pattern and the thermal stability of polymers (Zohuriaan and Shokrolahi, 2004). This technique was applied to study the effect of drying method on the decomposition behaviour of grewia polysaccharide gum. Representative thermograms under lean oxygen (5% oxygen in nitrogen) atmosphere are shown in figure 3.4. Figure 3.5 shows a representative derivative thermogram while table 3.2 summarises the details of thermal behaviour and thermal stability data according to the primary thermograms (figure 3.4) and derivative thermograms (figure 3.5) for the various gum samples.

The results (figs. 3.4 and 3.5) show that heating at a rate of 10°C per minute from 30°C to a maximum of 500°C results in two mass loss events for the grewia polysaccharide gum samples.

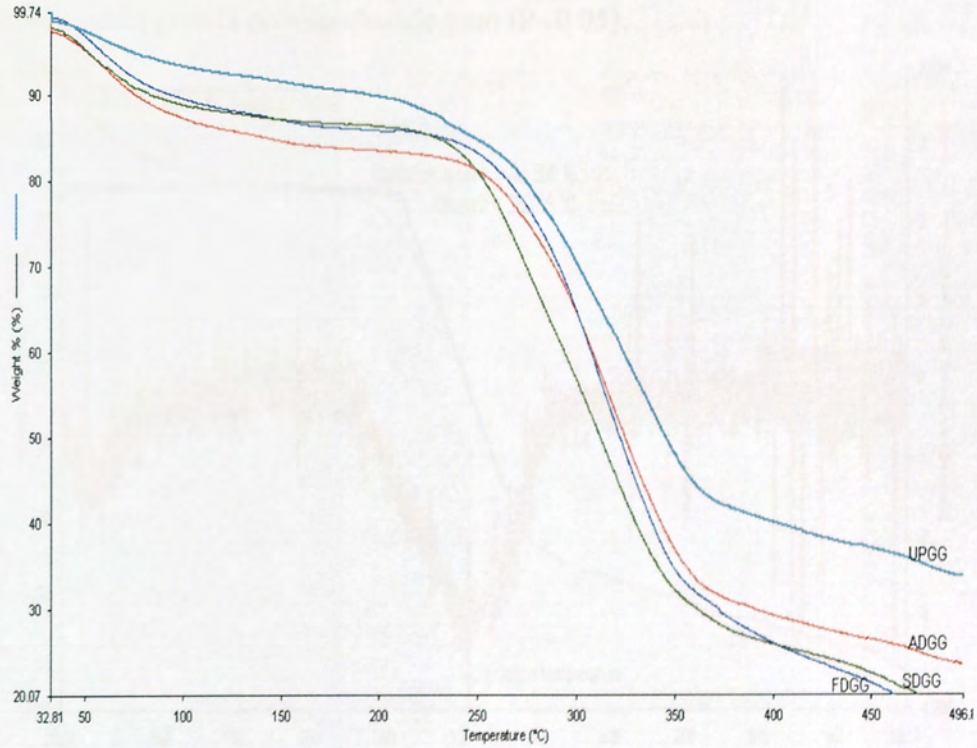


Figure 3.4: Thermograms of freeze-dried grewia gum (FDGG), air-dried grewia gum (ADGG), spray-dried grewia gum (SDGG) and unprocessed grewia gum (UPGG)

The first mass loss taking place between 30° - 140°C may be attributed to the loss of adsorbed and structural water of biopolymers as discussed by other authors (Kittur *et al.*, 2002; Vendruscolo *et al.*, 2009) or due to desorption of moisture as hydrogen

bound water to the polysaccharide structure. This represents the moisture content of the polysaccharide gum. The results indicate that purification increases the moisture carrying capacity of grewia polysaccharide gum. Okafor *et al.*, (2001), reported a moisture content of 7.8, 13.8 and 13.5% for grewia gum, tragacanth and acacia respectively. A moisture content of 12.5-16.0% has also been reported for acacia (Verbeken *et al.*, 2003).

The second weight loss event with an onset of over 250°C resulted in a weight loss of about 60%, has been attributed to thermal decomposition or oxidation of the polysaccharide (Varma *et al.*, 1997) and is described by a weight loss onset and oxidation temperature. The temperature when oxidation just begins is termed the weight loss onset while the temperature of maximum rate of oxidation is termed oxidation temperature. The air-dried, freeze-dried and spray-dried samples show no variation in their oxidation temperatures ($P>0.05$). These however have an oxidation temperature which is significantly lower than the unprocessed (crude and pulverised inner stem bark) grewia polysaccharide gum ($P<0.05$).

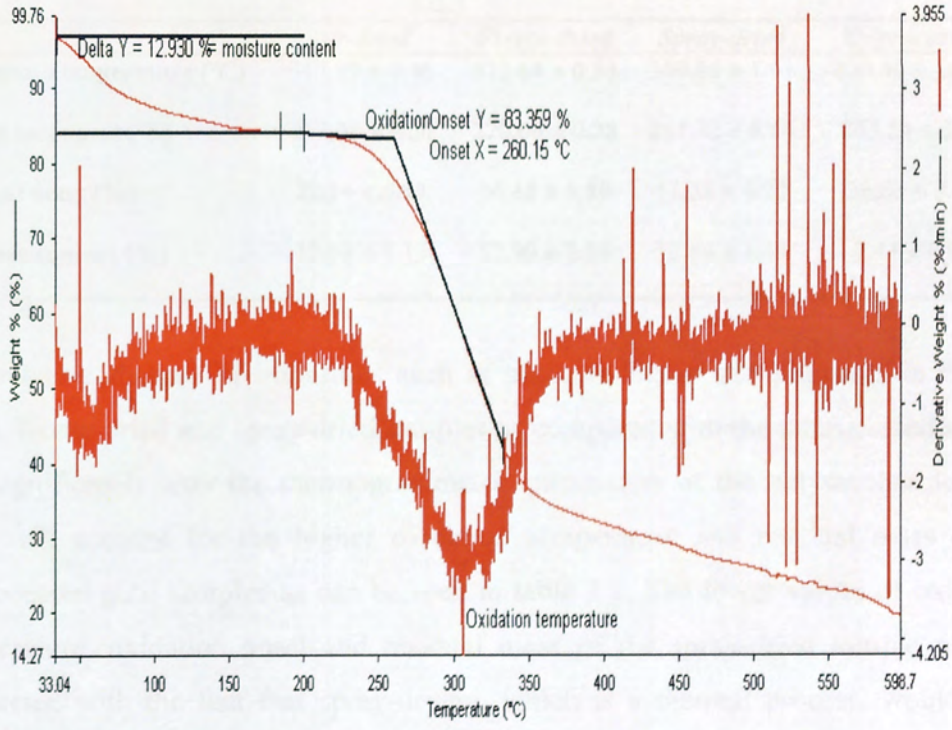


Figure 3.5: Representative thermogravimetric parameter derivatisation for grewia gum samples

The results also show a significant difference in the onset of oxidation or weight loss between the air-dried and the spray-dried sample, and between the freeze-dried and the

spray-dried sample ($P<0.05$). The weight loss onset was the same for both air-dried and freeze-dried samples ($P>0.05$). It can be noted also that the onset of oxidation was same for the air-dried, freeze-dried and unprocessed sample ($P>0.05$), but there is significant difference between the oxidation or weight loss onset of the spray-dried and the unprocessed sample ($P<0.05$). The residual mass was the same for all processed samples ($P>0.05$). The residual mass observed for the unprocessed sample was higher than the air-dried ($P<0.01$), and more significantly higher than the freeze-dried and the spray-dried samples ($P<0.001$). Specimen weight stability at the end of heating implies that all of the carbon in each sample is burnt off leaving behind metal oxides or ash (Zohuriaan and Shokrolahi, 2004). In this case however, heating of the samples up to 500°C did not result in sample weight stability and this implies that the residual mass for samples shown in table 3.2 does not represent the ash content of the samples.

Table 3.2: Thermogravimetric parameters from grewia gum samples (n=3, mean ± s.d.)

	Air-dried	*Freeze-dried*	*Spray-dried*	*Unprocessed*
Oxidation Temperature (°C)	317.97 ± 2.46	312.64 ± 0.53	309.91 ± 1.01	330.40 ± 10.48
Weight loss onset (°C)	267.08 ± 6.24	270.06 ± 0.28	251.32 ± 6.54	263.54 ± 6.02
Residual mass (%)	22.14 ± 5.43	16.48 ± 5.80	11.38 ± 4.22	36.63 ± 3.69
Moisture content (%)	12.68 ± 1.13	12.90 ± 0.98	12.64 ± 1.48	7.44 ± 0.82

The relative absence of impurities such as proteins, lignin and pigments in the air-dried, freeze-dried and spray-dried samples as compared with the unprocessed sample can significantly alter the thermogravimetric parameters of the polysaccharide gum. This will account for the higher oxidation temperature and residual mass of the unprocessed gum samples as can be seen in table 3.2. The lower values of oxidation temperature, oxidation onset and residual mass of the spray-dried sample may be connected with the fact that spray-drying, which is a thermal process, would have induced an initial degradation of the polysaccharide structure and made it more susceptible to subsequent thermal degradation as compared to the other samples.

The moisture content of the polysaccharide gum samples vary significantly ($P<0.01$) from the moisture content of the same samples determined using the moisture balance (MA 40, Sartorius, Germany) (Table 3.1). Although instrumental sensitivity may

account for some variation in readings between equipment, this does not totally explain the differences. It would seem that the interval between the determinations may in fact account for the differences. The moisture balance (MA 40, Sautorius, Germany) was used to determine the moisture content of the samples soon after extraction and purification of the polysaccharide gum samples. This implies that this represents the moisture content of the material immediately after processing of the polysaccharide gum. However, thermogrametric analysis of the samples was carried out some months later and the samples might have equilibrated to atmospheric conditions. Consequently, the air-dried sample gained moisture from the environment while the freeze-dried and spray-dried samples lost moisture to the environment. The result is that the moisture content of all three purified samples at the time of the thermogravimetric analysis was the same ($P>0.05$) except for the crude sample. The results show that extraction and purification increases the moisture sorption of the polysaccharide samples. It is expected that the extraction and purification process will rid the crude polysaccharide gum sample of hydrophobic matter such as wax. Consequently, the purified samples will be more hydrophilic and/or hygroscopic than the unpurified gum.

3.3.4.2 Differential scanning calorimetry

Physical and chemical changes that occur in a polysaccharide during thermal processing can be monitored using differential scanning calorimetery (DSC). This method yields thermograms that are unique for a given polysaccharide (Vinod *et al.*, 2007). It has been reported that structural and functional group differences in polysaccharide gums influence the thermal behaviour and affect the transition temperature, T_g (Zohuriaan and Shokrolahi, 2004). The temperature at which a material becomes glassy and brittle on cooling or soft on heating is referred to as the glass transition temperature, T_g.

The DSC traces of grewia polysaccharide gum are shown in figure 3.6(*a-c*). Two endothermic events were determined for the air-dried and the spray-dried polysaccharide samples but only one endothermic event was obvious for the freeze-dried grewia polysaccharide gum sample. The first endothermic event occurs between 50° and 140°C. This is attributable to moisture desorption and agrees with the results from the thermogravimetric analysis. The second endothermic event occurred between 140° and 160°C.

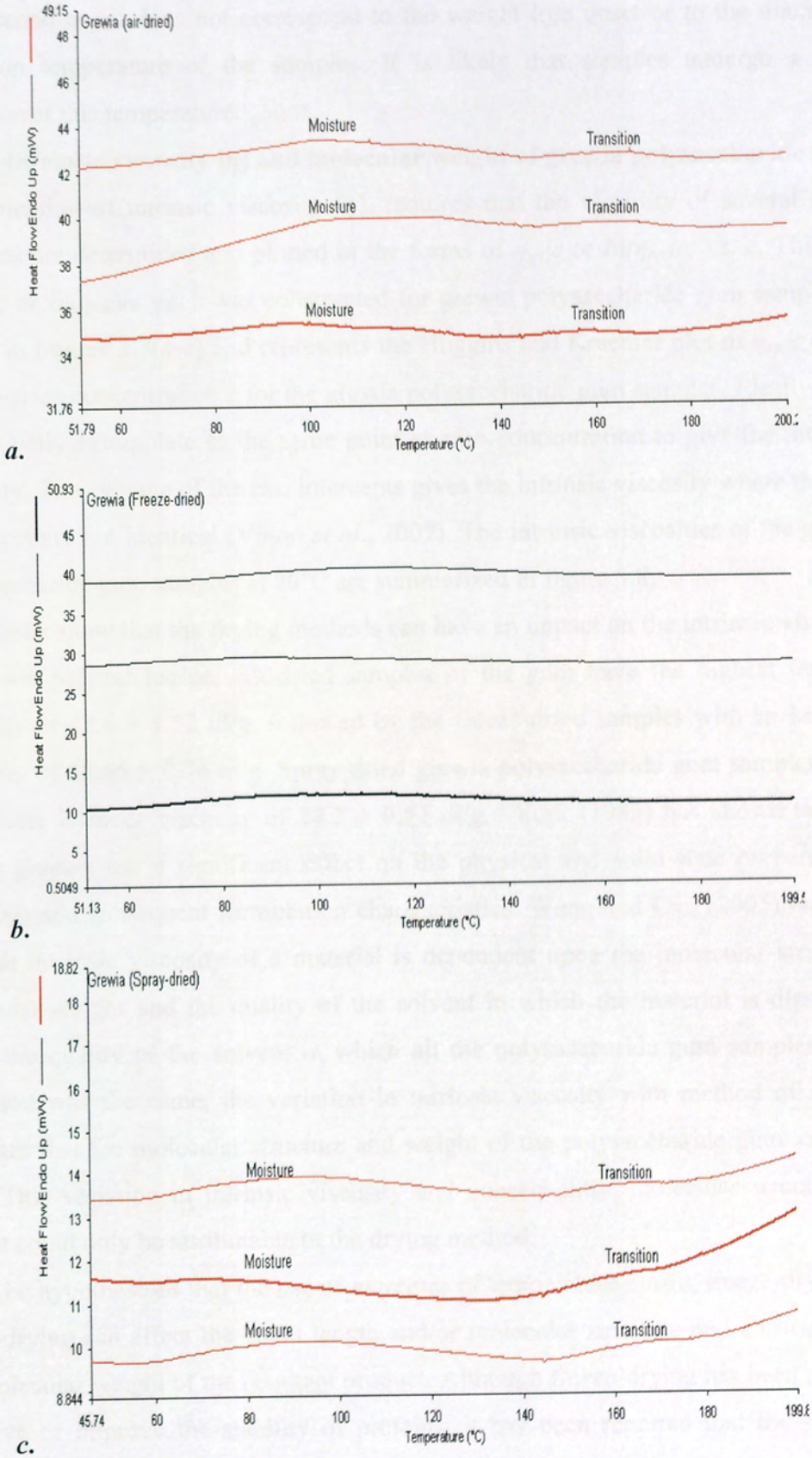


Figure 3.6: DSC scan of grewia polysaccharide gum at 10°C/min upto 200°C for *a*) air-dried, *b*) freeze-dried, and *c*) spray-dried samples

This second event does not correspond to the weight loss onset or to the maximum oxidation temperature of the samples. It is likely that samples undergo a glass transition at this temperature.

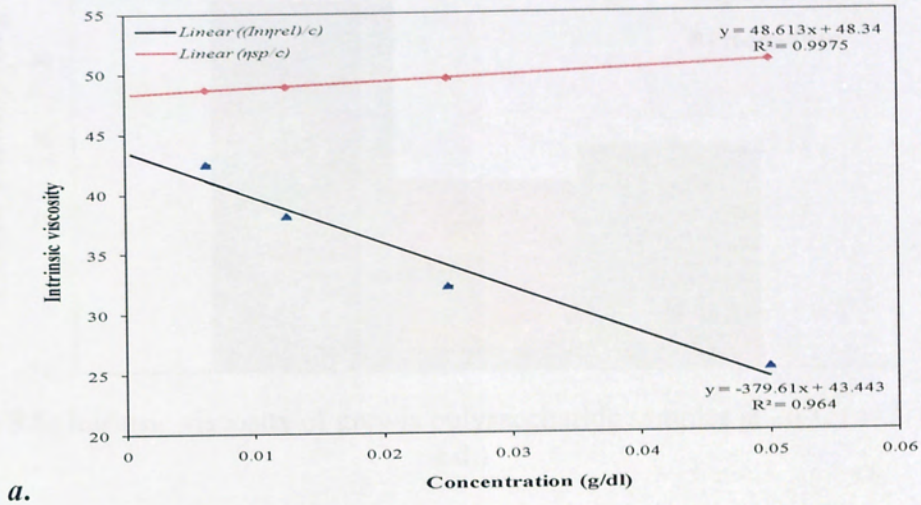
3.3.5 Intrinsic viscosity [η] and molecular weight of grewia polysaccharide gum

Determination of intrinsic viscosity [η], requires that the viscosity of several dilute solutions are determined and plotted in the forms of η_{sp}/c or $In(\eta_{rel})/c$ vs. c. This plot of η_{sp}/c or $In(\eta_{rel})/c$ vs. c was constructed for grewia polysaccharide gum samples as shown in figures 3.7(a-c) and represents the Huggins and Kraemer plot of η_{sp}/c or (In η_{rel})/c verses concentration c for the grewia polysaccharide gum samples. Ideally, both lines usually extrapolate to the same point at zero concentration to give the intrinsic viscosity. The average of the two intercepts gives the intrinsic viscosity where the two intercepts are not identical (Vinod *et al.*, 2007). The intrinsic viscosities of the grewia polysaccharide gum samples at 20°C are summarized in figure 3.8.

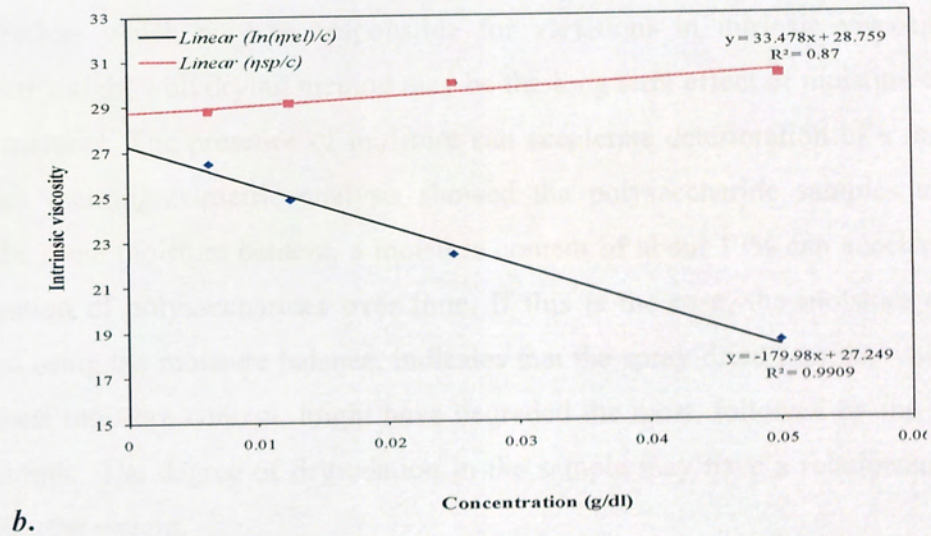
The results show that the drying methods can have an impact on the intrinsic viscosity of grewia polysaccharide. Air-dried samples of the gum have the highest intrinsic viscosity of 47.6 ± 1.52 dl/g, followed by the freeze-dried samples with an intrinsic viscosity of 28.65 ± 0.76 dl/g. Spray-dried grewia polysaccharide gum samples have the lowest intrinsic viscosity of 24.3 ± 0.52 dl/g. York (1983) has shown that the drying process has a significant effect on the physical and solid-state properties of materials and subsequent formulation characteristics. Wang and Cui, (2005) reported that the intrinsic viscosity of a material is dependent upon the molecular structure, molecular weight and the quality of the solvent in which the material is dissolved. Since the quality of the solvent in which all the polysaccharide gum samples were dispersed was the same, the variation in intrinsic viscosity with method of drying indicates that the molecular structure and weight of the polysaccharide gum samples vary. This variation in intrinsic viscosity and consequently, molecular structure or weight could only be attributable to the drying method.

It can be hypothesised that the use of extremes of temperature during freeze-drying or spray-drying can affect the chain length and/or molecular structure and consequently the molecular weight of the resultant product. Although freeze-drying has been used to preserve or improve the stability of proteins, it has been reported that the process could also lead to degradation of the protein (Bhatnagar *et al.*, 2007). Similar effect

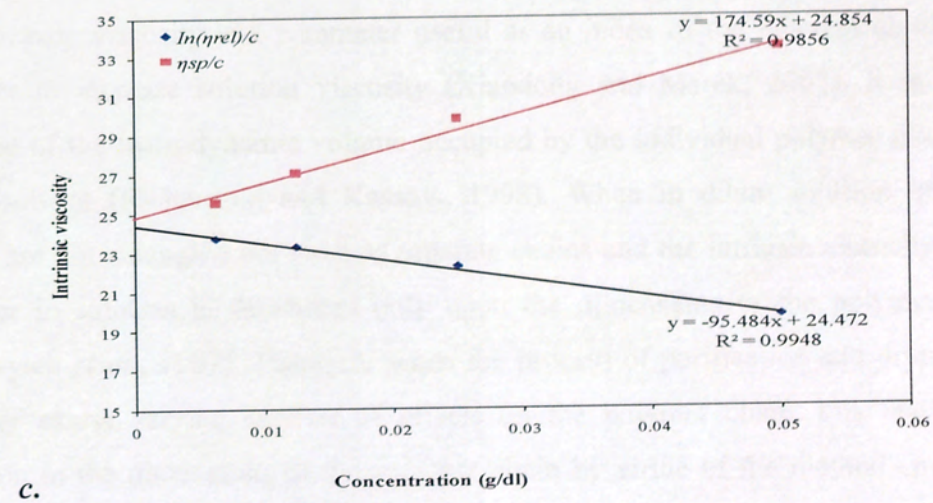
may be seen in polysaccharides. It is also known that very high temperature conditions can affect the molecular weight of polymers (Kokofuta *et al.*, 1978).



a.



b.



c.

Figure 3.7: Representative plot of η_{sp}/c or $(\ln \eta_{rel})/c$ verses concentration c, according to the Huggins and Kraemer equations for *a*) air-dried, *b*) freeze-dried, and *c*) spray-dried grewia polysaccharide gum samples (n=3, mean)

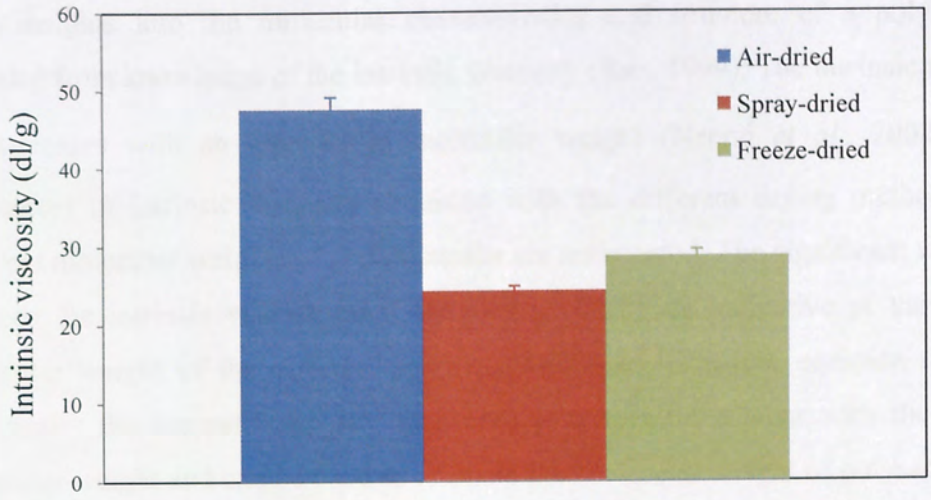


Figure 3.8: Intrinsic viscosity of grewia polysaccharide samples at 20°C (n=3, mean ± s.d.)

Other factors which may be responsible for variations in intrinsic viscosity and molecular weight with drying method may be the long term effect of moisture content on the material. The presence of moisture can accelerate deterioration of a material. Although thermogravimetric analysis showed the polysaccharide samples to have about the same moisture content, a moisture content of about 13% can accelerate the deterioration of polysaccharides over time. If this is the case, the moisture content recorded using the moisture balance, indicates that the spray-dried sample, which had the highest moisture content, might have degraded the most, followed by the freeze-dried sample. The degree of degradation in the sample may have a relationship with the molecular weight.

The intrinsic viscosity is a parameter useful as an index of the inherent ability of a polymer to increase solution viscosity (Xiaodong and Marek, 2007). It is also a measure of the hydrodynamic volume occupied by the individual polymer chain in a given solvent (Richardson and Kasapis, 1998). When in dilute solution, polymer chains are not entangled but exist as separate chains and the intrinsic viscosity of the polymer in solution is dependent only upon the dimensions of the polymer chain (Khouryieh *et al.*, 2007). Therefore when the process of purification and drying of a polymer exerts varying degrees of effects on the polymer chain, this may cause variation in the dimensions of the polymer chain by virtue of the method employed during purification and drying. The variations in polymer chain dimensions will in turn result in variable intrinsic viscosity.

Deep insights into the molecular characteristics and structure of a polymer are provided from knowledge of the intrinsic viscosity (Rao, 1999). The intrinsic viscosity [η] increases with an increase in molecular weight (Neeraj *et al.*, 2002). From differences in intrinsic viscosity observed with the different drying methods used, different molecular weights of grewia mollis are anticipated. The significant variations between the intrinsic viscosities of samples ($P<0.01$) are indicative of variation in molecular weight of the different samples. The Mark-Houwink equation (*equation 1.2*) relates the intrinsic viscosity measured in a specific solvent with the average molecular weight and can be used to estimate the molecular weight of polysaccharides (Cui, 2005). This requires that the constants K and α be known. These are determined by use of absolute methods such as sedimentation and osmometry. GPC was used to evaluate the molecular weight of the grewia polysaccharide samples.

Figure 3.9 shows a plot of the screen used to compute the GPC results. It shows the operator selected baseline and integration limits as well as the refractive index chromatogram and calibration curve.

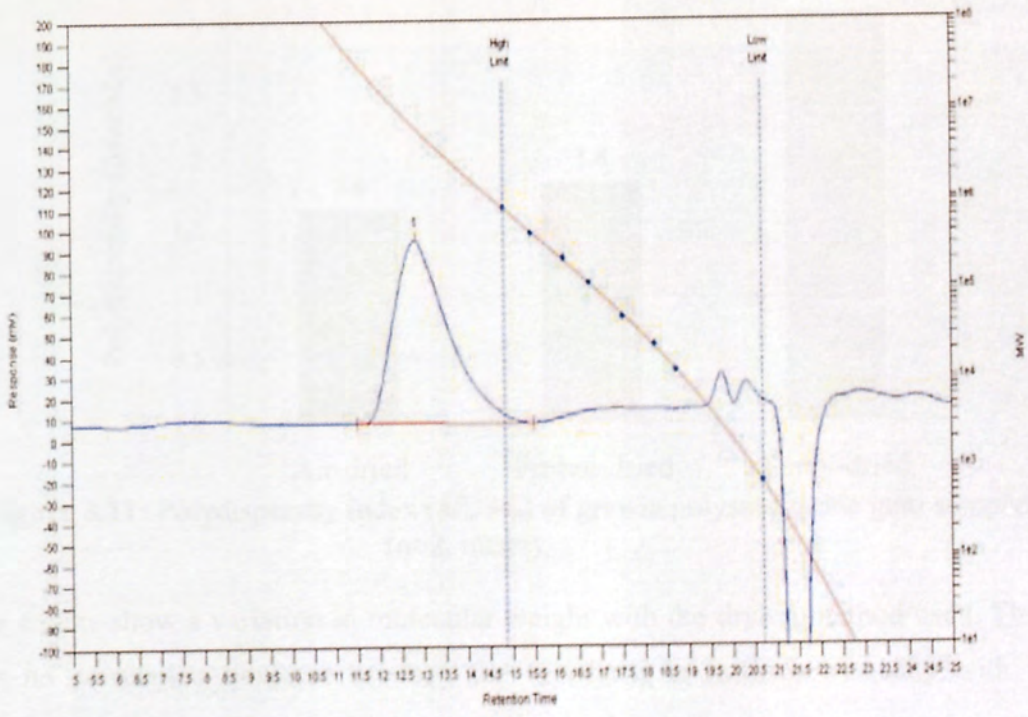


Figure 3.9: A chromatogram and calibration curve for GPC of grewia polysaccharide gum

The results of the study are expressed as the 'pullulan polysaccharide equivalent' molecular weight and are reported in figure 3.10 as the computed average molecular

weights (M_w), number average molecular weight (M_n), and polydispersity index (M_w/M_n) in figure 3.11.

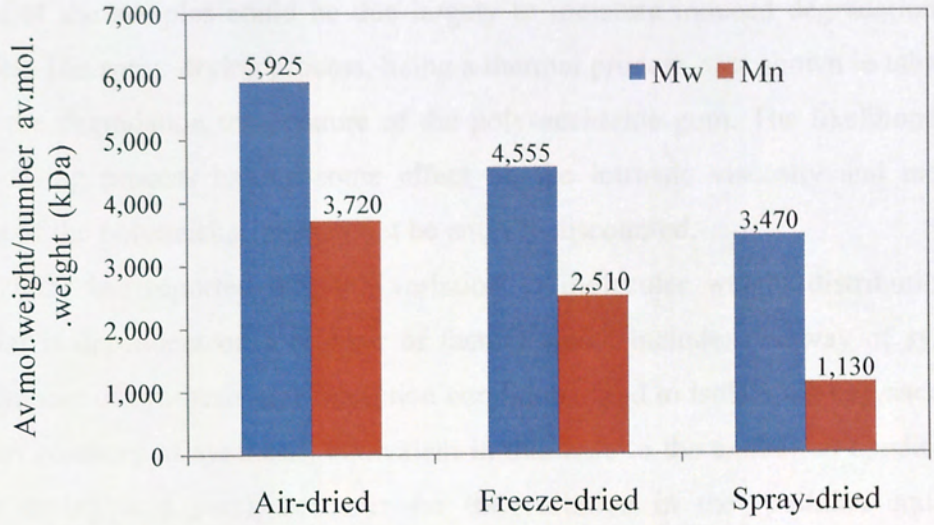


Figure 3.10: Weight average molecular weight (M_w) and number average molecular weight (M_n) of grewia polysaccharide gum (n=2, mean)

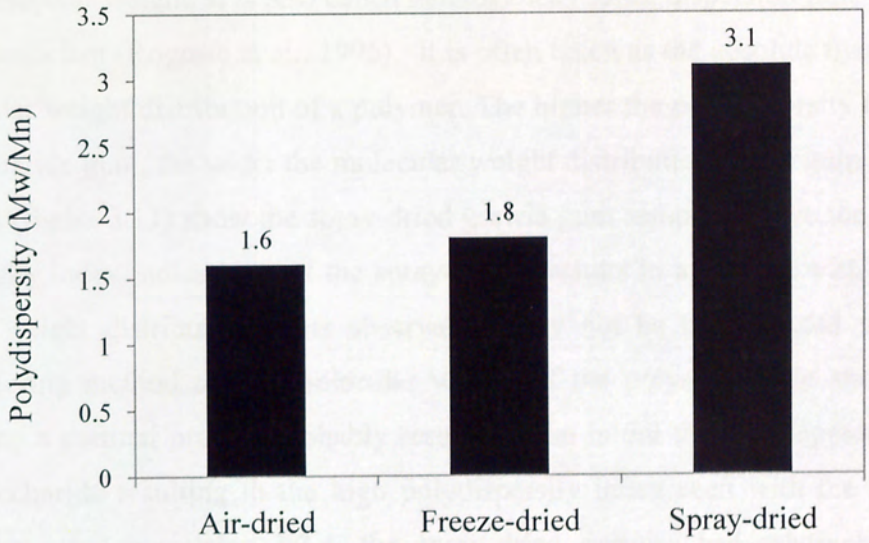


Figure 3.11: Polydispersity index (M_w/M_n) of grewia polysaccharide gum samples (n=2, mean)

The results show a variation in molecular weight with the drying method used. There was no correlation however between the variations in intrinsic viscosity with the variations in the molecular weight according to Mark-Houwink equation (*equation 1.2*) due in part to the fact that the GPC result is only indicative. The GPC result was only indicative because the method will only give the molecular weight of the polymer in solution. Polymer solubility, however, is relatively unaffected by the drying method (table 3.1) and other factors may contribute to the differences in molecular weight of

the various samples; the moisture content of the polysaccharide gum samples may influence degradation and the disparity between intrinsic viscosity and molecular weight of the samples could be due largely to moisture induced degradation of the samples. The spray-drying process, being a thermal process, was shown in table 3.2 to lower the degradation temperature of the polysaccharide gum. The likelihood of the spray-drying process having some effect on the intrinsic viscosity and molecular weight of the polysaccharide may not be entirely discounted.

Cui (2005) has reported that the variation in molecular weight distribution of a material is dependent on a number of factors which include: pathway of synthesis, environment of synthesis and extraction conditions used to isolate the polysaccharide. The environment of synthesis, equivalent in this case to the extraction conditions (of which drying is a part), accounts for the variation in the 'pullulan equivalent' molecular weight of the grewia polysaccharide gum samples.

The polydispersity index is the ratio of the average molecular weight to the number average molecular weight. It is also called heterogeneity ratio, dispersion ratio or non-uniform coefficient (Rogosic et al., 1996). It is often taken as the absolute measure of the molecular weight distribution of a polymer. The higher the polydispersity index of a polysaccharide gum, the wider the molecular weight distribution of the gum sample. The results (figure 3.11) show the spray-dried grewia gum sample to have the highest polydispersity index indicating that the spray-drying results in a product with a wider molecular weight distribution. This observation may not be unconnected with the effect of drying method on the molecular weight of the polysaccharide and spray-drying being a thermal process probably resulted in an initial thermal degradation of the polysaccharide resulting in the high polydispersity index seen with the sample. Similarly as noted in section 3.3.4, the spray-dried samples had relatively lower thermal stability compared to the air-dried or the freeze-dried samples.

3.3.6 FT-IR spectroscopy

The FT-IR spectra of air-dried, freeze-dried and spray-dried samples of grewia polysaccharide gum are presented in figure 3.12. The spectra indicate no major differences between the samples and show that drying method has no effect on the functional groups and type of bonds present in the polysaccharide gum samples. The samples all exhibit the typical bands and peaks characteristic of polysaccharides.

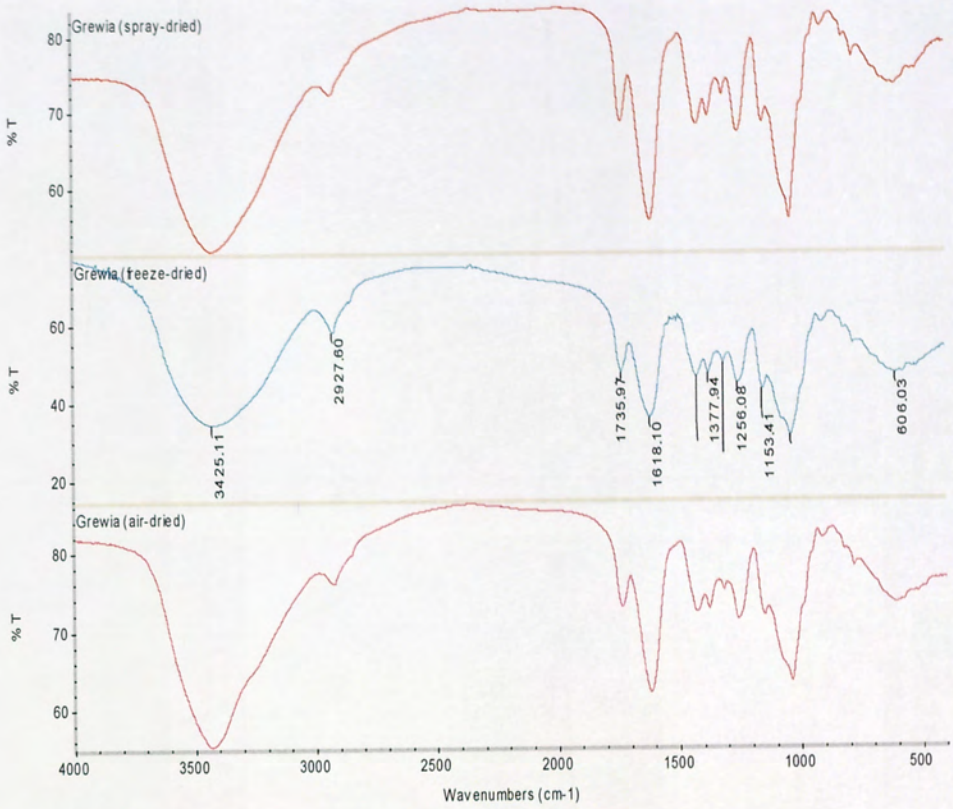


Figure 3.12: FT-IR spectra of air-dried grewia gum, freeze-dried grewia gum, and spray-dried grewia gum.

The broad band occurring at about 3425 cm^{-1}, results from the presence of hydroxyl (-*OH*) groups. The peak obtained at about 2927 cm^{-1} results from stretching modes of the *C-H* bonds of methyl groups (-CH_3). Absorption bands around 1618 and 1420 cm^{-1} are typical of carboxylate groups of uronic residues (Figueiro *et al.*, 2004). Natural gums usually contain fractions of uronic acid units which usually impart a weakly anionic character to the gum macromolecule (Wang *et al.*, 2003). The region between 1500 and 1800 cm^{-1} is typically used to detect the presence of carboxylic groups. Absorption peaks at 1735 cm^{-1} and 1256 cm^{-1} are typical of acetyl groups. The absorption band between 500 and 900 cm^{-1} may represents the finger print region for grewia polysaccharide gum. Carbohydrates generally have the finger print region occurring between 500 and 1500 cm^{-1} (Fillippove, 1992; Cui *et al.*, 2007).

3.3.7 Solid-state NMR

The solid-state NMR spectra of the grewia polysaccharide gum samples are shown in figures 3.13(*a-c*).

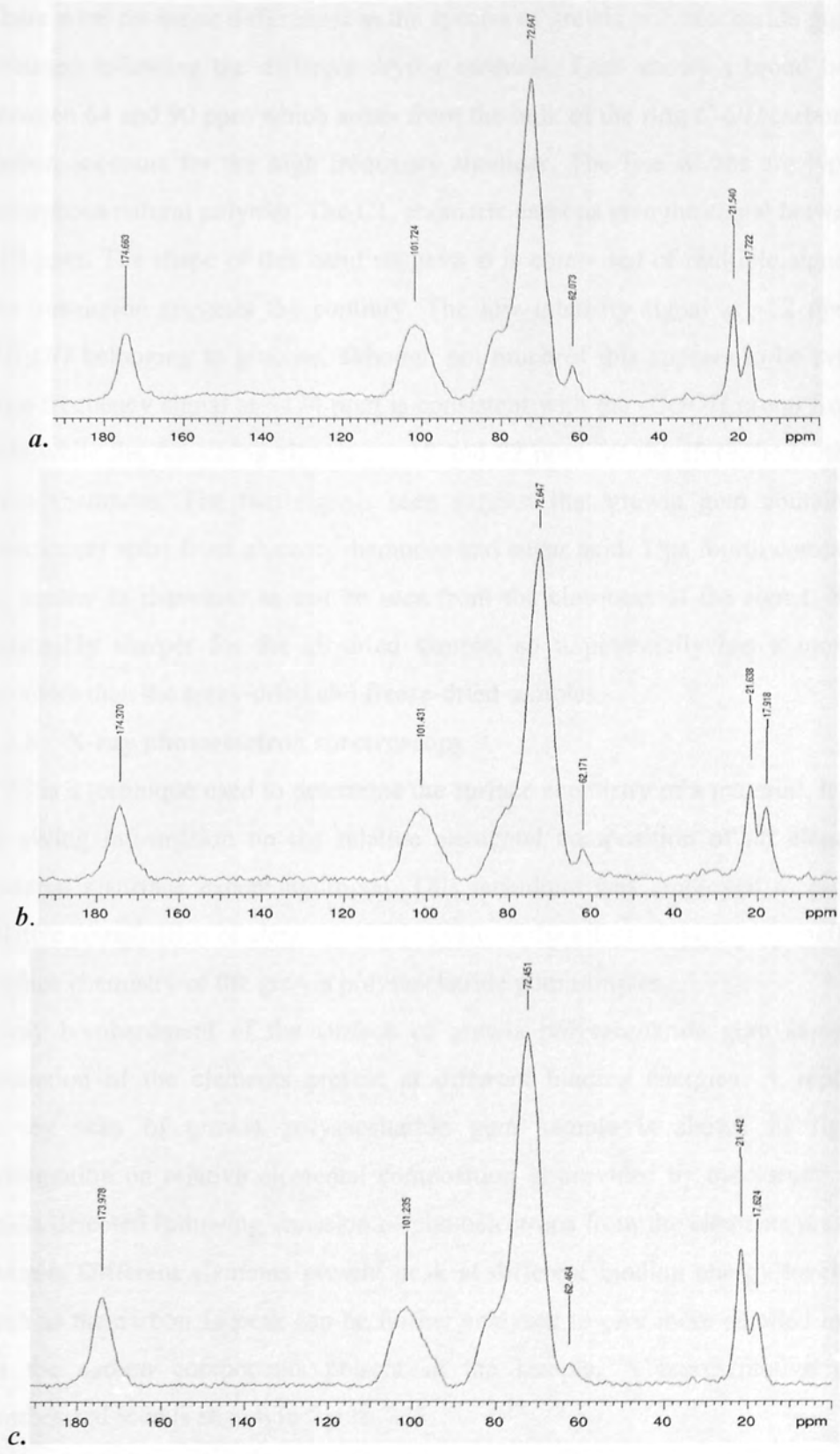


Figure 3.13: Solid State ^{13}C NMR of *a*) air-dried, *b*) freeze-dried, and *c*) spray-dried grewia polysaccharide gum

There were no major differences in the spectra of grewia polysaccharide gum samples obtained following the different drying methods. Each shows a broad band signal between 64 and 90 ppm which arises from the bulk of the ring *C-OH* carbons. The C4 carbon accounts for the high frequency shoulder. The line widths are typical of an amorphous natural polymer. The C1, anomeric carbons give the signal between 90 and 110 ppm. The shape of this band suggests it is composed of multiple signals but the low resolution suggests the contrary. The low intensity signal at ~62 ppm is the -CH_2OH belonging to glucose, although not much of this appears to be present. The high frequency signal at ~174 ppm is consistent with the *-COOH* group from a sugar acid while the remaining signals at ~18 and 22 ppm are therefore the methyl groups from rhamnose. The two signals seen suggest that grewia gum contain a fourth component apart from glucose, rhamnose and sugar acid. This fourth component may be similar to rhamnose as can be seen from the closeness of the signal. Signals are noticeably sharper for the air-dried sample, so it potentially has a more ordered structure than the spray-dried and freeze-dried samples.

3.3.8 X-ray photoelectron spectroscopy

XPS is a technique used to determine the surface chemistry of a material. It is capable of giving information on the relative elemental composition of all elements on a material's surface except hydrogen. This technique was employed to establish the relative composition of elements and to evaluate the effect of the drying method on the surface chemistry of the grewia polysaccharide gum samples.

X-ray bombardment of the surface of grewia polysaccharide gum samples cause excitation of the elements present at different binding energies. A representative survey scan of grewia polysaccharide gum sample is shown in figure 3.14. Information on relative elemental composition is provided by the survey scan from peaks detected following emission of photoelectrons from the elements present in the sample. Different elements present peak at different binding energy levels. A peak such as the carbon 1s peak can be further analysed to give more detailed information on the carbon components present in the sample. A representative carbon 1s synthesised scan is shown in figure 3.15.

Figure 3.16 shows the effect of drying technique on the synthesised carbon 1s spectra of the unprocessed (crude pulverised inner stem bark) and treated (purified and air-dried, freeze-dried or spray-dried) samples of grewia polysaccharide gum. The scan

shows that no two samples were exactly the same in terms of the relative composition of carbon components on the surface of the samples.

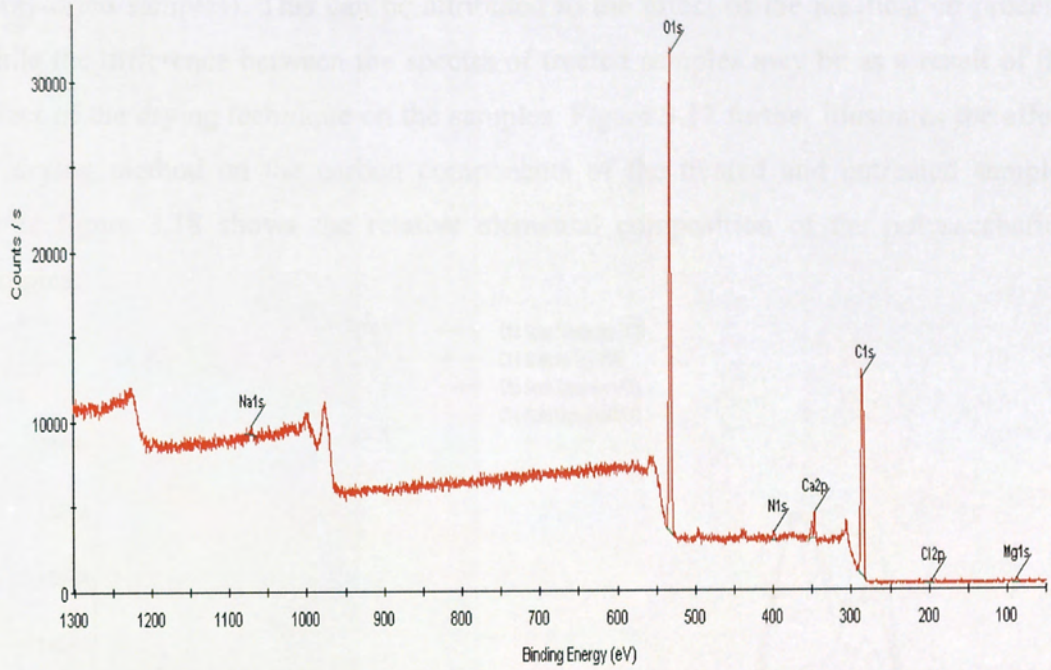


Figure 3.14: Representative XPS survey scan of grewia polysaccharide gum

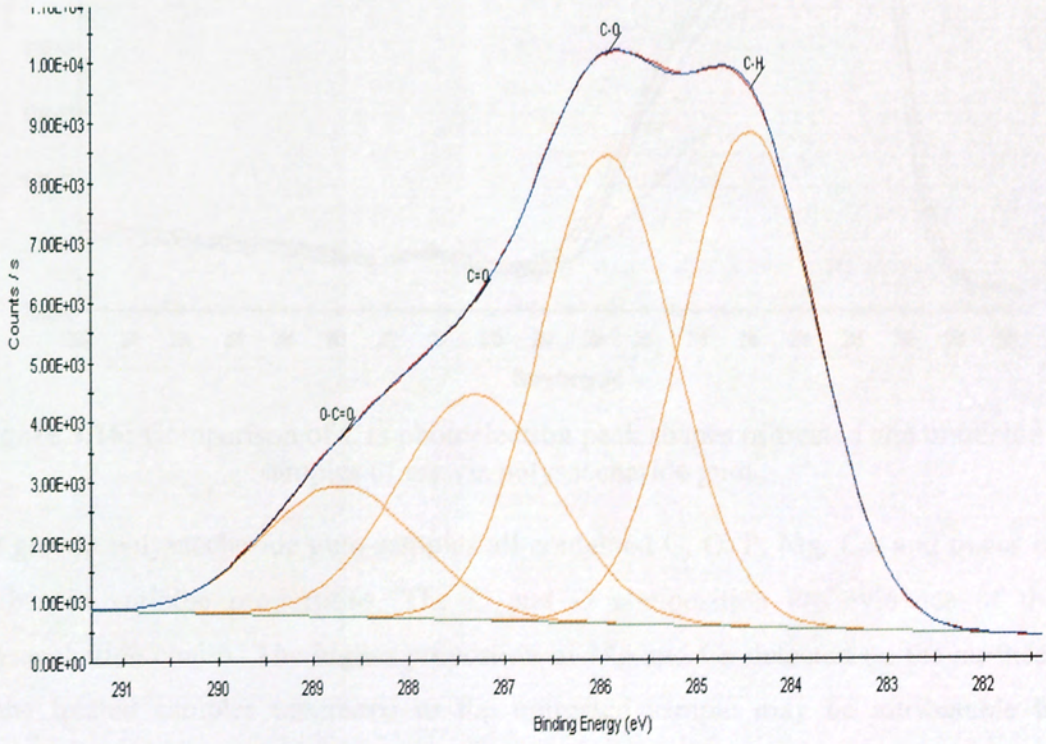


Figure 3.15: Representative carbon 1s synthesized scans of grewia polysaccharide gum

It can be seen that the spectra of the untreated sample (crude polysaccharide gum) differs greatly from the spectra of the treated samples (air-dried, freeze-dried and spray-dried samples). This can be attributed to the effect of the purification process, while the difference between the spectra of treated samples may be as a result of the effect of the drying technique on the samples. Figure 3.17 further illustrates the effect of drying method on the carbon components of the treated and untreated samples while figure 3.18 shows the relative elemental composition of the polysaccharide samples.

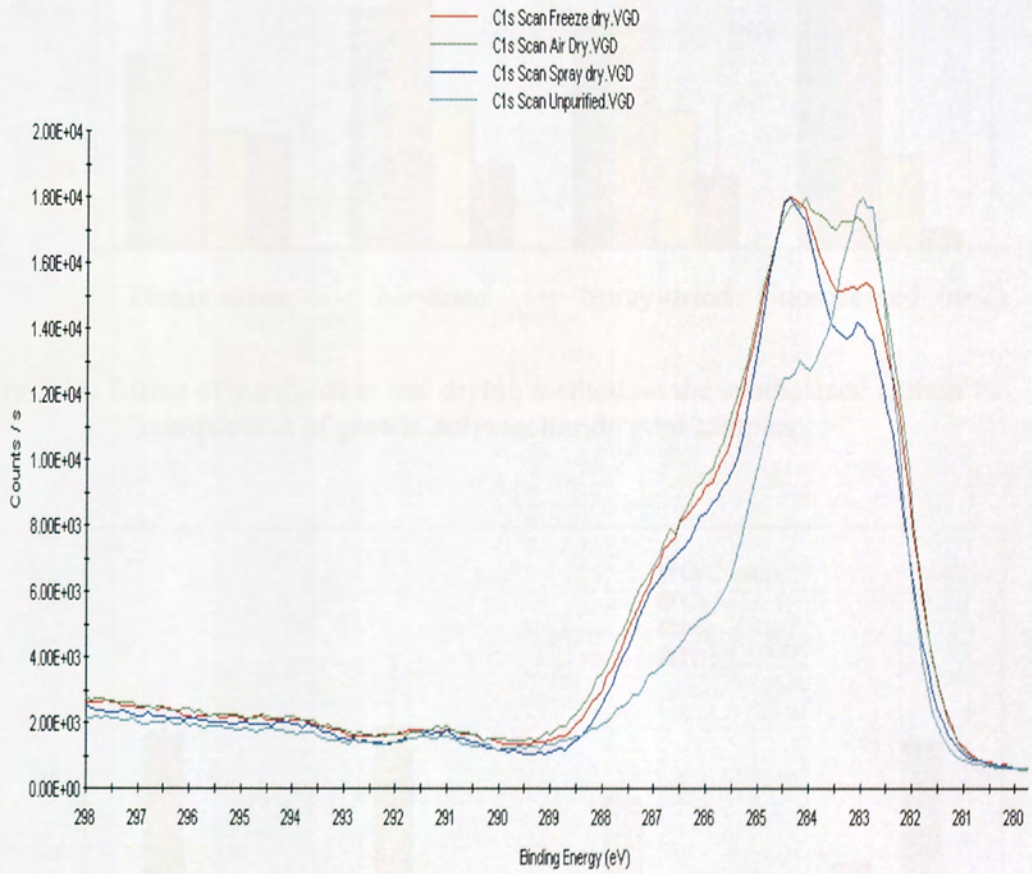


Figure 3.16: Comparison of C1s photoelectron peak shapes of treated and untreated samples of grewia polysaccharide gum

The grewia polysaccharide gum samples all contained C, O, P, Mg, Ca, and traces of Na but in variable proportions. The C and O composition are evidence of the polysaccharide chains. The higher proportion of Mg and Ca detected on the surfaces of the treated samples compared to the untreated sample may be attributable to segregation of these elements to the material surface after treatment or it may be that there was little or no loss of Mg and Ca during extraction and purification of the

polysaccharide gum. The nitrogen content is an indication of the presence of protein impurities, but may also indicate the presence of amino sugars.

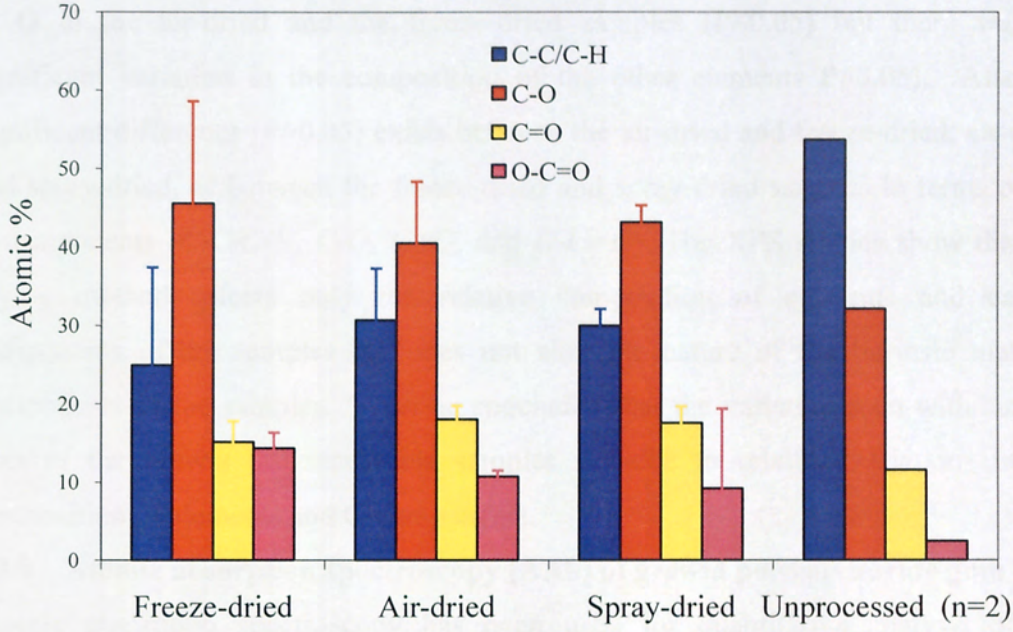


Figure 3.17: Effect of purification and drying method on the synthesized carbon 1s components of grewia polysaccharide gum samples

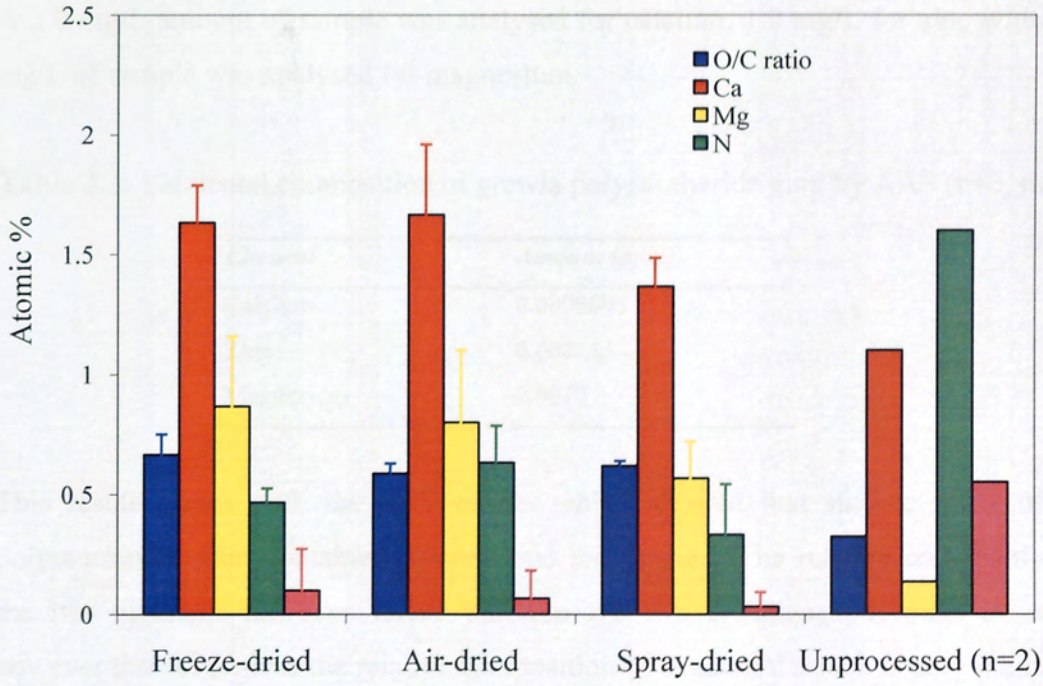


Figure 3.18: Effect of purification and drying method on the elemental composition of grewia polysaccharide gum

There was no significant difference in the elemental composition between the air-dried and spray-dried samples or between the freeze-dried and spray-dried samples ($P>0.05$). There were observed differences however, in the relative composition of C or O in the air-dried and the freeze-dried samples ($P<0.05$) but there was no significant variation in the composition of the other elements $P>0.05$). Also, no significant difference ($P>0.05$) exists between the air-dried and freeze-dried, air-dried and spray-dried, or between the freeze-dried and spray-dried samples in terms of the C-components (*C-C/C-H*, *C-O*, *C=O*, and *O-C=O*). The XPS studies show that the drying method affects only the relative composition of elements and carbon components of the samples but does not alter the nature of the intrinsic material components of the samples. It can be concluded that the variation seen with surface scan of the grewia polysaccharide samples are due to relative variations in the composition of elements and C-components.

3.3.9 Atomic absorption spectroscopy (AAS) of grewia polysaccharide gum

Atomic absorption spectroscopy has been used for quantitative analysis of the elemental composition of liquid materials. The elemental composition of grewia polysaccharide evaluated by AAS is shown in table 3.3. The analysis showed that grewia polysaccharide gum does not contain detectable amounts of Fe, K, Na and Cu. A 2.0 mg/L amount of sample was analysed for calcium, 1.0 mg/L for zinc while 0.3 mg/L of sample was analysed for magnesium.

Table 3.3: Elemental composition of grewia polysaccharide gum by AAS (n=3, mean)

Element	*Amount (ppm)*
Calcium	0.0000694
Zinc	0.000243
Magnesium	0.0017

This result agrees with the XPS results which showed that surface scans of the polysaccharide gum contained calcium and magnesium. The relative composition of the two elements however varied between the two techniques. It must be noted however that XPS gives the relative composition of a material which is only indicative and does not give the absolute quantities of components present. Atomic absorption spectroscopy gives the absolute amounts or quantity of each component present in a

material. Furthermore, XPS technique showed the presence of phosphorus, nitrogen and traces of sodium, but not the presence of zinc. The ability of XPS to detect the presence of the sodium may be due to relatively higher sensitivity of the technique. Since the concentration and distribution of elements vary across the surface and depth of a material, it is likely that the use of depth profiling for the XPS analysis of the sample instead of surface profiling could yield results that show the presence of zinc in the polysaccharide gum. Okafor *et al.*, (2001) has reported the presence of traces of metals (Ca, K, Na, Mg, Zn, Fe) in grewia polysaccharide gum. This study however indicates, as confirmed by both XPS and AAS analysis, that grewia polysaccharide gum does not contain potassium (K) and iron (Fe).

3.3.10 Monosaccharide composition by GC analysis

Hydrolysis of grewia polysaccharide gum using 2M TFA (see section 2.13.2) yielded no detectable monosaccharides on the GC and indicates that the procedure was not effective in hydrolysing the polysaccharide. The GC chromatogram of grewia gum hydrolysed according to the method in section 2.13.1 is shown in figure 3.19. The chromatogram indicates that grewia polysaccharide gum is composed of five neutral sugars eluting at various retention times.

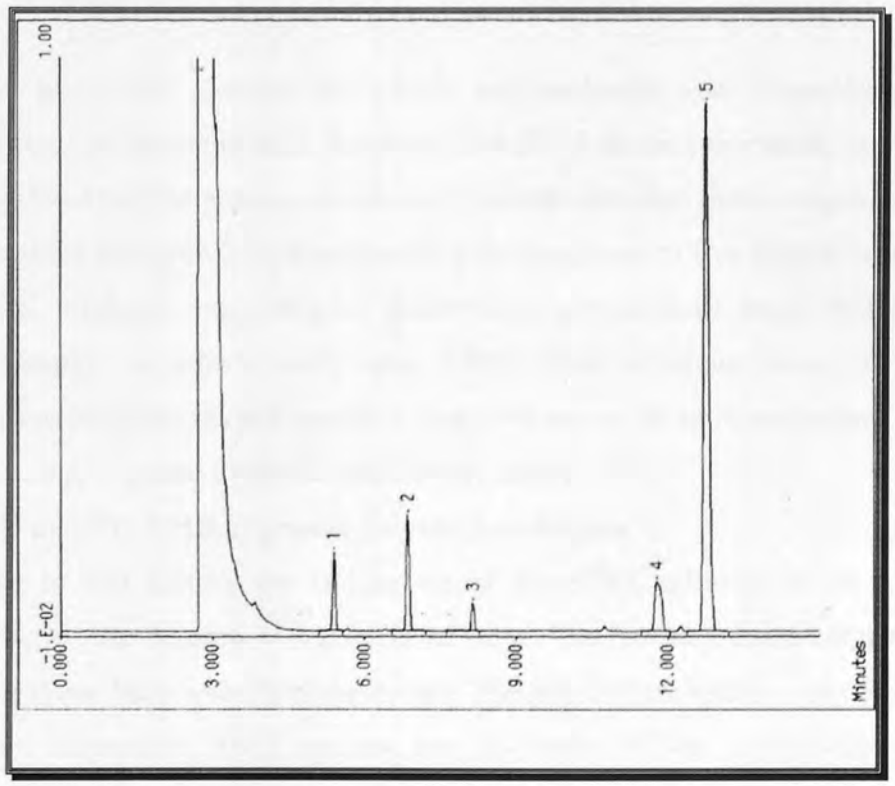


Figure 3.19: GC chromatograms of alditol acetates from grewia polysaccharide gum after hydrolysis with 2N H_2SO_4

Eluting at a retention time of 3.0 minutes is the solvent, ethyl acetate. The retention time of the sugar standards used for the analysis is shown in table 3.4*a*, while the retention time of the eluates shown in figure 3.19 is given in table 3.4*b*. The retention times indicate that the eluates in figure 3.19 contain glucose, rhamnose, arabinose, xylose and galactose.

Table 3.4*a*: The retention time (min) of sugar standards

Sugar standard	*Mean retension time (min)*
Rhamnose	5.4
Arabinose	6.9
Xylose	8.2
Galactose	11.8
Glucose	12.8

Table 3.4*b*: The retention time (min) of eluates and their relative concentration

Eluate	*Mean retention time*	*Relative concentration (%)*
1	5.41	6.2±0.71
2	6.86	12.71±2.65
3	8.16	2.72±0.49
4	11.81	9.61±0.86
5	12.79	67.14±5.85

Okafor *et al.*, (2001) reported that grewia polysaccharide gum comprises glucose, rhamnose and galacturonic acid. Solid-state NMR of the polysaccharide (see section 3.3.7) indicated that the polysaccharide may contain more than three components. This result confirms that grewia polysaccharide gum comprises of five neutral sugars. The use of GC produces more detailed information compared to paper or thin layer chromatography (Kharbade and Joshi, 1995). The technique however has the disadvantage of giving several anomeric peaks for any of the monosacharides, and the additional steps required to obtain volatile derivatives.

3.3.11 ^{1}H and ^{13}C NMR of grewia polysaccharide gum

According to Cui (2005), the assignment of the NMR spectrum of an unknown polysaccharide can be done in a number of steps. The first step is the comparison of the obtained spectrum with literature values. This can be followed by use of 2D NMR techniques. Simulated NMR spectra can be useful if the composition of the polysaccharide is known.

The ^{13}C and ^{1}H NMR spectra of grewia polysaccharide gum are shown in figures 3.20 and 3.21, respectively. It can be seen that the ^{1}H NMR spectrum is crowded in a narrow region between 3 to 5 ppm. This is typical for polysaccharides and makes the interpretation of the ^{1}H NMR spectra difficult especially when the polysaccharide contains many similar sugar residues (Cui, 2005). The ^{13}C NMR has weaker signals but has significant advantage over the ^{1}H NMR in the analysis of polysaccharides because the chemical shifts are spread out over a broader range and overcome the overlapping problem seen with ^{1}H NMR.

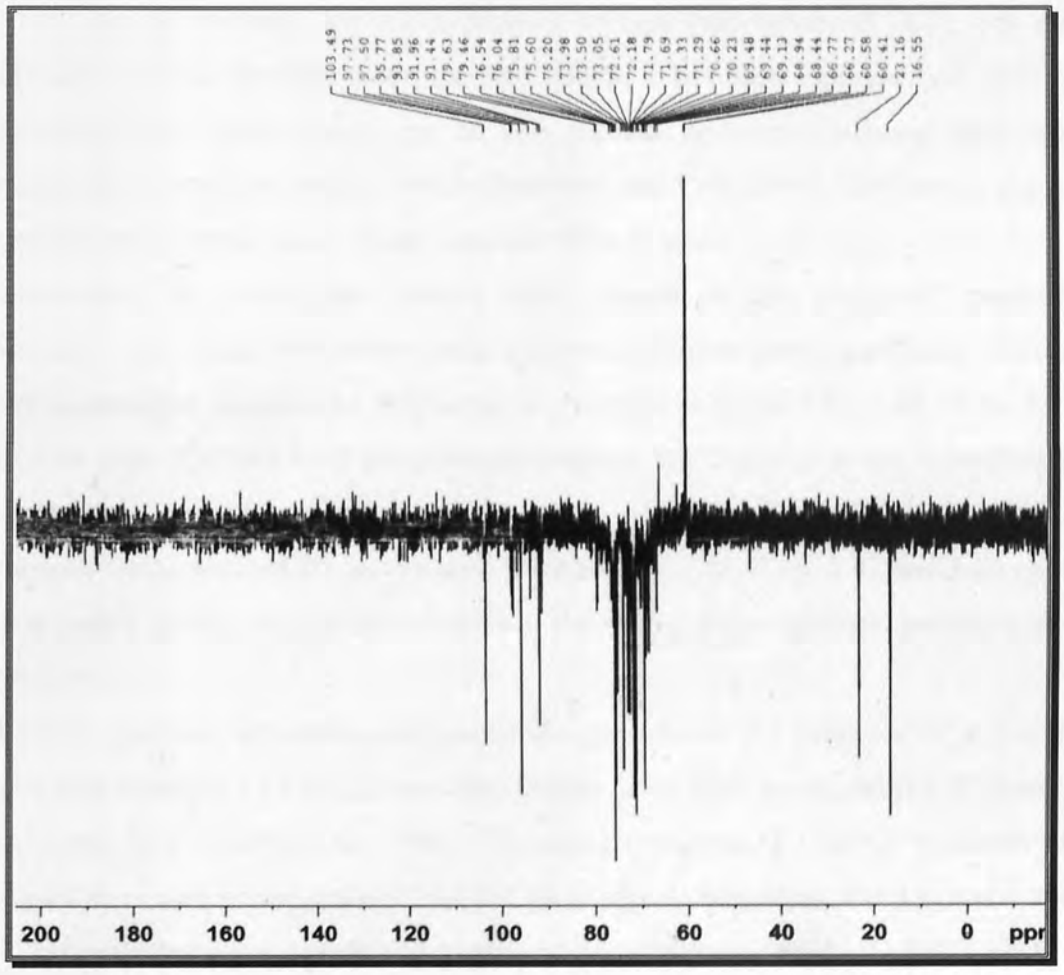


Figure 3.20: ^{13}C NMR of grewia polysaccharide gum hydrolysed with 2N H_2SO_4 and dissolved in D_2O

It can be seen from the ^{13}C NMR spectrum that grewia polysaccharide gum contains deoxygenated sugars. This is evident by the *-CH_3* signals appearing in the much higher field 15 to 20 ppm (Cui, 2005). This *-CH_3* may be attributable to the methyl group of the rhamnose sugar unit (see figure 1.1) and agrees with results from solid state ^{13}C NMR and GC analysis of the polysaccharide gum (section 3.3.7. and 3.3.10). The

signal between 20 to 30 ppm is thought to be a -CH_2 linked to *OH* which pushes the signal slightly downfield. The -CH_2OH may be attributable to the glucose or galactose sugar units. Signals from anomeric carbons of the monosaccharide components appear in the 90 to 110 ppm (Cui, 2005). The α- anomeric carbons are mostly in the region of 95 to 103 ppm whereas the β-anomeric carbons are in the region of 101 to 105 ppm. The results indicate the presence of 5 (or more) anomeric carbons which may be attributable to the five neutral sugar components of the polysaccharide (see section 3.3.10). The absence of signals from carboxyl carbons in the lower field between 170 to 180 ppm is evidence that the hydrolysis process (see section 2.12.1), was not sufficient to cause cleavage of uronic acid bonds. It has been reported that samples containing acidic acid residues can be very difficult to hydrolyse using traditional methods as reported in section 2.12.1 (Brummer and Cui, 2005). The signals due to non-anomeric carbons C_2-C_5 appear between 60 to 85 ppm.

Furthermore, ^{1}H NMR peak queries were entered on the biological magnetic resonance data bank (www.bmrb.wisc.edu/metabolomics/query_metab.php) which returned database matches for D-glucose at chemical shifts of 4.62, 3.67, 3.45, 3.41 and 3.36 ppm. The data bank also returned matches for D-galactose and L-arabinose. Matches for D-galactose were at chemical shifts of 4.57, 3.91, 3.83, 3.78, 3.67 and 3.66 ppm while matches for L-arabinose were at 3.91, 3.83, 3.78, 3.67 and 3.66 ppm. These results further confirm the presence of the neutral sugars glucose, arabinose and galactose.

^{1}H NMR spectrum of grewia polysaccharide gum shows the presence of a *C*-CH_3 group with signal at 1.15 to 1.21 ppm and further down field, the signal at 1.82 ppm is attributable to $COOCH_3$ (Cui, 2005). The signals between 3.1 to 4.3 ppm can be assigned to non-anomeric protons (H_2- H_6) while signals between 4.3 to 4.8, and 4.9 to 5.5 ppm arise from β-anomeric and α-anomeric protons respectively (Cui, 2005). The signals between 3.0 to 3.8 ppm have also been assigned to -*O*-CH_3. Again, the -CH_3 indicates the presence of methylated sugars and agrees with the ^{13}C NMR spectrum which together confirms that rhamnose is one of the sugar components of grewia polysaccharide gum (also see section 3.3.10). Matches for galacturonic acid and L-arabinose were returned at 103.49, 75.6, 73.98, 73.05, 72.61, 72.18, 70.66 ppm, and 75.2, 71.29, 68.94 ppm, respectively (www.bmrb.wisc.edu/metabolomics/).

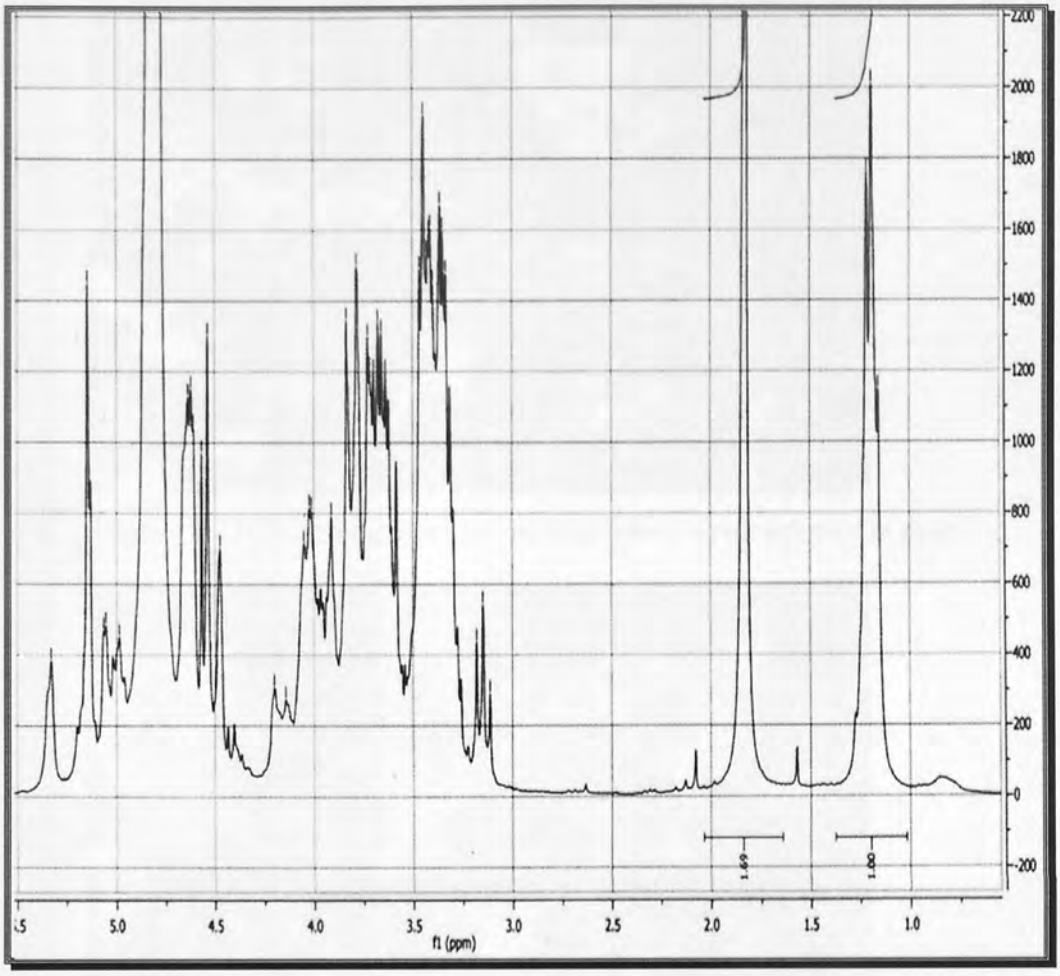


Figure 3.21: ^{1}H NMR of grewia polysaccharide gum hydrolysed with 2N H_2SO_4 and dissolved in D_2O

3.3.12 Scanning electron micrograph of grewia gum samples

The scanning electron micrographs of grewia gum showing the amorphous nature and the effect of drying technique on particle morphology of the polysaccharide gum powder are shown in figure 3.22 (air-dried), 3.23 (spray-dried) and 3.24 (freeze-dried). The micrographs show that spray-drying produced grewia polysaccharide powder that is smaller in size and with a more uniform size distribution than the freeze-dried or air-dried polysaccharide gum samples. Aggregation of particles seen with the spray dried sample is probably due to the presence of adsorb moisture. And although this may also be the case for the freeze-dried and the air-dried samples, the fact that these samples were powdered by use of mortar and pestle also mean that size-reduction may not be efficient or sufficient enough to effect a more uniform size distribution.

Figure 3.22: Scanning electron micrograph of air-dried grewia gum

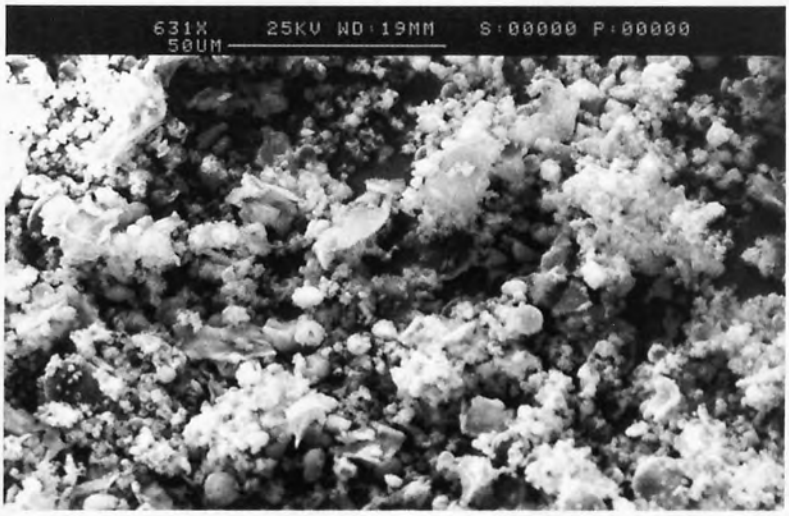


Figure 3.23: Scanning electron micrograph of spray-dried grewia gum

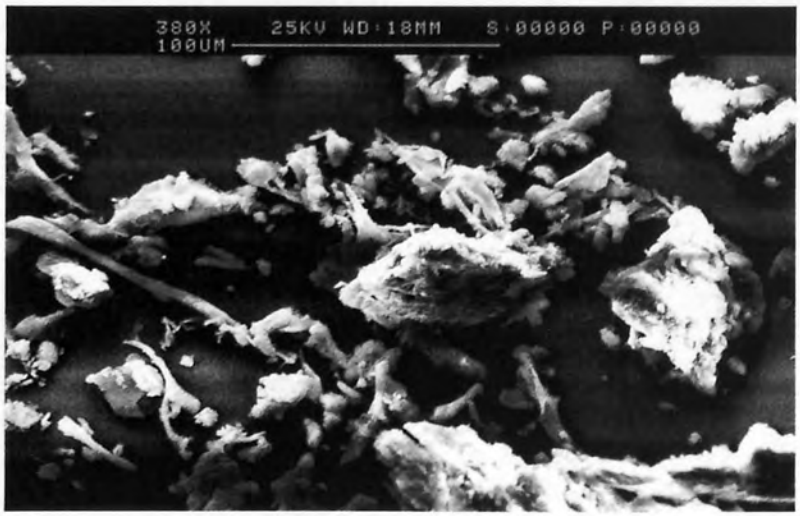


Figure 3.24: Scanning electron micrograph of freeze-dried grewia gum

3.4 CONCLUSION

Grewia polysaccharide gum can be extracted according to the method reported. FT-IR, and solid-state NMR confirmed the presence of a number of monosaccharide sugars and the amorphous nature of the gum. This was further confirmed by carbon component analysis using XPS. GC analysis of monosaccharide composition showed that the polysaccharide is composed of five neutral sugars which include glucose, rhamnose, arabinose, xylose and galactose with over 60% of the monosaccharide composition as glucose. GC analysis of the gum was supported by ^{1}H and ^{13}C NMR as containing glucose, rhamnose, arabinose and galactose as the neutral sugars.

The drying method employed during the processing of a polysaccharide can have some profound effects on the physicochemical properties and formulation characteristics of pharmaceutical excipients (York, 1983). The study shows that aqueous dispersions of the aid-dried grewia polysaccharide gum are more viscous than the freeze-dried or spray-dried polysaccharide gum. The drying method does not however affect the flow behaviour of the polysaccharide gum as all samples exhibited typical pseudoplastic (shear-thinning) flow behaviour. Solid-state NMR and FT-IR of the gum samples show that drying method has no obvious effect on the resultant spectra and indicates that drying technique does not change the nature of the polysaccharide gum but does have an effect on the relative composition of the carbon components and/ or elemental composition of the polysaccharide gum samples as indicated by XPS results.

The variation in intrinsic viscosity and molecular weight of the polysaccharide gum with drying method does indicate that the drying method could have some effect on both intrinsic viscosity and molecular weight. It was however noted earlier (see section 3.3.5) that other factors may be accountable for this observation.

CHAPTER FOUR

FILM-FORMING, SWELLING AND COMPRESSION CHARACTERISTICS OF GREWIA POLYSACCHARIDE GUM

4.1 INTRODUCTION

In recent times there has been a renewed interest in films made from natural polymers (Mali *et al.*, 2002; Mathew and Abraham, 2008) obtained from polysaccharides, lipids and proteins (Irissin-Mangata *et al.*, 2001). This is because these are renewable resources and are environmentally friendly. Edible or biodegradable films from natural polysaccharides have the potential to replace their synthetic counterparts as packaging materials and also as coating for solid dosage forms (Alves *et al.*, 2007).

Like proteins, natural polysaccharides are formed by various sugar units or monosaccharides which are capable of promoting intra- and inter-molecular bonds. They therefore have a large variation in their functional properties (Jangchud and Chinnan, 1999). Intermolecular interactions between polysaccharide chains based on hydrogen bonding, hydrophobic interactions and electrostatic forces can result in brittle films (Sothornvit and Krochta, 2001). Consequently, plasticisers must be added to modify the mechanical properties of the films. These compounds decrease inter- and intramolecular attractive forces and increase chain mobility thereby improving flexibility (Sa'nchez *et al.*, 1998; Irissin-Mangata *et al.*, 2001; Vanin *et al.*, 2005; Dai *et al.*, 2010). In most literature, polyols such as glycerol, sorbitol, polyethylene glycol and ethylene glycol have been used as plasticisers for films from carbohydrate sources (Lourdin *et al.*, 1997). According to Rodriguez *et al.*, (2006), the incorporation of these additives may however cause very significant changes in the barrier properties of the films. The choice of a plasticizer is based on compatibility between plasticizer and polysaccharide, permanence in the formed film, and efficiency of effect (Sothornvit and Krochta, 2001). It has also been reported that the extent and degree of modification of film properties is dependent on the type of plasticizer used (Dai *et al.*, 2010).

Natural polymers have come to enjoy wide acceptability for use in oral prolonged release compressed tablets as matrix systems. In order for these natural polymers to effectively function as matrix systems, they must possess a good degree of compactibility and compressibility in their dry powder form. A number of relationships based on transformation of classical stress-strain or force-displacement relationships where either the compaction pressure or the volume is transformed, have been used to describe the compressibility of powders (Kawakita and Ludde, 1970; Celik, 1992). Most of these equations are however of limited practical value

(Hassanpour and Ghadiri, 2004). In the development and formulation of tablets, the characterization of the compressibility of a material powder should achieve the goal of predicting the strength of the resultant compact from force-displacement curves and derived parameters.

Walker (1923) reported the first work on compression which related the relative volume of assembly (V) to the logarithm of pressure (P) on the bed using the relation (*equation 4.1*).

$$V = a_1 - lnP \qquad \text{--- (Equation 4.1)}$$

A model for the reduction of porosity of a bed during compaction in the form of a first order rate process relating the pressure (P) to the porosity ($\boldsymbol{\varepsilon}$) was proposed by Shapiro (1944) in the following form (*equation 4.2*).

$$-\frac{d\varepsilon}{dP} = \kappa\varepsilon \qquad \text{----- (Equation 4.2)}$$

After integrating the equation from initial porosity (ε_o) to the final porosity ($\boldsymbol{\varepsilon}$), Shapiro came out with the following equation (*equation 4.3*).

$$ln\frac{1}{\varepsilon} = In\frac{1}{\varepsilon_o} + KP \qquad \text{----- (Equation 4.3)}$$

Later, Heckel (1961) showed the existence of a linear portion of the curve in which the slope (K) was inversely related to the hardness of metal powders. Substituting porosity ($\boldsymbol{\varepsilon}$) with relative density (D) in the *equation 4.3*, the resulting *equation 4.4* is known as the Heckel equation.

$$ln\frac{1}{1-D} = A + KP \qquad \text{------ (Equation 4.4)}$$

When relating the relative density D of a powder bed during compression to the applied pressure P, the Heckel equation is often used. The slope of the straight-line portion, K, is the reciprocal of the yield pressure P_y, of the material (Emeje *et al.*, 2006). This material constant has been reported to be influenced by experimental conditions thus resulting in different yield pressures being reported for the same material (Kiekens *et al.*, 2004). However, Heckel analysis (determination of the P_y value) remains one of the most widely used techniques for the study of the compaction behaviour of pharmaceutical compacts despite the fact that its practical application has been questioned (Sonnergaard, 1999).

An important functional property of polysaccharide gums is their ability to hydrate and swell to form highly viscous solutions or dispersions. This physical property

underlies their use as viscosity imparting or suspending agents and their use as matrix formers in prolonged release solid dosage forms. The hydration rate and degree of swelling is very critical in their successful application and is dependent on a number of factors such as pH and temperature. The water absorption characteristics of polysaccharide gums can have a significant influence on the transport of medicaments across the gel layers (Peniche *et al.*, 1997)

In this study, the compactibility and swelling of grewia polysaccharide gum was evaluated to demonstrate the suitability of the polysaccharide as a polymer matrix in controlled release tablets. Also, the mechanical properties of films of grewia polysaccharide gum and the effect of plasticizer on the mechanical properties of the films was evaluated.

4.2 MATERIALS AND METHODS

All the materials used for this study were procured from Sigma-Aldrich unless otherwise stated. All equipment used is quoted in the text.

4.2.1 Preparation of grewia polysaccharide gum films

The mechanical properties of films prepared using the air-dried grewia polysaccharide gum sample were evaluated. Grewia polysaccharide gum dispersion (1% or 2% w/v) was prepared and hydrated overnight in order to rid the dispersion of trapped air bubbles. Similar dispersions were made using pullulan and guar gum. Pullulan was prepared by heating in distilled water at 60°C while stirring continuously. Thereafter it was allowed to settle overnight to remove entrapped air.. The solutions or dispersions so prepared were cast into petri dishes (15 mm in diameter) and allowed to dry in an oven at 37°C. The films were conditioned for 48 hours at 55% relative humidity using $CaCl_2$ in a desiccator and the effect of plasticizer on the mechanical properties of the films was evaluated. Grewia polysaccharide gum films containing PEG 200 in the ratios 5:1, 2:1 and 1:1 were prepared and cast similarly into petri dishes. The films were allowed to dry and conditioning was carried out as described previously, the films' mechanical properties were evaluated.

The films were cut into rectangular shapes of 10 mm width and 30 mm length. The mechanical properties of the films were determined using a Hounsfield tensiometer (Tenius Olsen, UK). The software was QMAT software with settings adjusted to an

extension range of 2000 mm, gauge length 20 mm, speed 20 mm/minute, and approach speed of 2 mm/minute. The load range was 5000 N.

4.2.2 Water uptake

Direct compression of 200 mg discs of air-dried or freeze-dried grewia polysaccharide gum was carried out using a KBr press (Specac 15.011, Germany) at 5 KgF/cm^2 for 2 minutes. Each accurately weighed disc was placed into a glass vial containing 10 ml of distilled water maintained at 10°, 30° or 50°C in a water bath using a thermostat. The water uptake *W*, was measured at 15 minute intervals by draining and removing excess water from the surface of the swollen gels and accurately determining the weight gained. A fresh amount of distilled water was replaced into the vial after every reading.

The water uptake (*W*) was calculated using the formula:

$$W = \frac{M - M_o}{M_o} \qquad \text{------ (Equation 4.5)}$$

Where, *Mo* is the weight of the dry disc and *M* is that of the swollen sample at time *t*.

4.2.3 Preparation of grewia polysaccharide gum compacts

Grewia polysaccharide gum (500 mg) was carefully weighed on an analytical balance and manually transferred into a 13 mm die of a KBr press. Compression of the polysaccharide powder into tablets was carried out at 3, 4, 5, 6 or 7 KgF/cm^2, maintained for 60 seconds. Thereafter, the thickness and diameter of the compacts were measured in triplicate using a digital slide calliper (Starett, The Starett Co, MA). The apparent density was determined by geometric calculation while true density was determined using a Multipycnometer (MVP- D160-E Quaterchrome Instruments, USA).

4.3 RESULTS AND DISCUSSION

4.3.1 Mechanical properties of grewia polysaccharide gum films

The mechanical properties of films containing 1% w/v dispersions of grewia polysaccharide gum, pullulan and guar gum are shown in table 4.1.

Grewia polysaccharide gum and guar gum films without plasticizer were brittle and difficult to handle in contrast with pullulan films which were highly flexible. The results (table 4.1) show that grewia polysaccharide gum films have a tensile strength

which is not significantly lower than pullulan films ($P>0.05$), but have a lower elastic modulus than those of pullulan and guar gum ($P<0.05$).

Table 4.1: Mechanical properties of 1% w/v polysaccharide films (n=5, mean ± s.d.)

	Tensile strength (MPa)	*Maximum force (N)*	*Elastic modulus (N/mm^2)*
Grewia	19.22±3.61	9.1±2.18	2.13±0.12
Pullulan	22.31±8.02	6.69±2.41	3.33±0.00
Guar	35.79±2.5	12.53±0.87	2.86±0.00

The effect of adding polyethylene glycol (*MW* 200) as a plasticizer to grewia polysaccharide gum films is shown in table 4.2. As the concentration of plasticizer increases the films become less brittle with a more translucent appearance. The results show that the tensile strength of grewia gum films decreases with increasing concentrations of polyethylene glycol. The addition of 20% PEG to grewia polysaccharide gum (ratio 5:1) decreased the tensile strength by about 30%. Further increases in the concentration of the plasticizer resulted in further decreases in the tensile strength of the films. Similar results were observed for the elastic modulus which decreased from 2.13 to 1.97 N/mm^2 with the addition of 20% PEG and further to 1.62 N/mm^2 with the addition of 100% (by weight grewia gum) of PEG.

These results are in line with similar studies on the effects of plasticizer concentration on the mechanical properties of films (Yamauchi and Yamauchi, 2002; Watkinson and Mohsen 2003; Sobral *et al.*, 2005; Moore *et al.*, 2006) showing that PEG is capable of plasticising polysaccharide films and the effect is concentration dependent.

Table 4.2: The effect of PEG (*MW*=200) on the mechanical properties of 1% w/v grewia polysaccharide gum films (n=5, mean ± s.d.)

	Tensile strength (MPa)	*Maximum force (N)*	*Elastic modulus (N/mm^2)*
Grewia	19.22±3.61	9.1±2.18	2.13±0.12
Grewia+PEG (5:1)	13.52±0.87	6.87±0.49	1.97±0.02
Grewia+PEG (2:1)	11.95±0.65	7.17±0.39	1.67±0.0
Grewia+PEG (1:1)	8.79±0.42	5.46±0.61	1.62±0.11

4.3.2 Heckel analysis of grewia polysaccharide gum compacts

Powder compaction is a volume reduction process, and the Heckel equation is also based on volume change of a powder column during compression. The plots give a

general impression of the densification process of the powder column. The apparent density (ρA) of the polymer compacts was calculated geometrically while the true density (ρT) of the air-dried or the freeze-dried grewia polysaccharide gum was used as determined in section 3.2.5 and 3.3.2. The relative density, D of the compacts was calculated according to the formula:

$$D = \rho A/\rho T \qquad \text{--------- (}\textit{Equation 4.1}\text{)}$$

A plot of *ln 1/1-D* against compression pressure P gives the Heckel plot for the given sample (figure 4.1).

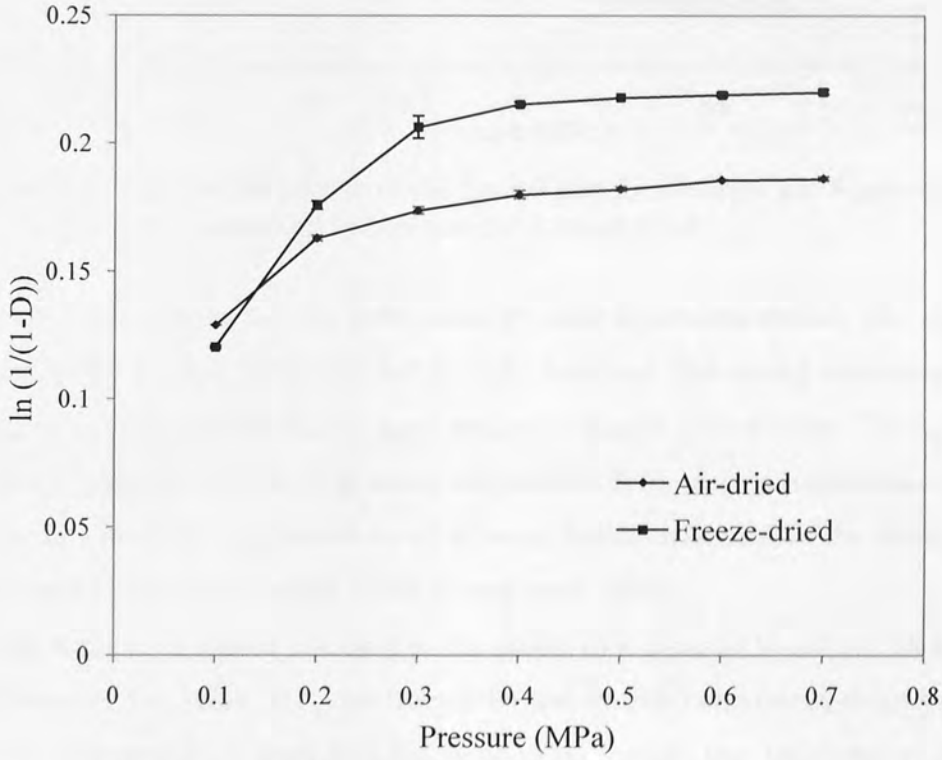


Figure 4.1: The Heckel plot for freeze-dried and air-dried grewia polysaccharide gum compacts (n=3, mean ± s.d.)

Heckel (1961) showed the existence of a linear portion of the curve in which the slope (K) was inversely related to the hardness of metal powders. This straight line portion of the Heckel plot *ln 1/1-D* against pressure (P) is shown in figure 4.2. To explain the deformation characteristic of grewia polysaccharide gum Heckel constants were derived from the plots (Table 4.3). The slope of the straight-line portion, K, is the reciprocal of the yield pressure P_y, of the material (Emeje *et al.*, 2006). The mean yield pressure, P_y, values were calculated from the slope of the linear line constructed over the compression pressure range (figure 4.2).

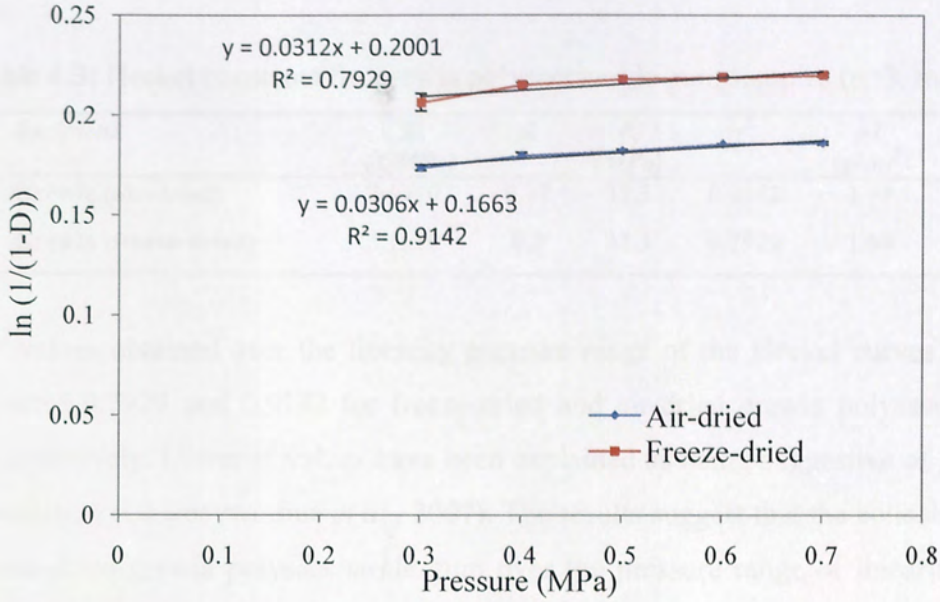


Figure 4.2: Straight line portion of the Heckel plot for air-dried and freeze-dried grewia polysaccharide gum (n=3, mean ± s.d.)

The Heckel plots (figure 4.1) for both materials were somewhat similar. The slope of the straight line portion of the Heckel plot (K) indicates that during compression or compaction grewia polysaccharide gum undergoes plastic deformation. The constant K for the polysaccharide gum was same irrespective of the drying technique. Greater plasticity in a material is indicated by an increase in the value of K of the Heckel plot (Paronen and Lilla, 1996; Heckel, 1996; Zhang *et al.*, 2003).

The yield pressure is related inversely to the ability of a material to deform plastically under pressure. The value of P_y determined for the air-dried and freeze-dried samples of grewia polysaccharide gum was the same. This implies that the onset of plastic deformation in the two samples of grewia polysaccharide gum occurred at the same pressure (Emeje *et al.*, 2006) and yield pressure is independent of experimental conditions (Kiekens *et al.*, 2004).

This indicates that the drying conditions have no effect on the yield pressure of grewia polysaccharide and consequently both air-dried and freeze-dried samples have the same onset of plastic deformation. It has been reported (Ohwoavworhua *et al.*, 2007) that lower value of P_y (i.e. larger value of the slope K) can be correlated with the crushing strength of tablets; lower values of P_y usually indicating harder tablets. This implies that variation in tablet hardness may be insignificant when the freeze-dried or air-dried samples of grewia polysaccharide gum are used as binder in tablets.

Table 4.3: Heckel constants for grewia polysaccharide gum samples (n=3, mean)

Excipient	*K* (*1/MPa*)	*A*	P_y (*MPa*)	r^2	ρT (g/cm^3)
Grewia (air-dried)	$3.1x10^{-2}$	0.17	32.3	0.9142	1.98
Grewia (freeze-dried)	$3.1x10^{-2}$	0.2	32.3	0.7929	1.68

The r^2 values obtained over the linearity pressure range of the Heckel curves in this study were 0.7929 and 0.9142 for freeze-dried and air-dried grewia polysaccharide gum respectively. Lower r^2 values have been explained as being suggestive of greater fragmentation (Ohwoavworhua *et al.*, 2007). The results suggest that the consolidation of freeze-dried grewia polysaccharide gum over the pressure range of linearity may have involved fragmentation as well as plastic deformation of the primary particles. For air-dried grewia polysaccharide gum with a larger r^2 value, it is probable that plastic deformation of the predominant primary particles contributed overwhelmingly to the formation of compacts.

4.3.3 Effect of temperature on water uptake of grewia polysaccharide gum

The water uptake profiles for polysaccharide gum samples at the different temperatures studied are shown in figure 4.3-4.5.

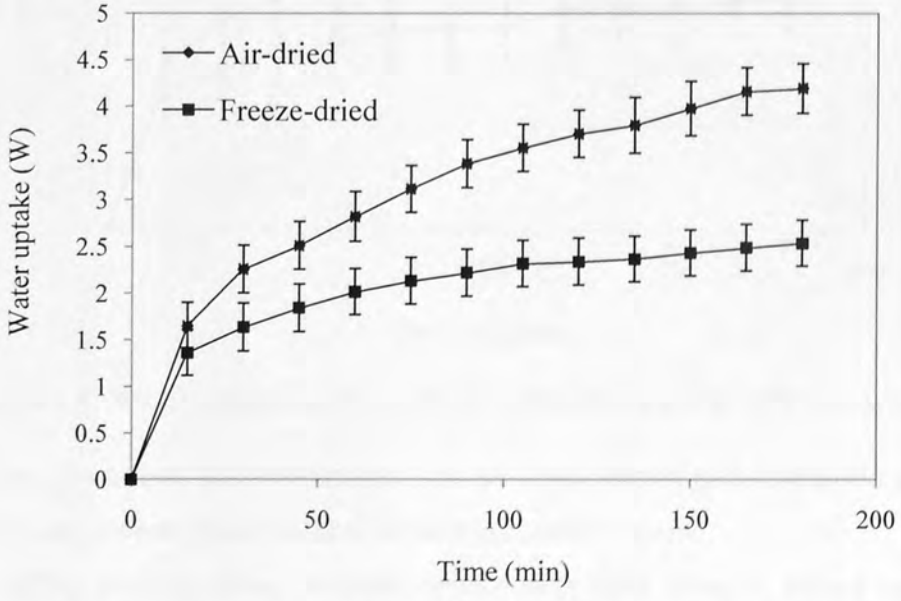


Figure 4.3: Water sorption profile of gels in distilled water at 10°C (n=3, mean ± s.d.)

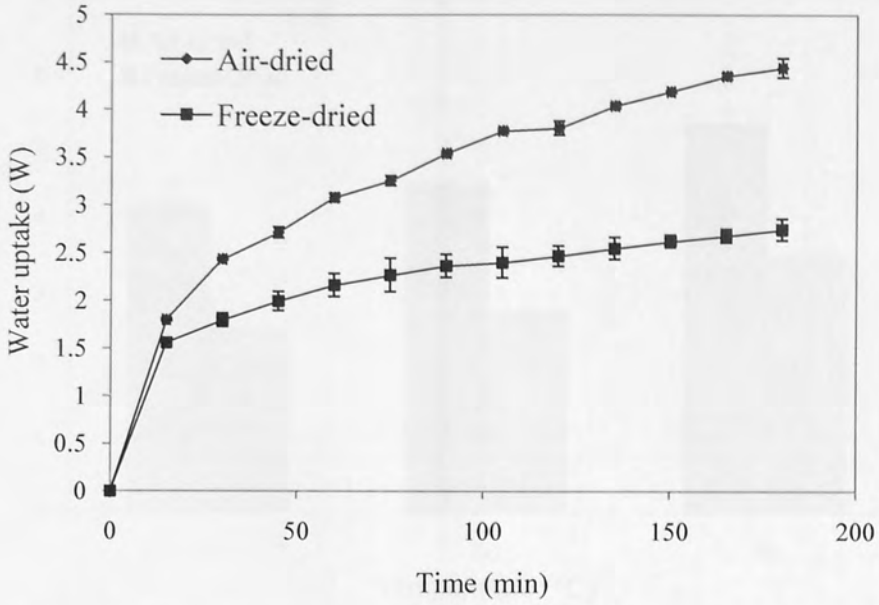


Figure 4.4: Water sorption profile of gels in distilled water at 30°C (n=3, mean ± s.d.)

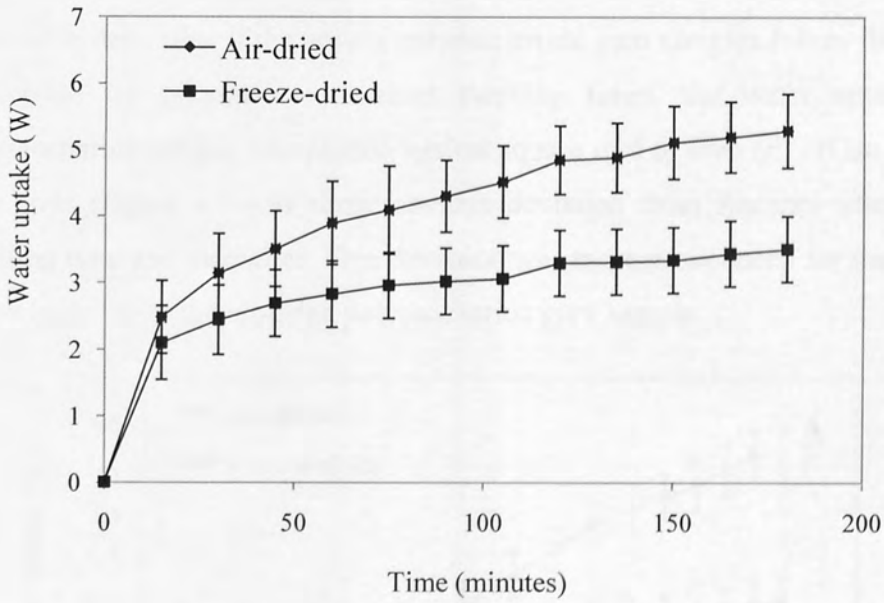


Figure 4.5: Water sorption profile of gels in distilled water at 50°C (n=3, mean ± s.d.)

At any given time and temperature, the air-dried grewia polysaccharide gum sample swells and absorbs more water than the freeze-dried sample.

The effect of temperature on water uptake over three hours is shown in figure 4.6. Water uptake increased with increasing temperature from 10°C to 50°C. This is in contrast to "thermo-shrinking" gels that swell at low temperatures and de-swell at higher temperatures (Gan *et al.*, 2001).

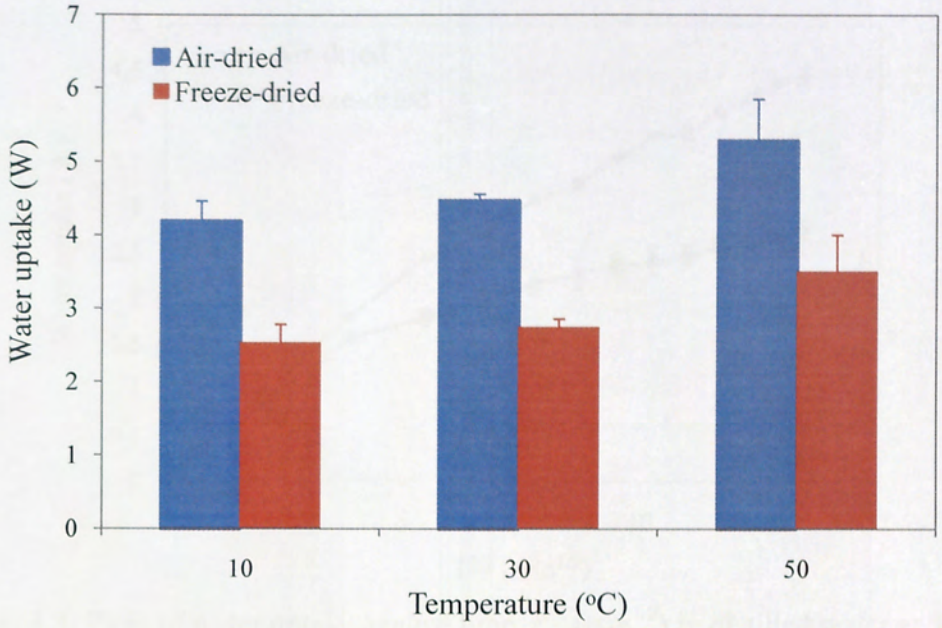


Figure 4.6: Effect of temperature on equilibrium water uptake for air-dried and freeze-dried polymer gels in distilled water after 3 hours (n=3, mean ± s.d.)

In order to determine if the grewia polysaccharide gum samples follow the mechanism of release for polymers with short swelling times, the water uptake for each polysaccharide sample was plotted against square root of time ($t^{1/2}$) (Gan *et al.*, 2001). The plots (figure 4.7-4.9) show obvious deviation from linearity after 30 min of swelling time and thereafter. This deviation was more pronounced for the freeze-dried as compared with the air-dried polysaccharide gum sample.

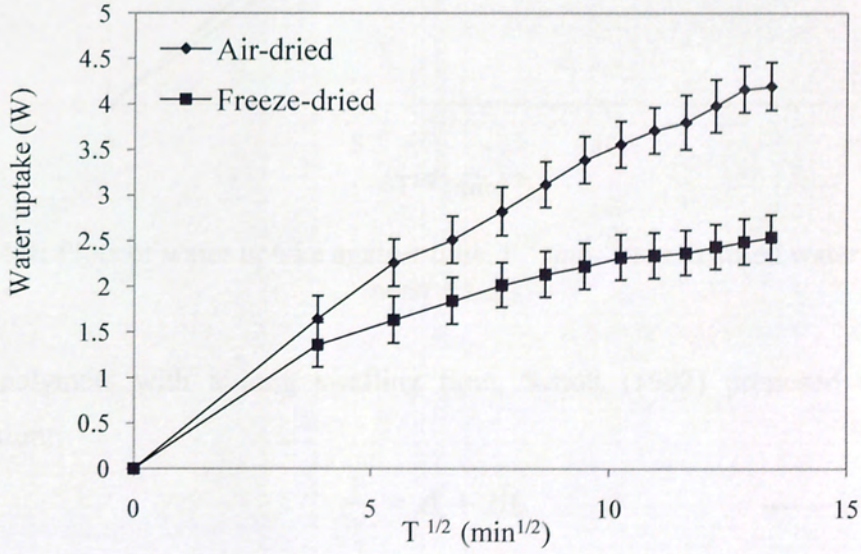


Fig. 4.7: Plot of water uptake against time, $t^{1/2}$ ($min^{1/2}$) in distilled water at 10°C (n=3, mean ± s.d.)

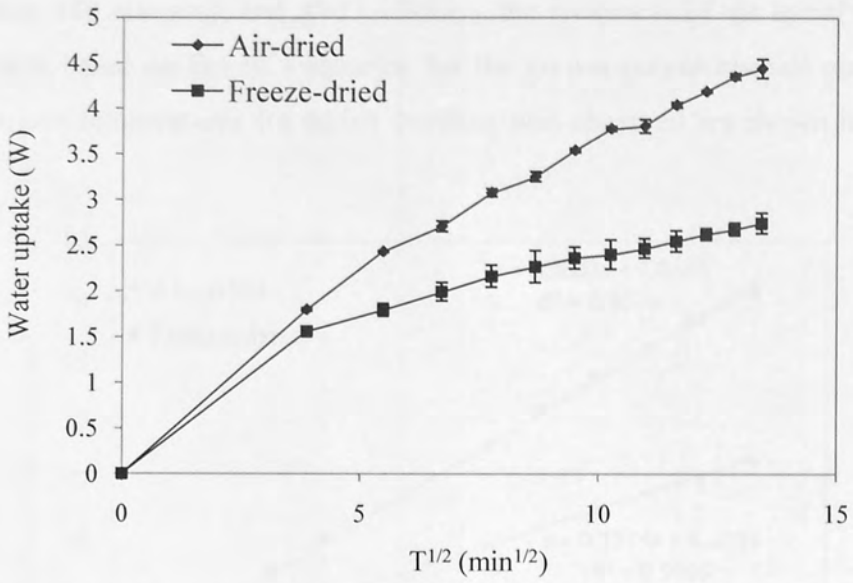


Fig. 4.8: Plots of water uptake against time, $t^{1/2}$ ($min^{1/2}$) in distilled water at 30°C (n=3, mean ± s.d.)

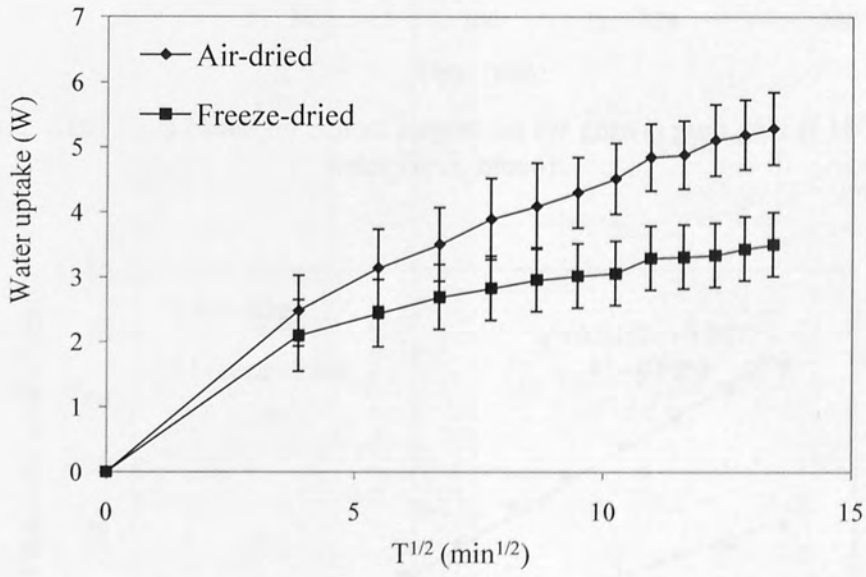


Fig. 4.9: Plots of water uptake against time, $t^{1/2}$ ($min^{1/2}$) in distilled water at 50°C (n=3, mean ± s.d.)

For polymers with a long swelling time, Schott (1992) proposed the following equation:

$$\frac{t}{W} = A + Bt \qquad \text{------- (Equation 4.6)}$$

The above equation was applied to the data set shown in figure 4.3-4.5 where W is the water uptake at time t, $B = 1/W_\infty$ (W_∞ is the water uptake at infinite time, which in this

case was 180 minutes), and $A=1/(dW/dt)_0$, the reciprocal of the initial swelling rate. The plots based on Schott's equation for the grewia polysaccharide gum samples at the various temperatures for which swelling was observed are shown in figure 4.10-4.12.

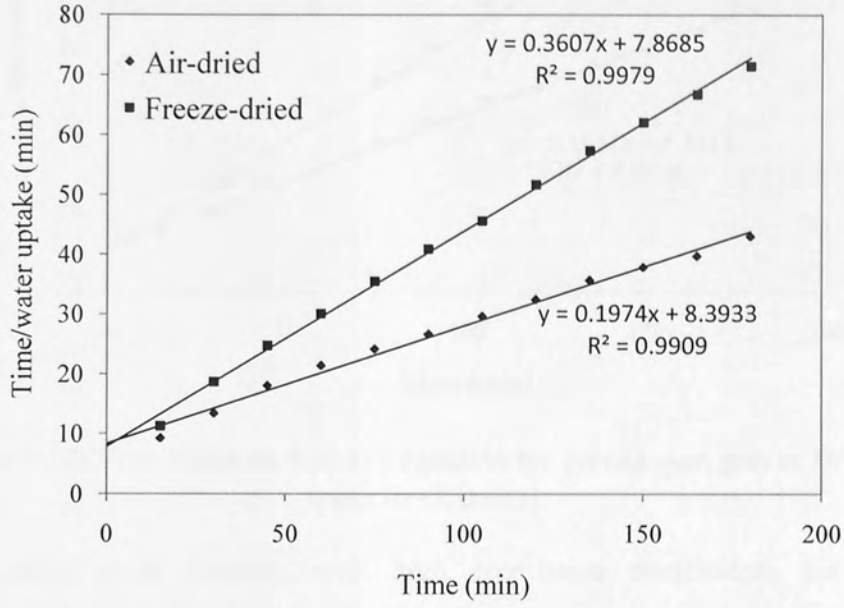


Figure 4.10: Plots based on Schott's equation for grewia gum gels at 10°C in distilled water (n=3, mean)

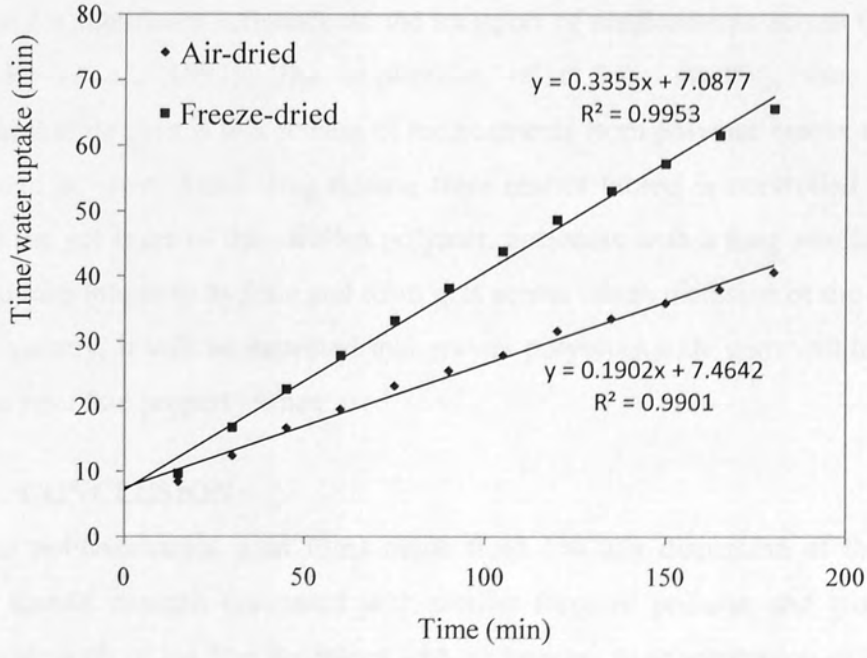


Figure 4.11: Plots based on Schott's equation for grewia gum gels at 30°C in distilled water (n=3, mean)

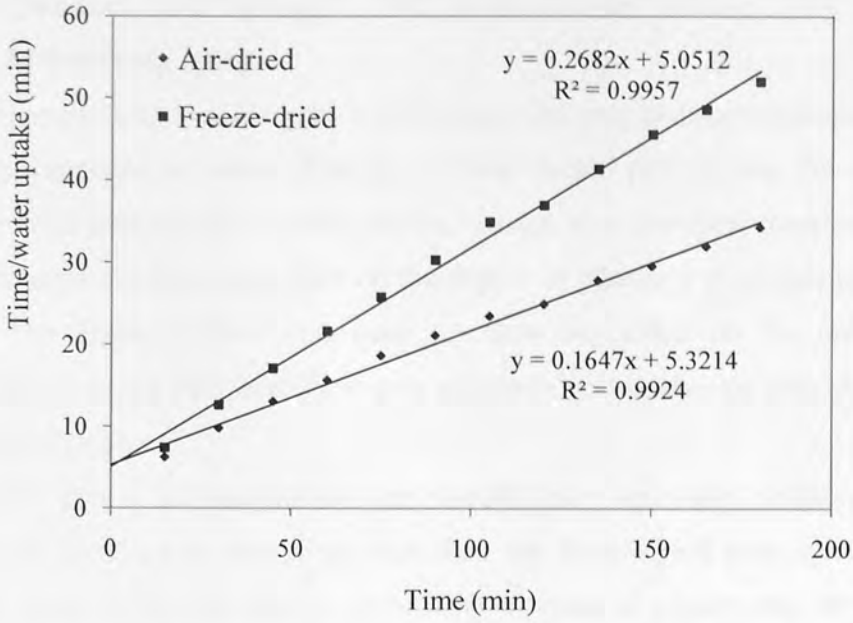


Figure 4.12: Plots based on Schott's equation for grewia gum gels at 50°C in distilled water (n=3, mean)

The results show linearity with high correlation coefficients for all swelling experiments conducted at 10°, 30° and 50°C. The good correlation indicates that both air-dried and freeze-dried grewia polysaccharide gum samples are polymers with a long swelling time. This water absorption characteristic of grewia polysaccharide gum can have a significant influence on the transport of medicaments across the gel layers (Peniche *et al.*, 1997). The implication of a long swelling time for grewia polysaccharide gum is that release of medicaments from polymer matrix tablets of the gum will be slow. Since drug release from matrix tablets is controlled by transport across the gel layer of the swollen polymer, polymers with a long swelling time will tend to take longer to hydrate and form gels across which diffusion of the drug occurs. Consequently, it will be expected that grewia polysaccharide gum will have a strong release retardant property when used.

4.4 CONCLUSION

Grewia polysaccharide gum films made from 1% w/v dispersion of the gum have lower tensile strength compared with similar films of pullulan and guar gum. The tensile strength of the film decreases with an increase in concentration of polyethylene glycol (molecular weight 200) used as plasticizer. Similar results have been reported in studies on the effects of plasticizer concentration on the mechanical properties of

films (Yamauchi and Yamauchi, 2002; Watkinson and Mohsen 2003; Sobral *et al.*, 2005; Moore *et al.*, 2006).

Heckel analysis shows that grewia polysaccharide gum undergoes plastic deformation during compression studies. The slope of the Heckel plot (k) was the same for both freeze-dried and air-dried grewia polysaccharide gum samples; therefore, the drying method does not have any effect on the degree of plasticity of grewia polysaccharide gum. The drying method also does not have any effect on the onset of plastic deformation of the polysaccharide gum with both polysaccharide gum samples having the same P_y value.

Air-dried grewia polysaccharide gum sample takes up water resulting in a higher degree of swelling at any given time than the freeze-dried polysaccharide sample. Water uptake increases with corresponding increase in temperature for both the air-dried and freeze-dried grewia polysaccharide gum samples. Plots based on Schott's equation (*equation 4.6*) show both samples of the gum give a high level of correlation indicating that both samples are polymers with long swelling time. This suggests that matrix tablet formulations of grewia polysaccharide gum will swell slowly and consequently release of the API from the tablet core will be sustained. Consequently, grewia polysaccharide gum may have release retardant properties suitable for oral controlled drug delivery.

CHAPTER FIVE

EVALUATION OF GREWIA POLYSACCHARIDE GUM AS EXCIPIENT IN IBUPROFEN ORAL SUSPENSION FORMULATION

5.1 INTRODUCTION

Pharmaceutical suspensions are solid dispersions of insoluble or sparingly-soluble drugs in an aqueous or oily vehicle. They are intended for oral administration, topical application or parenteral administration of drugs. Aerosol suspensions of finely divided or micronized drugs are also another class of pharmaceutical suspensions intended for inhalation.

Formulation of drugs as suspensions for oral administration is a convenient way to administer insoluble or sparingly soluble drugs to infants and the elderly that have difficulty swallowing tablets or capsules. Pharmaceutical oral suspensions are also intended to mask taste.

Pharmaceutical suspensions will settle when left to stand for a long time and this can happen during storage. The solid insoluble drug separates from the vehicle and settles to the bottom of the container. It is desirable that such a formulation re-suspend easily upon shaking to ensure homogeneity of dose. Re-dispersibility of the insoluble drug substance is therefore a critical requirement in the evaluation of suspensions. Loose networks of flocs (flocculated systems) settle rapidly, do not form cakes and are easily re-dispersible. Settling and aggregation may result in the formation of cakes that are difficult to re-suspend. This is a common occurrence in deflocculated systems which do not easily settle but are difficult to re-disperse once set.

It is also a critical requirement that the drug in suspension be homogeneously mixed and remain both physically and chemically stable during the shelf-life of the formulation. This is important because of the need to dispense a uniform and accurate dose of the medicament per portion of the suspension. Suspensions intended for parenteral and ocular administration have the additional requirement of sterility.

In the formulation of suspensions, wetting agents are used to promote wettability of the insoluble drug substance. Non-wetting of the insoluble drug can result in floating of the drug in the continuous phase, adversely affecting content uniformity. Also to prevent rapid settling of the dispersed phase, suspending agents are used, as viscosity imparting agents. They reduce caking and promote re-dispersibility.

The aim of this study was to compare the efficacy of grewia polysaccharide gum as a suspending agent in ibuprofen suspension with other suspending agents. Three suspending agents were used as standards: xanthan gum (XAN), acacia gum (ACA) and sodium carboxymethylcellulose (SCMC). The evaluation parameters were

viscosity, rheology, redispersibility, pourability, sedimentation volume, re-dispersibility, degree of flocculation, zeta potential and microbial load.

5.2 MATERIALS AND METHODS

All materials used for this study were procured from Sigma-Aldrich, UK except otherwise indicated, and include: xanthan gum (XAN), sodium carboxymethylcellulose (SCMC) (Molecular weight 250,000), acacia gum (ACA), ibuprofen (volume mean diameter = 76.5 ± 0.8 μm), glycerol, polysorbate 20 (Tween 20®) and sodium saccharin. Grewia polysaccharide gum was extracted in our laboratory according to the method of Tamoda (1977) with modification.

5.2.1 Suspension formulation

Ibuprofen suspension was formulated according to the batch formula shown in table 5.1.

Table 5.1: Batch formula for preparation of ibuprofen suspension

Ingredients	*Quantities*
Ibuprofen (g)	2.0
Suspending agent (% w/v)	0.5, 0.75 or 1.0
Glycerol (ml)	10.0
Sorbitol F 70wt% (ml)	10.0
Tween 20® (% v/v)	2.0
Sodium saccharin (g)	0.02
Distilled water to	100 ml

Accurately weighed sodium saccharin, suspending agent and ibuprofen were geometrically triturated with a mortar and pestle. Sorbitol, followed by glycerol was added while triturating continuously. Some amount of water was added to make it pourable and the content was then transferred into a 100 ml bottle and made up to 100 ml with rinsing from the mortar. This formulation contained 100 mg of ibuprofen per 5 ml of suspension. A total of 300 ml of formulation was made for each batch. Suspending agents evaluated were xanthan gum (XAN), acacia gum (ACA), sodium carboxymethyl cellulose (SCMC) and grewia polysaccharide gum (freeze-dried- (FDGG), and air-dried- (ADGG)). Sodium sacharin was used primarily as a sweetener, while glycerol and sorbitol F 70 wt% provided both flavour and sweetening. Tween 20®, a surfactant was used as wetting agent.

5.2.2 pH, viscosity and rheology

The pH of the suspension formulations were measured using a pH meter. The viscosity of the preparations made with the different suspending agents was measured using a viscometer (DV-1+version 5, Brookfield Engineering Labs, Stoughton-USA) at shear rates of 10, 20, 30, 50 and 100 rpm. The apparent viscosity was read in centipoises at a temperature of 20°C using spindle number 2.

5.2.3 Sedimentation volume

Three 50 ml portions of the preparations were poured separately into three 100 ml measuring cylinders and allowed to stand. Sedimentation volume was taken after 1, 2, 3 and 7 days, and thereafter at weekly intervals for 12 weeks. Sedimentation volume ratio, F was calculated according to *equation 1.17* (see page 74).

5.2.4 Redispersibility

A 35 ml portion of each batch of the formulations was transferred into capped cone tubes and evaluated for redispersibility at 7 day intervals. This was done by clamping the tube to a stand and then turning it through a ninety degree cycle. Redispersibility was taken as the number of inversions required to completely redisperse the formulation in the cone tube.

5.2.5 Degree of flocculation

To evaluate the degree of flocculation in the different batches of the formulations, 0.004 ml of 1 M potassium dihydrogen phosphate (KH_2PO_4) was added as a flocculating agent. The degree of flocculation was assessed by comparing the ultimate sedimentation volume after 12 weeks with the same formulations in which no flocculating agent was added.

Degree of flocculation, $\beta = F / F\alpha$ ------ (*Equation 5.1*)

Where, F is the ultimate sedimentation height in the flocculated system and,
$F\alpha$ is the ultimate sedimentation height in deflocculated system.

5.2.6 Zeta potential

The zeta potential of the formulated suspensions was determined on a zeta potential analyzer, ZetaPlus (Brookhaven Instruments Corporation, U.S.A). Approximately 1 ml of suspension was transferred into a plastic cuvette using a pipette and diluted with distilled water up to two-thirds of the cuvette. The Brookhaven zeta potential software was used with measurement parameters set to a temperature of 25°C and refractive

index (1.33). The zeta potential of the formulations was determined on day 0, 7, 14, 21 and day 28 of formulation.

5.2.7 Microbiological evaluation

Suspension formulations containing 1.0% w/v of suspending agents were analysed for microbial load. This was done on day 0 of formulation and on day 21 following storage. Nutrient broth was prepared by weighing 13.0 g of the powder and dissolving in 1 litre of distilled water before autoclaving at 121°C for 1 hour. Nutrient agar was prepared by transferring 28 g of the powder into 1 litre of distilled water and autoclaving at 121°C for 1 hour. Thereafter, 0.1, 0.01 or 0.001 ml of the suspension formulations were dispensed aseptically into pre-sterilised universal tubes followed by 10 ml of nutrient broth. Nutrient broth containing nil amount of sample was used as a negative control. These were then incubated in an oven at 37°C for 48 hours. At the end of the 48 hours a 1 ml portion was aseptically transferred into already prepared nutrient agar plates with spreading. The nutrient agar plates were then replaced into the oven and incubated for 48 hours. The results were interpreted according to the B.P (2010) method for evaluation of the microbiological quality of non-sterile pharmaceutical preparations and substances for pharmaceutical use.

5.3 RESULTS AND DISCUSSION

5.3.1 Appearance and pourability

All XAN-containing suspension formulations formed dispersed systems in which the particles remained discrete over the entire duration of the study. ACA or SCMC-containing suspension formulations rapidly sedimented within hours of formulation with a clear and transparent supernatant liquid. ADGG or FDGG-containing formulations did not sediment as quickly as the ACA or SCMC-containing formulations and the sedimentation volumes were larger. The supernatant liquid of the ADGG or FDGG containing formulations were cloudy. The cloudy appearance of the supernatant liquid was due to the presence of colloidal particles that remain dispersed (Figure 5.1).

All the suspension formulations were pourable except XAN-containing suspension formulations at a concentration of 1.0% w/v which were not readily pourable.

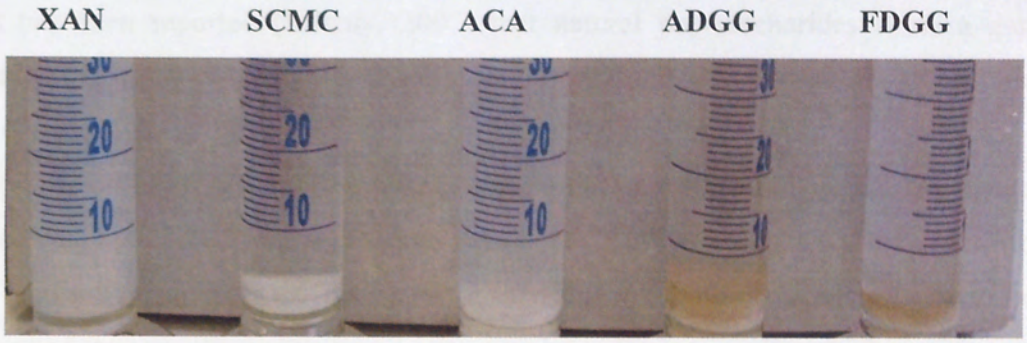


Figure 5.1: Ibuprofen suspension formulations containing 1.0% w/v suspending agent

5.3.2 Viscosity and pH of the suspension formulations

Figure 5.2 compares the viscosity of the ibuprofen suspension formulations containing 0.75% w/v suspending agent at the beginning (day 0) and at the end (day 84) of the study. It can be seen that at the end of the study, the viscosity of all the formulations had reduced. The viscosity of XAN-containing formulations (0.75% w/v) exceeded the limit of the Brookfield viscometer at the settings used and could not be measured at the beginning or at the end of the study.

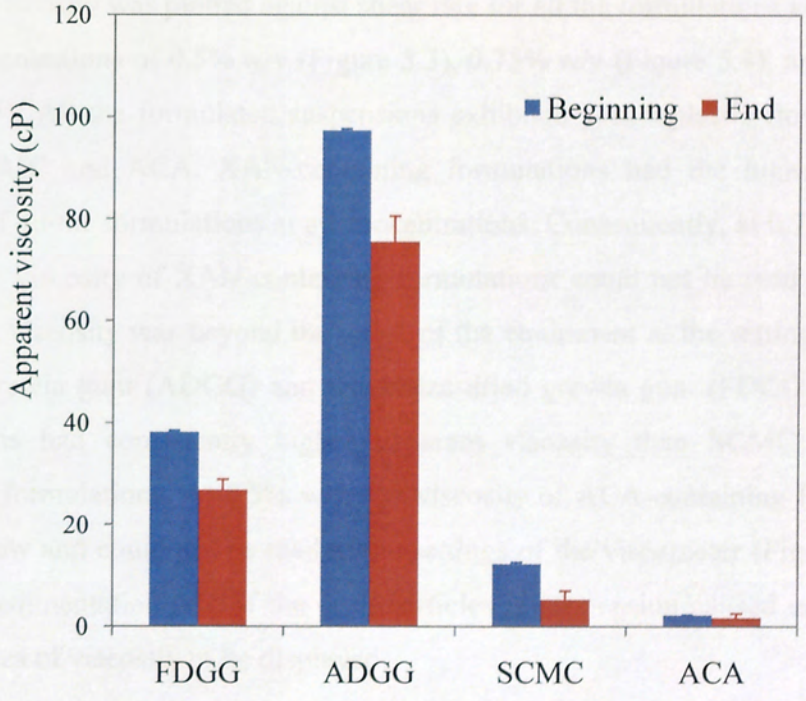


Figure 5.2: A comparison of the viscosity of ibuprofen suspension formulations at the beginning (day 0) and at the end (day 84) of the Study (n=3, mean ± s.d.)

It has been reported (Billany, 2007) that natural polysaccharides show a gradual reduction in the viscosity of their dispersions or solutions with age due to bacterial or mould growth.

The pH of the suspension formulations containing 1.0 % w/v of suspending agents on day 0 and day 28 of formulation was determined and the results are shown in table 5.2. The results show that the pH of the suspension formulations was stable over the 28 days of storage.

Table 5.2: pH of ibuprofen suspension formulations containing 1.0% w/v suspending agent

Suspension formulation	*Day 0*	*Day 28*
XAN	4.7±0.02	4.7±0.10
ACA	4.7±0.10	4.8±0.10
SCMC	5.4±0.10	5.4±0.03
FDGG	5.0±0.10	5.0±0.04
ADGG	4.9±0.03	5.0±0.04

5.3.3 Rheology of the suspension formulations

Apparent viscosity was plotted against shear rate for all the formulations at suspending agent concentrations of 0.5% w/v (Figure 5.3), 0.75% w/v (Figure 5.4), and 1.0% w/v (Figure 5.5). All the formulated suspensions exhibited pseudoplastic flow behaviour except SCMC and ACA. XAN-containing formulations had the highest apparent viscosity of all the formulations at all concentrations. Consequently, at 0.75 % and 1.0 % w/v, the viscosity of XAN-containing formulations could not be read because the suspension viscosity was beyond the range of the equipment at the settings used. The air-dried grewia gum (ADGG) and the freeze-dried grewia gum (FDGG)-containing formulations had consistently higher apparent viscosity than SCMC and ACA-containing formulations. At 0.5% w/v, the viscosity of ACA-containing formulations was very low and could not be read at the settings of the viscometer (Fig. 5.3). Also, the rapid sedimentation rate of the drug particles in suspension caused unsteady and erratic values of viscosity to be displayed.

It has been reported that a large number of suspensions exhibit pseudoplastic behaviour (Kittipongpatana and Sirithunyalug, 2006). This is a desirable property in the formulation of suspensions (Bilany, 2007). At all concentrations studied ACA, SCMC-containing formulations demonstrated mainly Newtonian flow behaviour as a result of the very low viscosities of the suspension formulations.

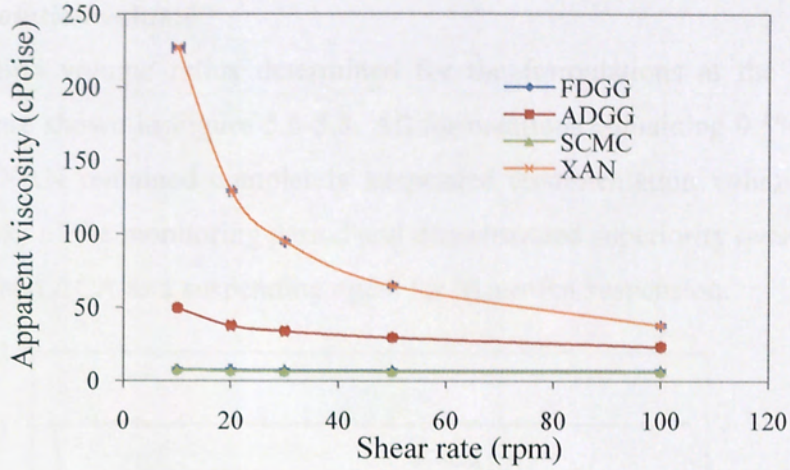


Figure 5.3: Rheological profiles of ibuprofen suspension formulations containing 0.5% w/v suspending agent (n=3, mean ± s.d.).

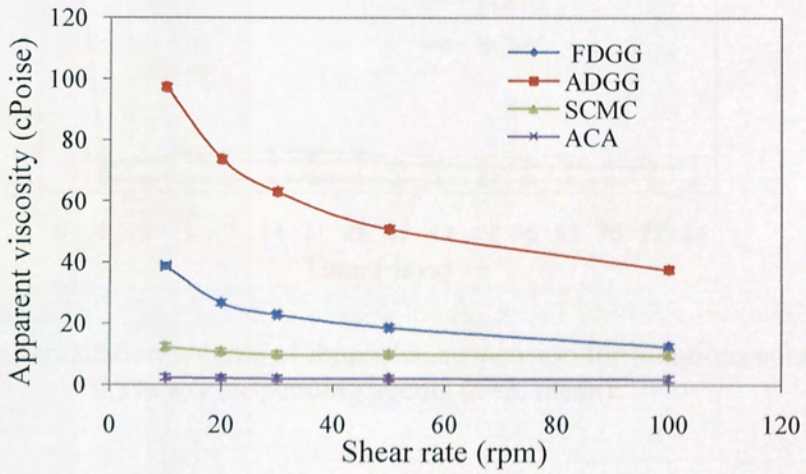


Figure 5.4: Rheological profiles of ibuprofen suspension formulations containing 0.75% w/v suspending agent (n=3, mean ± s.d).

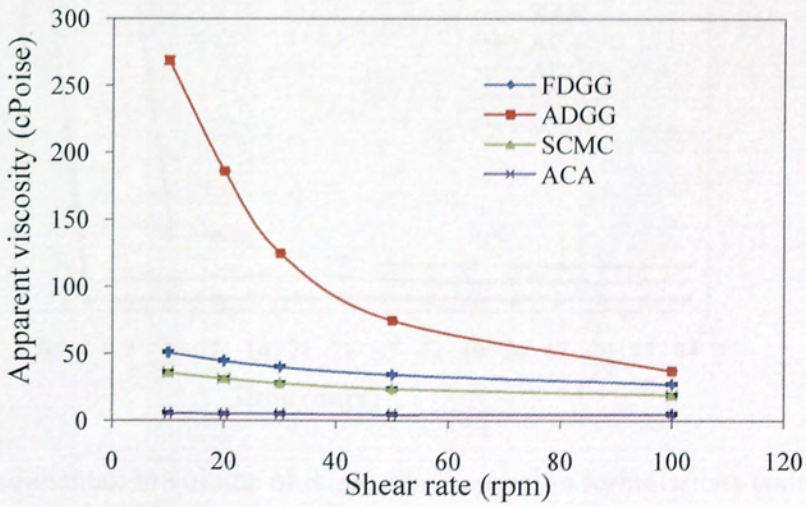


Figure 5.5: Rheological profiles of ibuprofen suspension formulations containing 1.0% w/v suspending agent (n=3, mean ± s.d.).

5.3.4 Sedimentation volume

The sedimentation volume ratios determined for the formulations at the different concentrations are shown in Figure 5.6-5.8. All formulations containing 0.5%, 0.75% and 1.0% w/v XAN remained completely suspended (sedimentation volume = 1.0) over the 18 weeks of the monitoring period and demonstrated superiority over ADGG, FDGG, SCMC and ACA as a suspending agent for ibuprofen suspension.

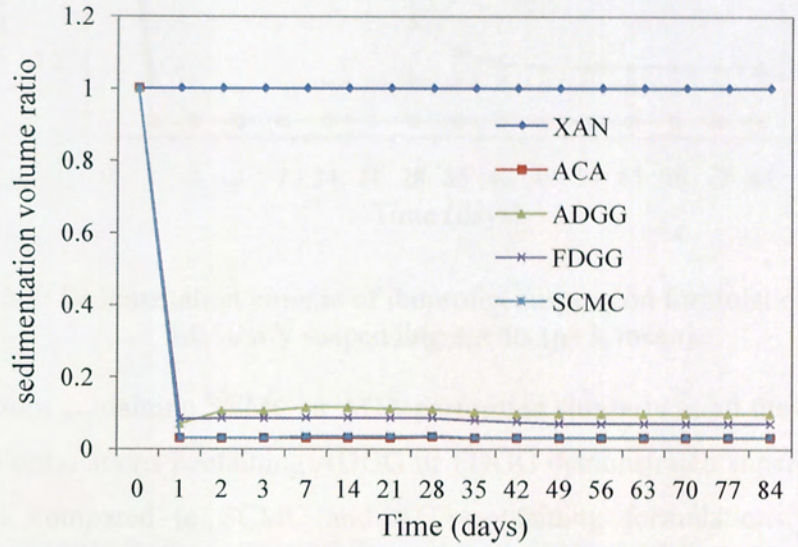


Figure 5.6: Sedimentation volume of ibuprofen suspension formulations containing 0.5% w/v suspending agents (n=3, mean).

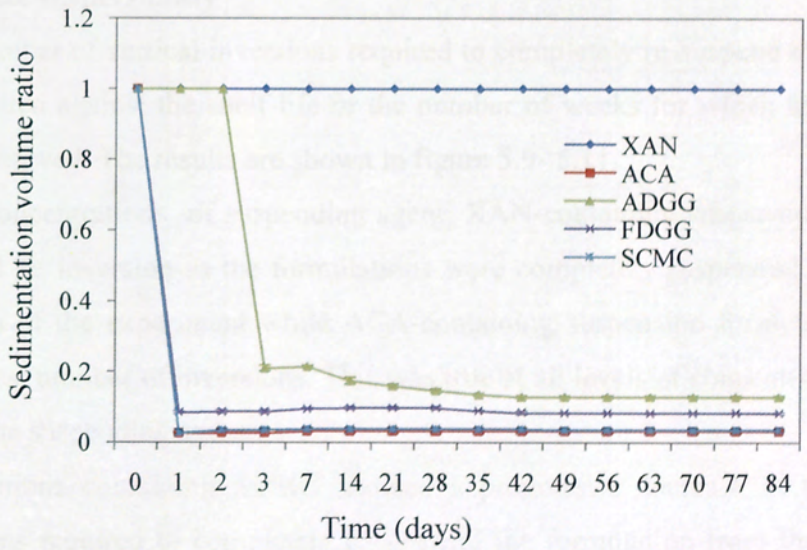


Figure 5.7: Sedimentation volume of ibuprofen suspension formulations containing 0.75% w/v suspending agents (n=3, mean).

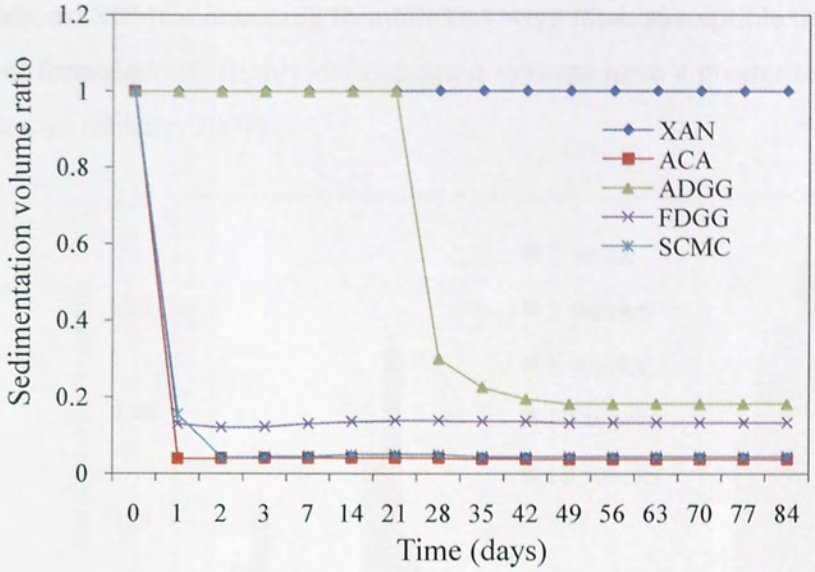


Figure 5.8: Sedimentation volume of ibuprofen suspension formulations containing 1.0 % w/v suspending agents (n=3, mean).

Formulations containing SCMC or ACA performed similarly at all the concentrations studied. Formulations containing ADGG or FDGG demonstrated superior suspending properties compared to SCMC and ACA-containing formulations. The ADGG-containing suspension formulations had higher sedimentation volumes than FDGG at 0.5%, 0.75% and 1.0% w/v, and were completely suspended for 21 days at 1.0% w/v.

5.3.5 Re-dispersibility

The number of vertical inversions required to completely re-suspend the formulations was plotted against the shelf life or the number of weeks for which the formulations were observed. The results are shown in figure 5.9- 5.11.

At all concentrations of suspending agent, XAN-containing suspension formulations required no inversion as the formulations were completely suspended throughout the duration of the experiment while ACA-containing suspension formulations required the lowest number of inversions. This was true at all levels of concentration and shelf-life of the suspending agent.

Formulations containing SCMC showed a progressive increase in the number of inversions required to completely re-suspend the formulation from the first week to the eighteenth week of the study. This was the case for all the concentrations at which SCMC was used as suspending agent. At the end of the study period, SCMC-containing suspension formulations were notably not as re-dispersible as the other

formulations. SCMC-containing formulations were more susceptible to caking than all the other formulations. Highly deflocculated systems have a greater tendency to cake upon storage (Bilany, 2007).

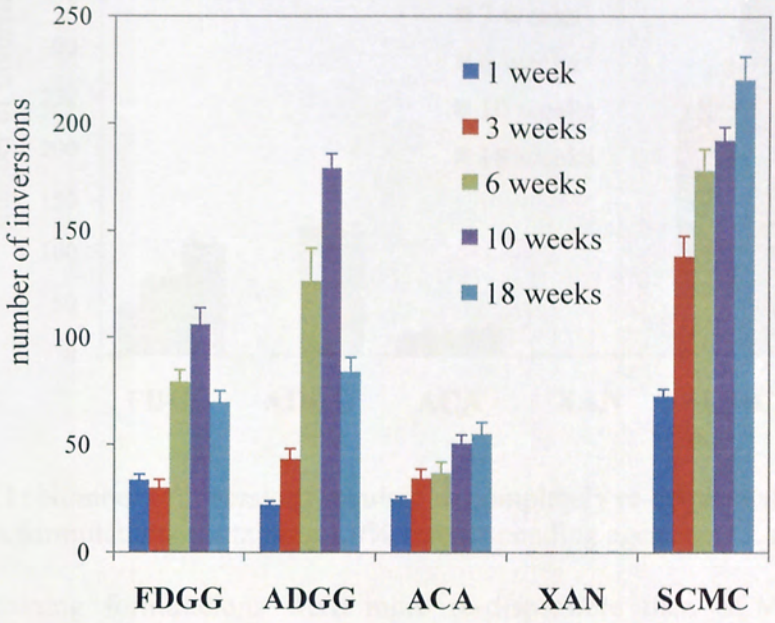


Figure 5.9: Number of inversions required to completely re-disperse the ibuprofen suspension formulations containing 0.5% w/v suspending agents (n=3, mean ± s.d.)

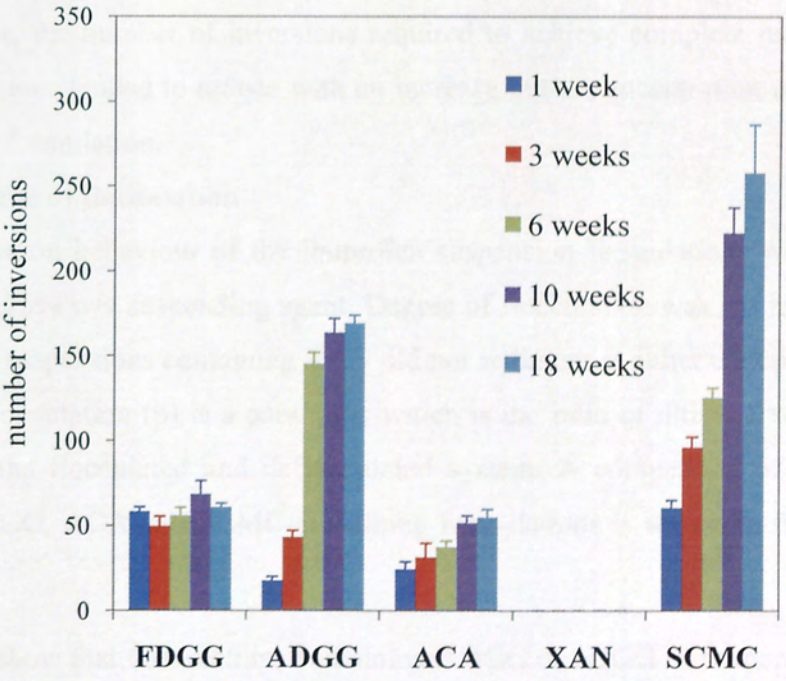


Figure 5.10: Number of Inversions required to completely re-disperse the ibuprofen suspension formulations containing 0.75% w/v suspending agents (n=3, mean ± s.d.)

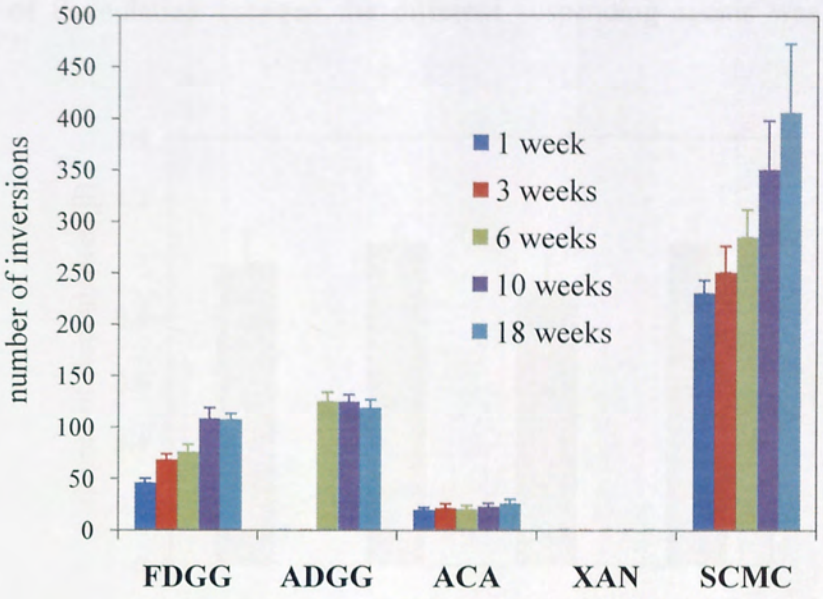


Figure 5.11: Number of Inversions required to completely re-disperse the ibuprofen suspension formulations containing 1.0% w/v suspending agents (n=3, mean ± s.d.)

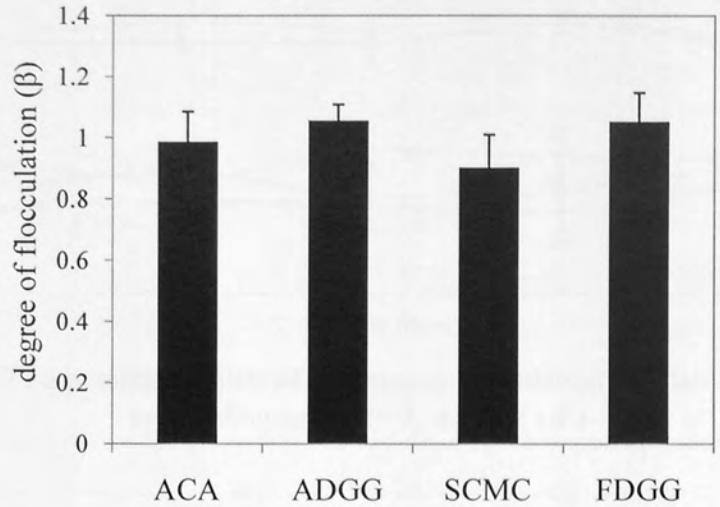
ADGG-containing formulations were more re-dispersible than SCMC-containing formulations. At 0.5% w/v ADGG or FDGG-containing suspension formulations required a higher number of inversions to completely re-disperse at the end of the tenth week of study (figure 5.9) compared to all the other weeks. For all the formulations, the number of inversions required to achieve complete redispersion of the formulations tended to reduce with an increase in the concentration of suspending agent in the formulation.

5.3.6 Degree of flocculation

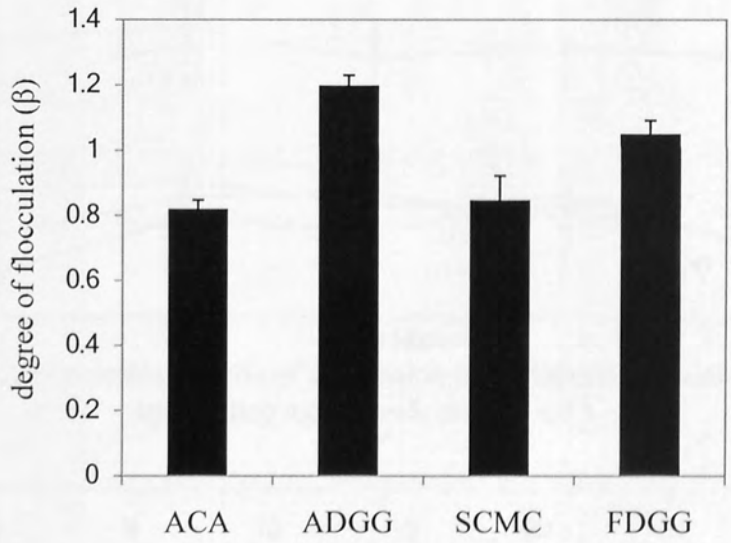
The flocculation behaviour of the ibuprofen suspension formulations was studied at 0.5% and 0.75% w/v suspending agent. Degree of flocculation was not read for XAN because the suspensions containing XAN did not sediment at either concentration. The degree of flocculation (β) is a parameter which is the ratio of ultimate sedimentation volume in the flocculated and deflocculated system. A comparison of β values of ADGG, FDGG, ACA and SCMC-containing formulations is shown in figure 5.12(*a*, *b*).

The results show that formulations containing ADGG or FDGG form more flocculated suspensions than formulations containing SCMC or ACA. At 0.5 % w/v, the variation

in degree of flocculation between the different suspending agents was significant (P>0.05).



a.



b.

Figure 5.12: Comparison of the degree of flocculation for ibuprofen suspension formulations containing *a*) 0.5% and *b*) 0.75% w/v suspending agent (n=3, mean ± s.d.)

5.3.7 Zeta potential of the suspension formulations

The zeta potential of ibuprofen in suspension without the addition of suspending agent was -5.2 ± 0.8 mV. The effect of suspending agents on the zeta potential profile of the suspension formulations is shown in figures 5.13-5.15.

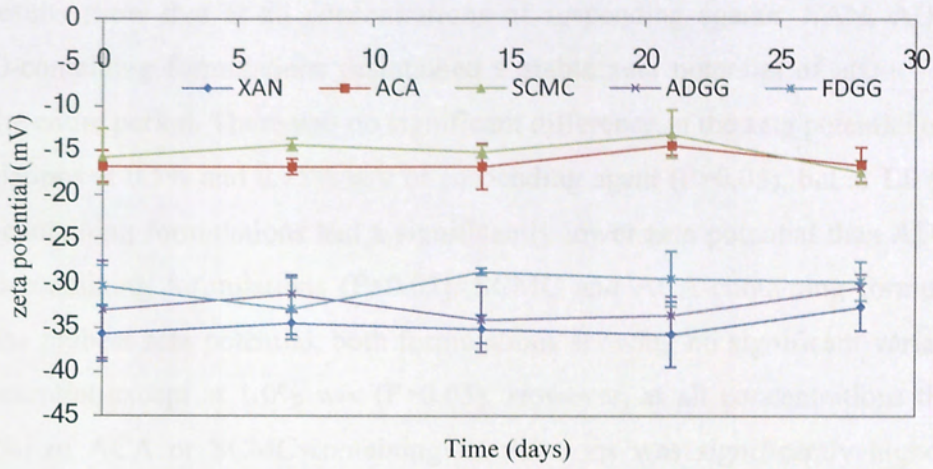


Figure 5.13: Zeta potential profile of suspension formulations containing 0.5% w/v suspending agent (n=3, mean ± s.d.)

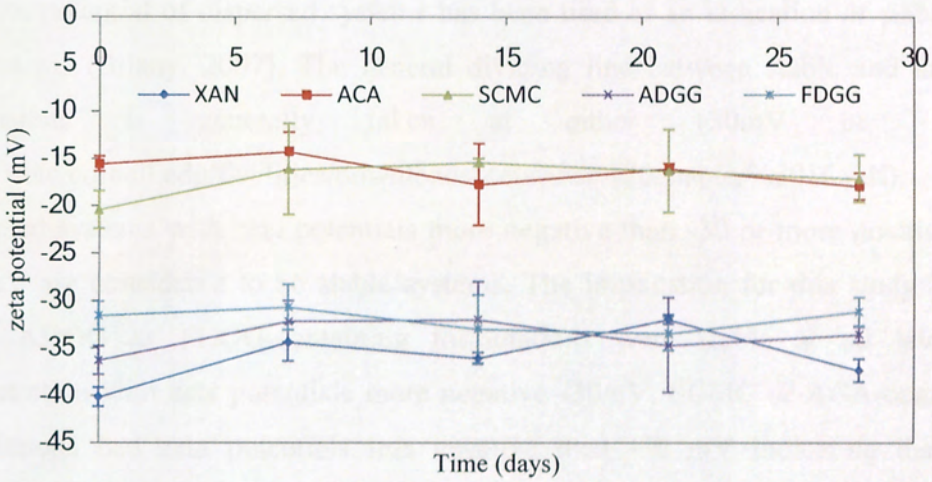


Figure 5.14: Zeta potential profile of suspension formulations containing 0.75% w/v suspending agent (n=3, mean ± s.d.)

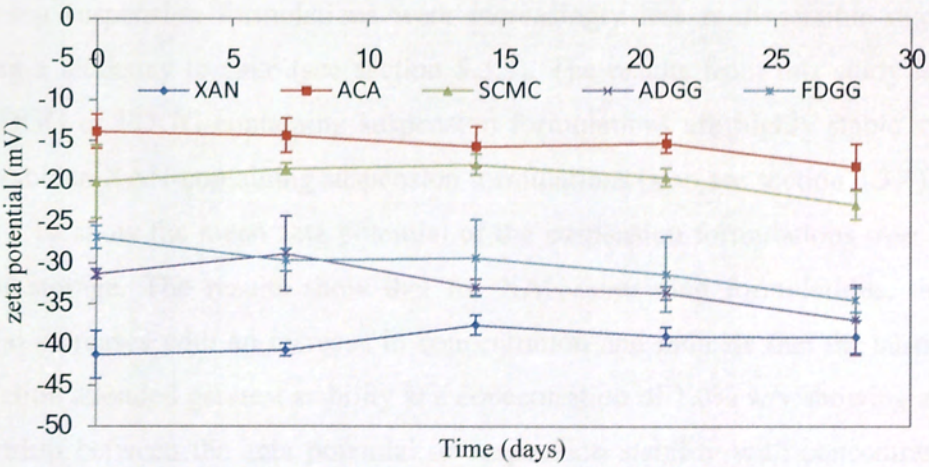


Figure 5.15: Zeta potential profile of suspension formulations containing 1.0% w/v suspending agent (n=3, mean ± s.d.)

The results show that at all concentrations of suspending agents, XAN, ADGG or FDGG-containing formulations maintained a stable zeta potential of about -30 mV over the entire period. There was no significant difference in the zeta potential of these formulations at 0.5% and 0.75% w/v of suspending agent ($P>0.05$), but at 1.0 % w/v, XAN-containing formulations had a significantly lower zeta potential than ADGG or FDGG-containing formulations ($P<0.05$). SCMC and ACA-containing formulations have the highest zeta potential, both formulations showing no significant variation in zeta potential except at 1.0% w/v ($P>0.05$). However, at all concentrations the zeta potential of ACA or SCMC-containing formulations was significantly higher than XAN, ADGG or FDGG-containing formulations ($P<0.05$). This may be attributable to different anionic characteristics of the polymers.

The zeta potential of dispersed systems has been used as an indication of stability of the systems (Bilany, 2007). The general dividing line between stable and unstable suspensions is generally taken at either +30mV or -30mV. (www.nbtc.cornell.edu/facilities/downloads/Zetasizer%20chapter%2016.pdf).

Dispersed systems with zeta potentials more negative than -30 or more positive than +30 mV are considered to be stable systems. The implication for this study is that, XAN, ADGG or FDGG-containing formulations were stable at all levels of concentration with zeta potentials more negative -30mV. SCMC or ACA-containing formulations had zeta potentials less negative than -30 mV indicating that both dispersed systems were unstable settling very rapidly after formulation (see section 5.3.1). While ACA-containing suspension formulations were re-dispersible, SCMC-containing suspension formulations were increasingly less re-dispersible over time showing a tendency to cake (see section 5.3.5). The results from this study indicate that ADGG or FDGG-containing suspension formulations are highly stable systems comparable to XAN-containing suspension formulations (also see section 5.3.4).

Figure 5.16 show the mean zeta potential of the suspension formulations over the 28 days of storage. The results show that for XAN-containing formulations, the zeta potential increases with an increase in concentration and indicate that the suspension formulation attended greatest stability at a concentration of 1.0% w/v showing a direct relationship between the zeta potential or suspension stability with concentration of suspending agent or viscosity of the suspension formulation. The same was also true for SCMC-containing formulations. At 0.75% and 1.0 % w/v, FDGG or ADGG-

containing formulations had lower zeta potentials and were therefore more stable than the 0.5% w/v formulations. The zeta potential for all ACA-containing formulations were the same ($P > 0.05$) irrespective of concentration and indicate that increasing the concentration of ACA from 0.5% to 1.0% did not significantly affect the stability of the system. Pearson *et al.*, (2004) have shown that zeta potential is constant at optimal floc character regardless of polymer concentration.

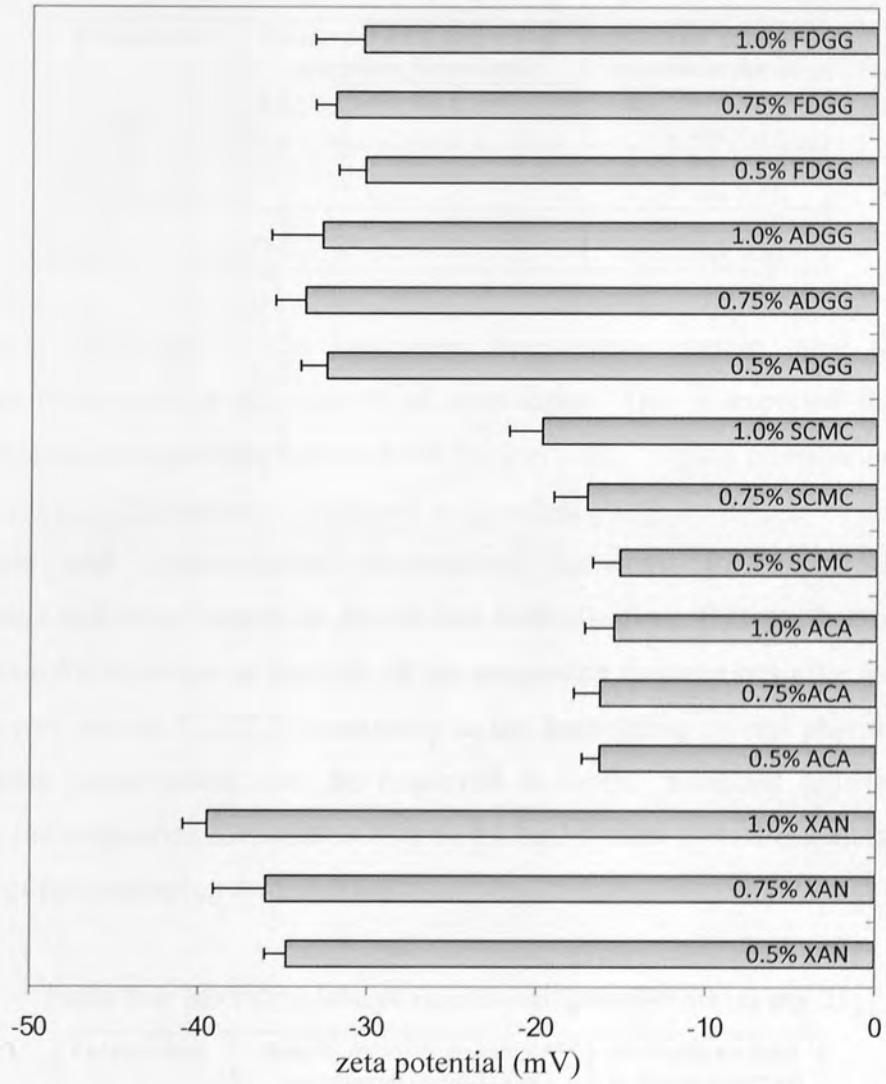


Figure 5.16: Mean zeta potential of suspension formulations over 28 days of storage

5.3.8 Microbiological evaluation

The ability of natural polysaccharide gums to increase solution viscosity accounts for their use as suspending agents in oral pharmaceutical suspensions. However, natural polysaccharides can show a gradual reduction in the viscosity of their dispersions or solutions with age due to bacterial or mould growth (Billany, 2007). The microbial

load and/or growth in suspension formulations containing 1.0% w/v of suspending agent was evaluated on day 0 and on day 21 of storage and the results are presented in table 5.2 and 5.3 respectively. Microbial growth evident by colony forming units or cloudiness and darkening of the nutrient agar is indicative of positive result while a clear and transparent nutrient agar is indicative of a negative result.

Table 5.3: Microbial load of suspension formulations on day 0

Formulation	*Results for each quantity of suspension formulation*			*Probable number of bacteria per ml of formulation*
	0.1 ml	0.01 ml	0.001 ml	
ADGG	+	-	-	$< 10^2$ and > 10
FDGG	+	+	-	$< 10^3$ and $> 10^2$
SCMC	+	-	-	$< 10^2$ and > 10
ACA	+	-	-	$< 10^2$ and > 10
XAN	+	-	-	$< 10^2$ and > 10

The results show that all the suspension formulations contain some degree of microbial contamination from day 0 of formulation. This is expected for natural polymers used as suspending agents. After 21 days (table 5.4) the probable number of bacteria per ml of formulation (estimated in accordance with the BPC 2010 method for non-sterile oral pharmaceutical suspensions) increased for each suspension formulation indicating microbial growth and multiplication. This result provides an explanation for reduction in viscosity of the suspension formulations after storage for 84 days (see section 5.3.2). Consequently in the formulation of oral pharmaceutical suspensions, preservatives may be employed to inhibit microbial growth and to preserve the suspension formulation. Figure 5.17(*a*,*b*), show typical microbial growth patterns of the suspension formulations.

Table 5.4: Microbial load of suspension formulations on day 21

Formulation	*Results for each quantity of suspension formulation*			*Probable number of bacteria per ml of formulation*
	0.1 ml	0.01 ml	0.001 ml	
ADGG	+	+	+	$>10^3$
FDGG	+	+	+	$>10^3$
SCMC	+	+	+	$>10^3$
ACA	+	+	+	$>10^3$
XAN	+	+	+	$>10^3$

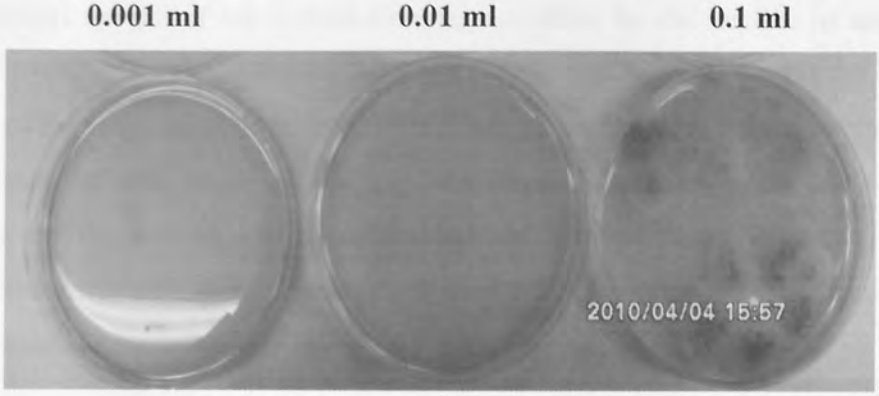


Figure 5.17*a*: Typical microbial growth pattern of the suspension formulations on nutrient agar on day 0.

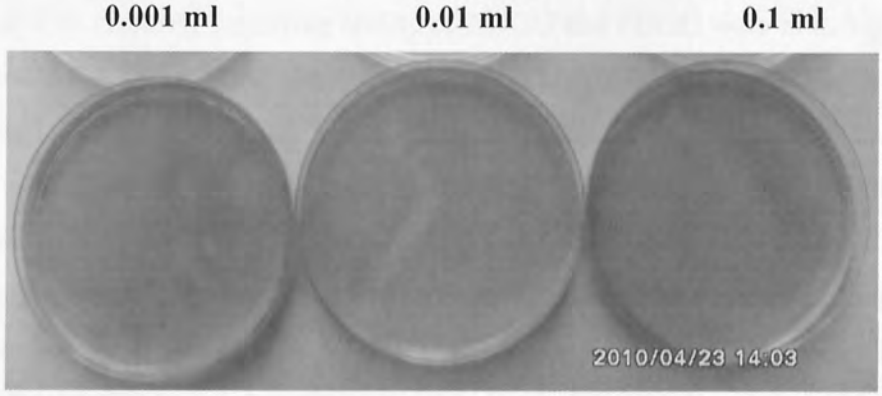


Figure 5.17*b*: Typical microbial growth pattern of the suspension formulations on nutrient agar on day 21.

Microbiological evaluation of dispersions of the air-dried or freeze-dried grewia polysaccharide gum (1.0% w/v), with no additives were also carried out and the results are shown in table 5.5. Although the grewia polysaccharide gum samples were not purified to sufficient pharmaceutical quality, microbial contamination may be minimized prior to use by irradiation of the samples.

Table 5.5: Microbial load of air-dried or freeze-dried grewia polysaccharide gum dispersions (1.0% w/v)

Dispersion	*Results for each quantity of suspension formulation*			*Probable number of bacteria per ml of formulation*
	0.1 ml	0.01 ml	0.001 ml	
Day 0				
ADGG	+	-	-	$< 10^2$ and > 10
FDGG	+	+	-	$< 10^3$ and $> 10^2$
Day 21				
ADGG	+	+	+	$>10^3$
FDGG	+	+	+	$>10^3$

The microbial growth of the formulations can account for the decline in suspension viscosity over time but the results also show that this had little effect on the zeta potential of the suspension formulations. It can be speculated that the microbial growth and viscosity at this stage of the shelf-life, had no effect on the electrokinetic properties of the suspension formulation. This implies that changes within the suspension formulation were not enough to alter the aggregation rate or flocculation of the suspension formulations.

5.4 CONCLUSION

ADGG and FDGG-containing suspensions exhibit pseudoplastic (shear-thinning) flow behaviour. The viscosity imparting ability of ADGG and FDGG were both higher than SCMC and ACA at the same concentration. This indicates that the insoluble drug in ADGG and FDGG-containing formulations can remain in suspension for longer storage time than SCMC and ACA-containing formulations. The sedimentation volume of ADGG and FDGG-containing formulations were higher than SCMC and ACA containing formulations. They are more re-dispersible and more flocculated than SCMC-containing formulations. ADGG containing formulations were however superior to FDGG-containing formulations in terms of viscosity, sedimentation volume and degree of flocculation. FDGG-containing formulations were more re-dispersible compared to ADGG-containing formulations.

The suspending ability of a polysaccharide gum is related to its ability to increase solution viscosity. They are therefore also called viscosity modifiers (Bilany, 2007). When the viscosity of the disperse medium is increased the sedimentation rate of the dispersed phase is reduced. Consequently, the degree to which a material enhances solution viscosity is a direct measure of its suspending ability and explains why XAN-containing formulations have greater suspending ability than all the other suspension formulations at any given concentration of suspending agent.

The zeta potential of the suspension formulations indicated that at all levels of concentration XAN, ADGG or FDGG-containing formulations were very stable compared to SCMC or ACA-containing formulations. 1.0 % w/v XAN-containing suspension formulation has the highest mean zeta potential and was the most stable of all the formulations.

Microbiological evaluation of the suspension formulations indicated bacterial or mould contamination from day 0 of formulation and showing evidence of growth after 21 days of formulation. Bacterial or mould growth and multiplication is expected for natural polymers and accounts for the gradual reduction in viscosity of suspension formulations. However, this has been overcome by the use of preservatives in oral pharmaceutical suspensions.

CHAPTER SIX

PREFORMULATION STUDIES

6.1 INTRODUCTION

During the development of solid dosage forms, large scale development trials are normally preceded by the assessment of possible incompatibilities between a drug and different excipients used in the formulation. Excipients are required to be medically inert but physical and chemical interactions with APIs are commonplace (Brown *et al.*, 1999). The screening of a novel excipient for possible incompatibilities is therefore an absolute requirement.

A number of techniques have been used for screening of drug-excipient mixtures for incompatibilities to include isothermal stress testing and thermal analysis (Brown *et al.*, 1999). Thermal analysis has the advantage over conventional isothermal stress testing in that long term storage of physical mixtures and chromatographic analysis are not required and only a few milligrams of sample is needed. However, the technique has been criticized as being inconclusive as moisture stress testing is usually not included and the temperature and heating rates used are not characteristic of normal storage conditions resulting in difficulty of interpretation and extrapolation of results (Brown *et al.*, 1999; Gaisford and O'Neil, 2006). Consequently, DSC should be used in conjunction with other techniques.

In this section, FTIR and/or thermal analysis using DSC was employed to determine if interactions occur between grewia polysaccharide gum and the active pharmaceutical ingredients, cimetidine or ibuprofen. Likely interactions between grewia polysaccharide gum and any of the excipients used in the formulation of the matrix tablets were also investigated. This was done by comparing the results obtained for the controls with physical mixtures of the drug and polymer or polymer and polymer. If the drug and the polymer interact, then the functional groups in the FT-IR spectra would show band shifts and broadening compared to the spectra for the pure drug and polymer (Silverstein *et al.*, 1991). When the FT-IR spectra or DSC traces of the physical mixtures are a summation of the characteristic traces obtained with the individual components of the physical mixtures, this indicates that there was no chemical interaction of the drug with polymer in physical mixes (Silverstein *et al.*, 1991; Gaisford and O'Neil, 2006).

6.2 MATERIALS AND METHODS

6.2.1 Materials

Ibuprofen and cimetidine were a gift from GSK Consumer Health. Ethyl cellulose (Ethocel® - standard 100FP Premium) and hydroxypropyl methylcellulose (Methocel® - K100 premium LVCR) were gifts from Colorcon, England. Metolose (Metolose® - 90SH-100SR, viscosity-100 mPa.s, substitution type-2208) was a gift from ShinEtsu Chem. Co Ltd, Japan. Colloidal silicon dioxide (Aerosil 200®) was a gift from Evonik, UK. Carboxy methylcellulose (CMC) (Blanose® – Type 7M1F-PHARM) was a gift from Aqualon, UK. Lactose monohydrate USP (Pharmatose®, grade DCL14) was a free gift from DMV-International, UK. Grewia polysaccharide gum (air-dried) was extracted from the crude bark in our laboratory (see section 2.3). All other materials were procured from Sigma-Aldrich, UK.

6.2.2 Fourier-transformed infra red spectroscopic analysis (FT-IR)

The details of methodology for FT-IR analysis of the samples are as detailed in section 2.10 except otherwise indicated.

6.2.3 Differential scanning calorimetry

Details of DSC analysis is as already provided in section 2.11 except otherwise indicated.

6.3 RESULTS AND DISCUSSION

6.3.1 Ibuprofen – excipient interactions

6.3.1.1 FT-IR analysis of ibuprofen- excipient blends (see section 2.10)

The FT-IR scan of ibuprofen gave characteristic absorption peaks of ibuprofen at 1710 and 2955 cm^{-1} which are caused by the carbonyl stretching vibration and the hydroxyl stretching vibration respectively. Interaction with ibuprofen primarily interferes with the position or intensity of these two peaks (Wu and McGinity, 2001; Maheshwari *et al.*, 2003).

Figure 6.1 shows the FT-IR spectrum of ibuprofen, Metolose® or the blend of ibuprofen and Metolose® (1:1). The figure shows typical absorption bands for Metolose® in the region 1150–1060 cm^{1} caused by ether *C-O-C* stretch, while secondary alcohols absorb strongly at 1075 cm^{-1}. The methoxy groups gave rise to an absorption band around 1200 –1175 cm^{-1} and methyl groups and *C-H* stretch can be seen around 2800 cm^{-1}. A carbonyl peak is seen at 1625 cm^{-1} and *OH* at around 3500

cm^{-1} (Gutafson *et al.*, 2003). The physical mixture of Metolose® with ibuprofen shows that the characteristic absorption peaks of ibuprofen at 1710 and 2955 cm^{-1} have not been altered by the combination. The absorption peak due to the *OH* of Metolose® at around 3500 cm^{-1} is still visible in the physical mixture. There is noticeably a physical presence of the spectral peaks of ibuprofen and Metolose® in the spectrum of the physical mixes especially in the region between 1200 –1175 cm^{-1} and the finger print region with no interference with the characteristic absorption peaks of either component. This confirms that no interaction occurred between Metolose® and ibuprofen when combined in a physical mixture.

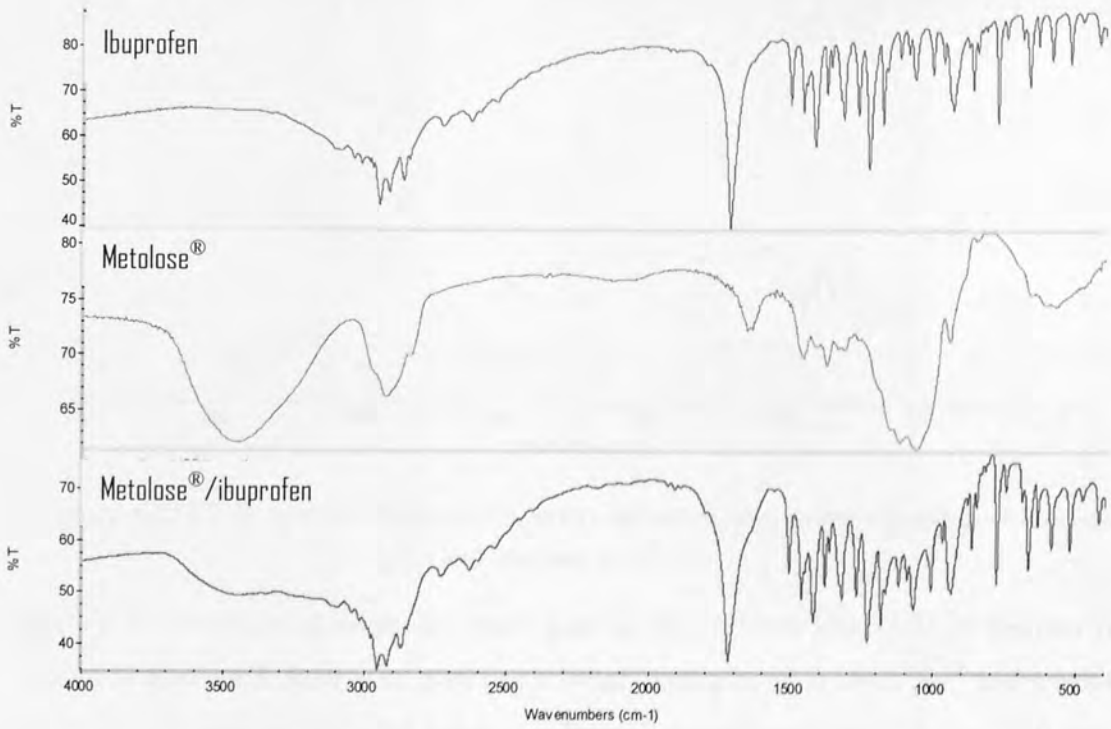


Figure 6.1: FTIR spectra of ibuprofen, Metolose®, and ibuprofen/ Metolose® physical mixture (1:1)

Figure 6.2 shows the spectrum of ethyl cellulose, ibuprofen or the physical mixes (1:1) of ibuprofen and ethyl cellulose. The characteristic absorption bands of ethyl cellulose can be seen around 3600-3300 cm^{-1} due to hydroxyl stretching vibrations. It also represents the intra- and intermolecular hydrogen bonding due to *OH* groups (Ravindra *et al.*, 1999). The asymmetric peaks around 2900 cm^{-1} have been associated with *C-H* stretching vibrations while the peak at 1375 cm^{-1} is due to CH_3 bending and the small peak near 1450 cm^{-1} is due to CH_2 bending. The broad distinct peak near

1100 cm^{-1} may be due to the *C-O-C* stretch in the cyclic ether (Suthar *et al.*, 2000; Desai *et al.*, 2006). The physical mix of ibuprofen and ethyl cellulose also shows no interference with the individuality of the spectrum of each component and the absorption peaks of ibuprofen at 1710 and 2955 cm^{-1}.

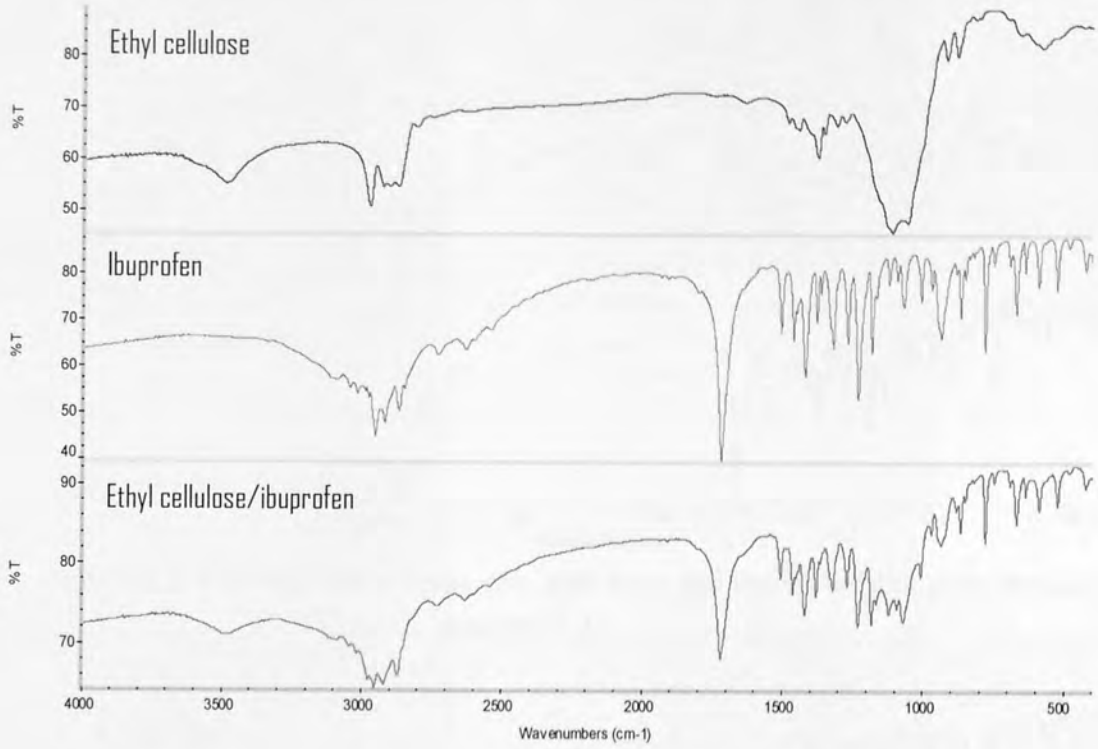


Figure 6.2: FTIR spectra of ibuprofen, ethyl cellulose, and ibuprofen/ethyl cellulose physical mixture (1:1)

The FT-IR spectrum of ibuprofen, guar gum or the physical mix (1:1) of the two is shown in figure 6.3. Pure guar gum has a broad, strong band at 3410 cm^{-1} and a band at 2900 cm^{-1}, indicating *C-OH* linkages. There is a band maximum in the 1800–900 cm^{-1} region and the peak at 1800-1600 cm^{-1} which has been previously assigned to vibrations of the *C–O–C* bonds of glycosidic bridges (Risica *et al.*, 2005). There was however no interference with the characteristic absorption peaks of ibuprofen when the guar gum and ibuprofen were physically mixed.

The FT-IR absorption bands of grewia polysaccharide gum, ibuprofen and their physical mixtures (1:1) are shown in figure 6.4. The absorption bands of grewia polysaccharide are typical for carbohydrates, the details of which have been given in section 3.3.6. It can be seen that a physical mixture of grewia gum with ibuprofen give FT-IR absorption peaks that are representative of the individual spectra of the

components. This result points to no obvious interaction between grewia polysaccharide gum and ibuprofen.

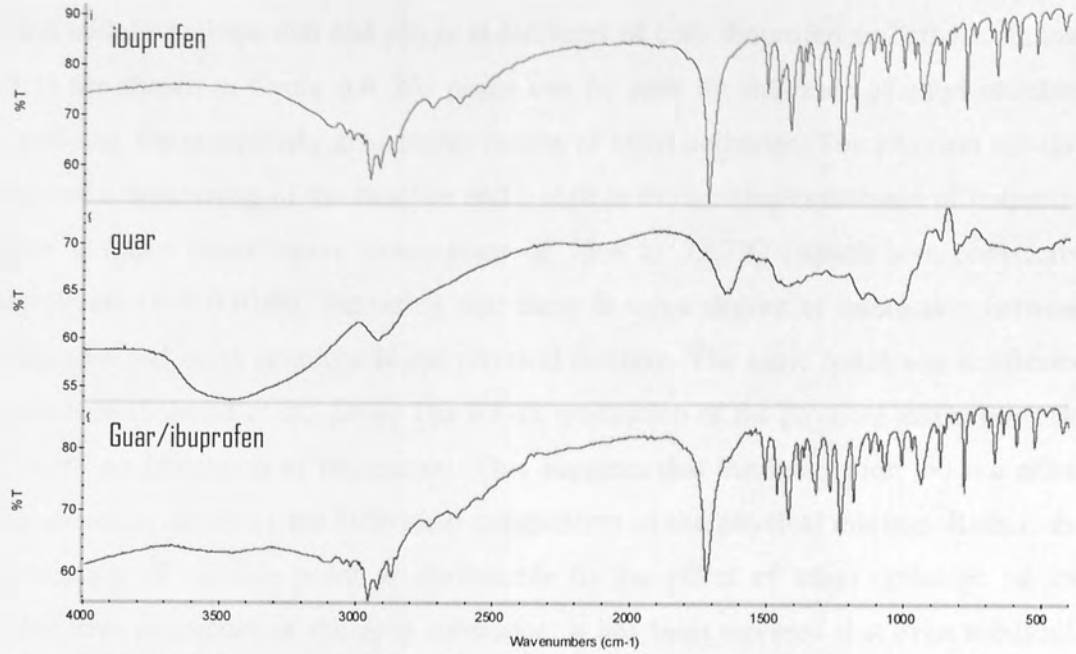


Figure 6.3: FTIR spectra of ibuprofen, guar gum, and ibuprofen/guar gum physical mixture (1:1)

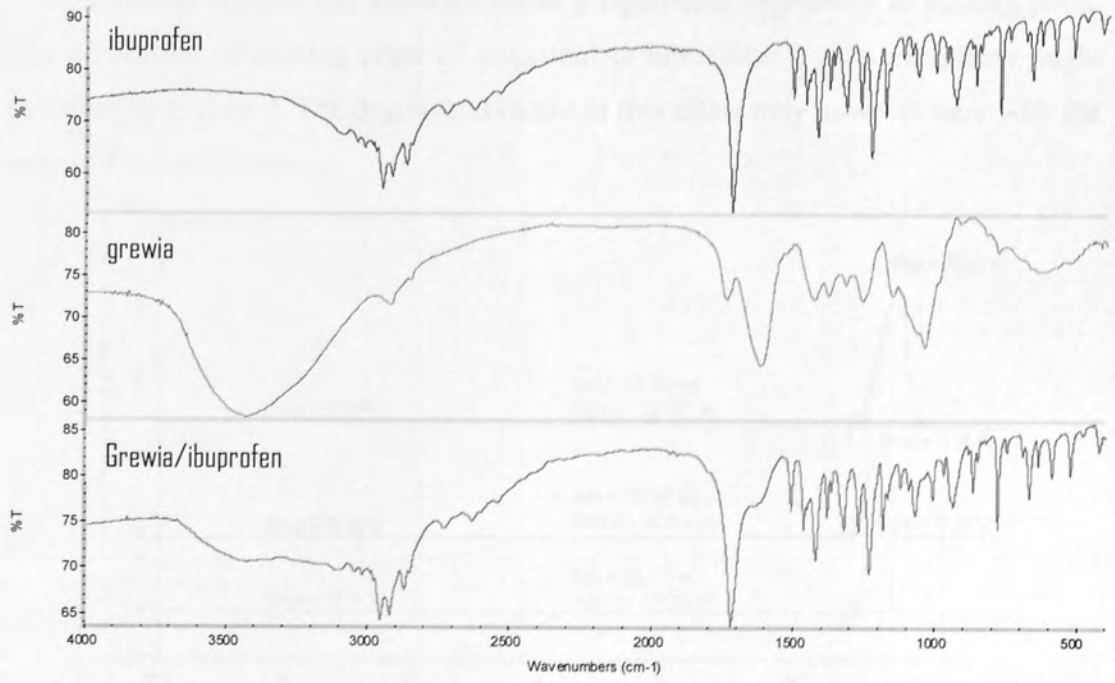


Figure 6.4: FTIR spectra of ibuprofen, grewia gum, and ibuprofen/grewia gum physical mixture (1:1)

6.3.1.2 Differential scanning calorimetry of ibuprofen- excipient blends

The DSC trace for ibuprofen showed a sharp melting endothermic peak at about 77°C with a linear onset temperature of 75°C as shown in figure 6.5. The DSC traces for ethyl cellulose, ibuprofen and physical mixtures of both ibuprofen and ethyl cellulose (1:1) are shown in figure 6.6. No peaks can be seen for the trace of ethyl cellulose signifying the completely amorphous nature of ethyl cellulose. The physical mixture showed a broadening of the baseline and a shift in the melting endotherm of ibuprofen from a mean linear onset temperature of 75.4 to 73.7°C (which was considered significant (P = 0.016)), indicating that there is some degree of interaction between ibuprofen and ethyl cellulose in the physical mixture. The same result was confirmed elsewhere (Schmid *et al.*, 2000) The FT-IR evaluation of the physical mixes however showed no indication of interaction. This suggests that the interaction did not affect the chemical nature of the individual components of the physical mixture. Rather, the depression of melting point is attributable to the effect of ethyl cellulose on the colligative properties of the drug substance. It has been reported that even miniscule amounts of impurity in a material will affect the colligative properties of that material (Gaisford and O'Neill, 2006). The equal proportion of ethyl cellulose with ibuprofen in the physical mixture can therefore cause a significant depression in melting point. The depression of melting point of ibuprofen or cimetidine by the excipients might therefore be expected. The degree and extent of this effect may however vary with the nature of the excipient.

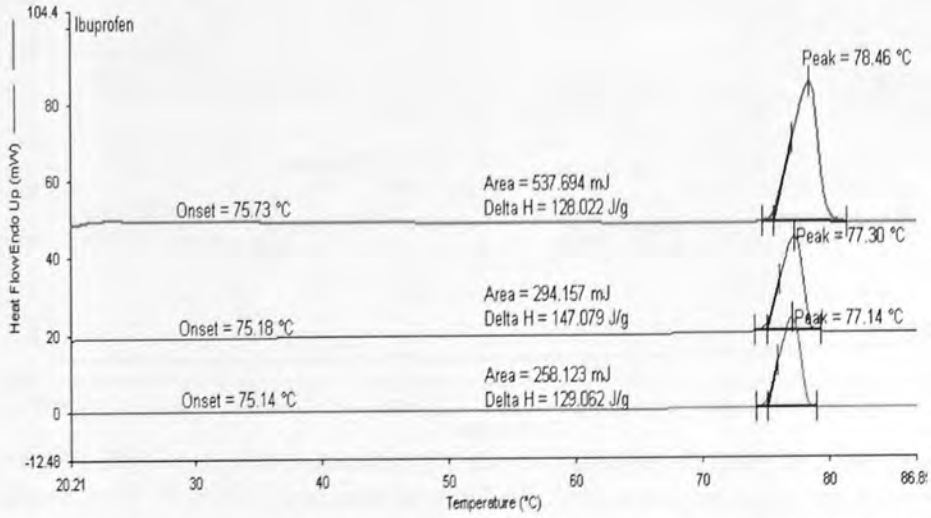


Figure 6.5: DSC traces of ibuprofen at 20° to 200°C and scan rate of 10°C/min under nitrogen atmosphere

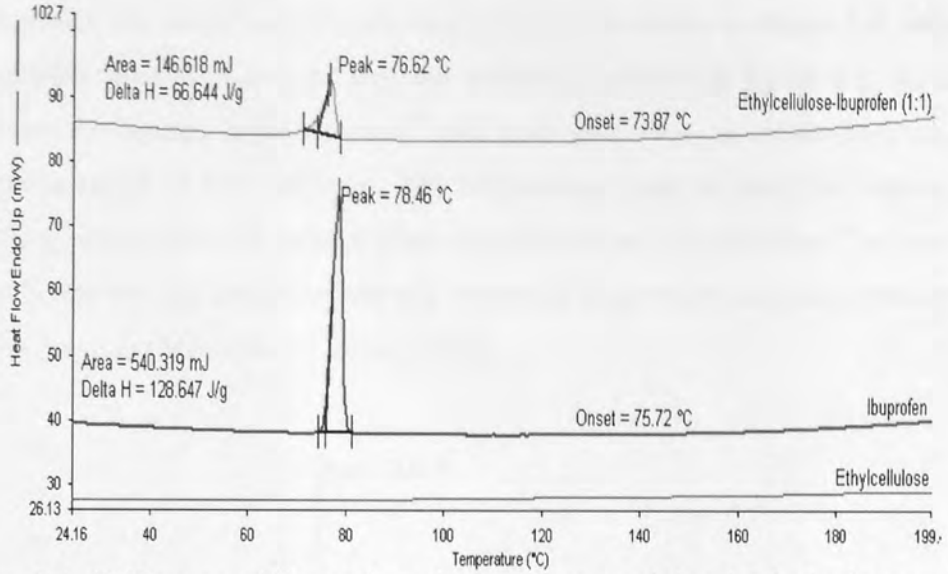


Figure 6.6: DSC traces of ibuprofen, ethyl cellulose, and the physical mixture at 20° to 200°C and scan rate of 10°C/min under nitrogen atmosphere

The DSC trace of grewia polysaccharide gum, ibuprofen and the physical mixtures shown in figure 6.7 indicate no interaction between grewia polysaccharide gum and ibuprofen. This is evidenced by the fact that the melting onset of ibuprofen remained unchanged. Also, as with ethyl cellulose, the melting peak area of ibuprofen is seen to decrease for the physical mixture. This is attributable to the physical presence of the amorphous polymer.

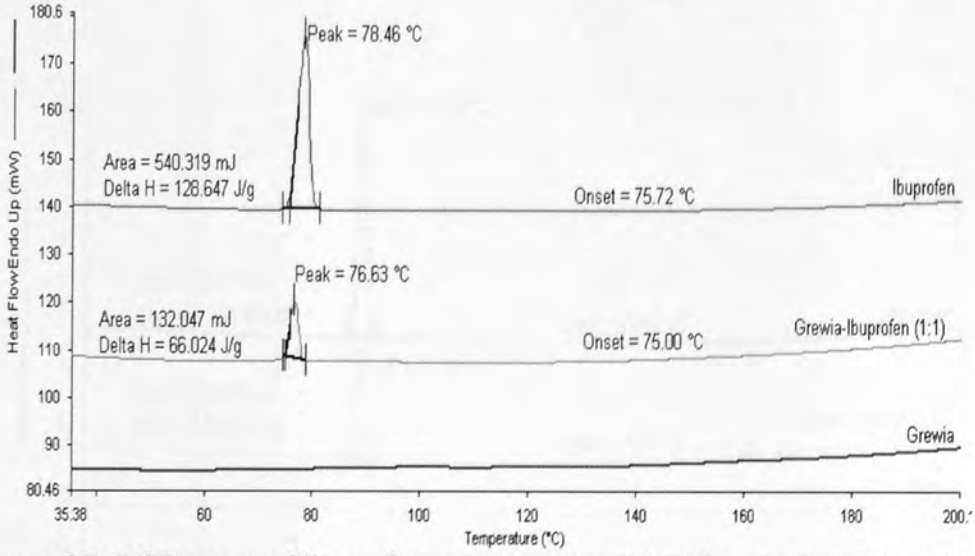


Figure 6.7: DSC traces of ibuprofen, grewia polysaccharide gum, and the physical mixture at 20° to 200°C and scan rate of 10°C/min under nitrogen atmosphere

As was the case with grewia polysaccharide gum or ethyl cellulose, there was no obvious interaction between ibuprofen and Metolose® or guar gum. The DSC traces

for ibuprofen, Metolose® and the physical mixture are shown in figure 6.8 while that of ibuprofen, guar gum and the physical mixture is shown in figure 6.9. As can be seen from the figures, both Metolose® and guar gum show no peaks indicating the amorphous nature of both polymers. The endothermic peak of ibuprofen representing its melting point could still be seen after physical mixing with Metolose® or guar gum. This indicates that the ibuprofen was not converted to an amorphous form after mixing with the polymer (Makhija and Vavia, 2002).

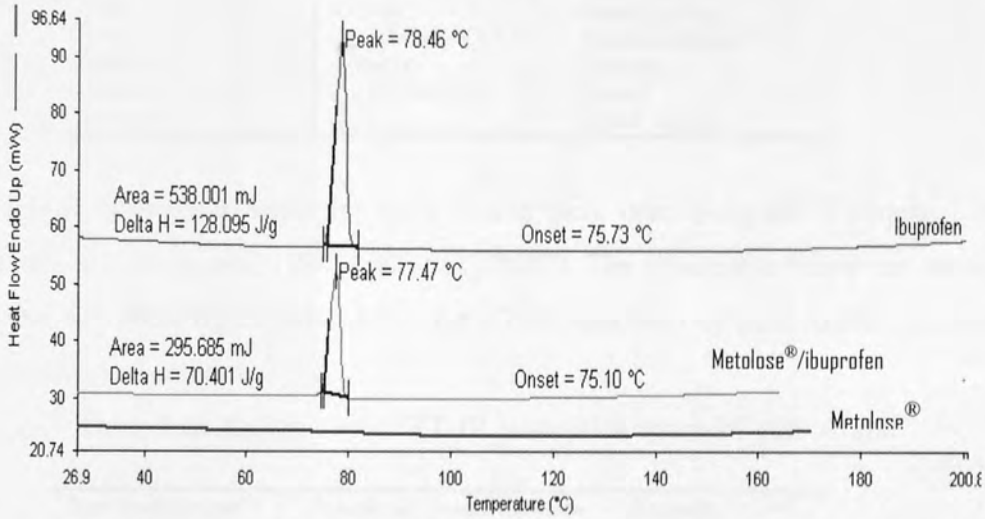


Figure 6.8: DSC traces of ibuprofen, Metolose®, and the physical mixture at 20° to 200°C and scan rate of 10°C/min under nitrogen atmosphere

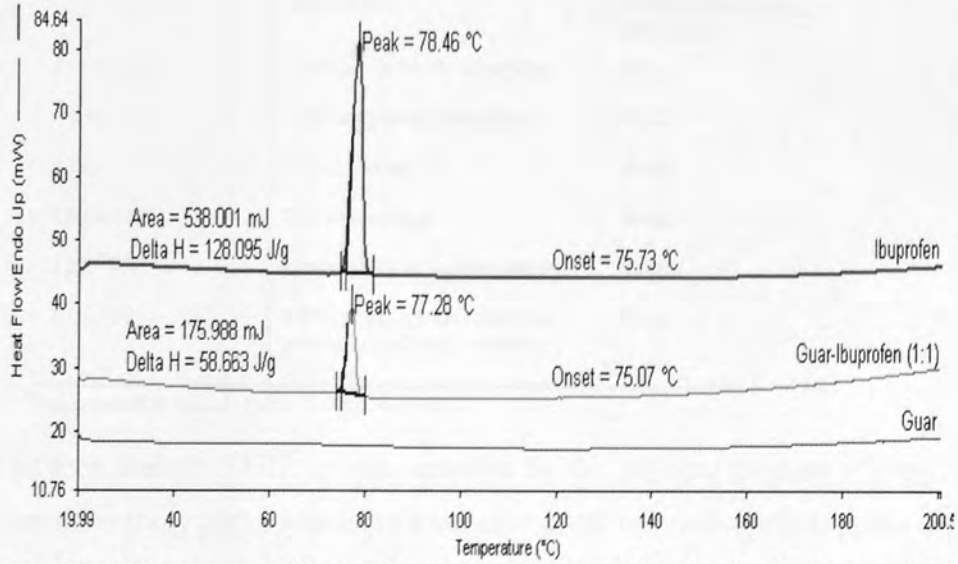


Figure 6.9: DSC traces of ibuprofen, guar gum, and the physical mixture at 20° to 200°C and scan rate of 10°C/min under nitrogen atmosphere

6.3.2 Cimetidine-excipient interactions

6.3.2.1 FT-IR analysis of cimetidine - excipient blends

The FT-IR absorption spectrum recorded for cimetidine has a double broad band at 3240 cm^{-1} assigned to *vibrational* (*NH*) of the associated *NH* group. The absorption bands have been assigned as shown in table 6.1 (Onoa *et al.*, 2002).

Table 6.1: Assignment of FT-IR absorption bands for cimetidine

Wave number (cm^{-1})	*Functional groups*	*Intensity*
3226-3147	ν(N-H)	Broad, strong
2187	ν(C≡N)	Medium, Strong
1622	ν(C=N)	Broad, Strong
1586	ν_{asym}(C=N-C=C)	Medium, strong
1506	σ (N-SH)	Medium
1456	ν_{sym} (C=N-C=C)	Strong
697-668	ν(C-S)	Broad, strong

The FT-IR absorption bands for gum Arabic have been assigned (Filippove, 1992; Engelsen, and Norgaard, 1996; Cui *et al.*, 2007). The absorption bands are shown in the table 6.2 while figure 6.10 shows the FT-IR spectrum of gum Arabic, cimetidine and the physical mixture.

Table 6.2: Assignment of FT-IR absorption bands of gum Arabic

Wave number (cm^{-1})	*Functional groups/vibration mode*	*Intensity*
3600–2500	O–H stretching	Broad, strong
3000–2800	C–H stretching, symmetric, asymmetric	Sharp, occasionally double overlapping with O–H
1630–1600	COO-asymmetric stretching	Strong
1400	COO-symmetric stretching	Weak
1380	C–H bending	Weak
1300–1000	C=O stretching	Weak
1200–900	Finger-print of carbohydrates [a,b]	Strong
830–500	CCO, COC, symmetrical and asymmetrical ring breathing vibration	Weak

[a]Engelsen and Norgaard (1996). [b]Filippove (1992).

It can be seen that the FT-IR spectra obtained for the physical mixture of gum Arabic and cimetidine show peaks which are a summation of the characteristic peaks obtained with the pure drug and pure polymer. This indicates that there was no chemical interaction of the drug with polymer in the physical mixture.

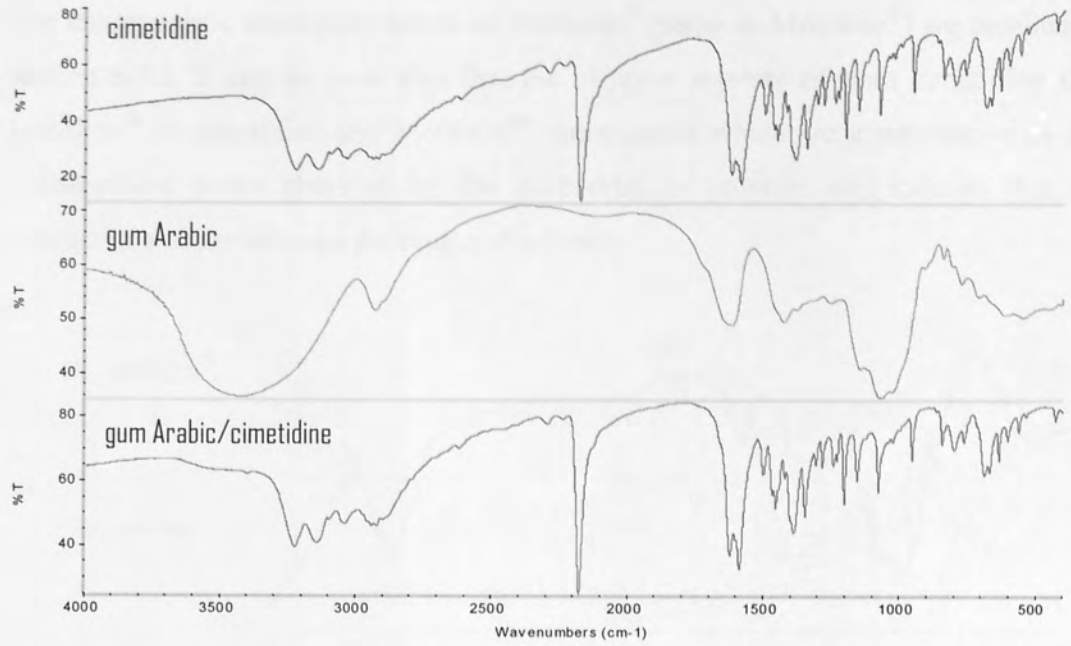


Figure 6.10: FT-IR spectra of cimetidine, gum Arabic, and cimetidine/gum Arabic physical mixture (1:1)

Figures 6.11 and 6.12 shows the FT-IR spectra of Metolose® and Methocel® respectively, along with the corresponding spectra for cimetidine and the physical mixtures.

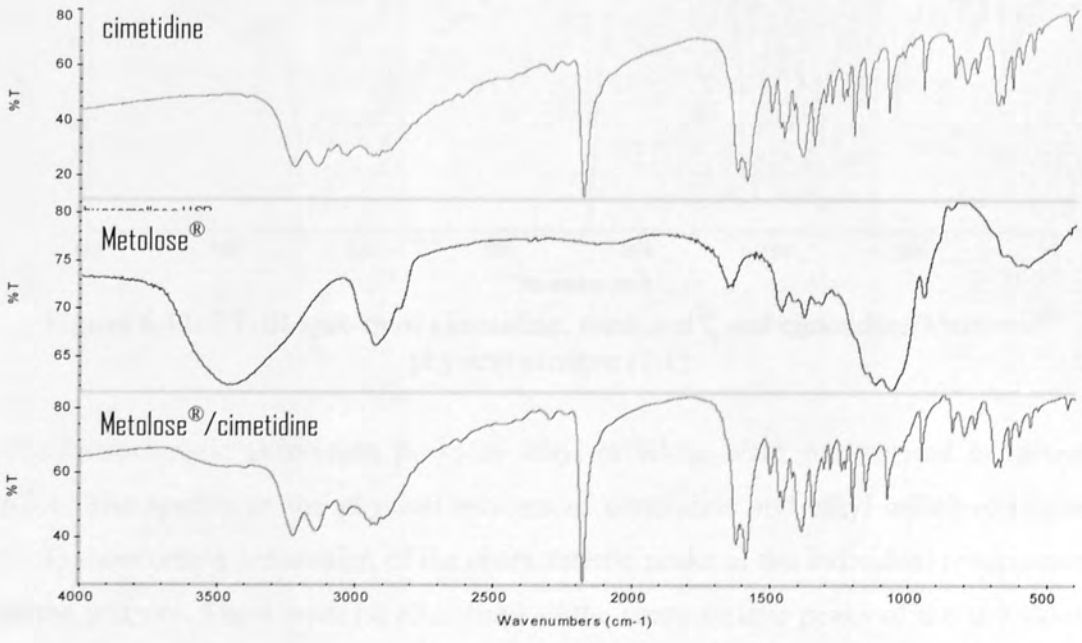


Figure 6.11: FT-IR spectra of cimetidine, Metolose®, and cimetidine/ Metolose® physical mixture (1:1)

The characteristic absorption bands of Methocel® (same as Metolose®) are detailed in section 6.3.1. It can be seen also that the physical mixture of both cimetidine and Metolose® or cimetidine and Methocel®, show peaks which are a summation of the characteristic peaks obtained for the pure drug or polymer and indicate that no interactions occur between the drug and polymer.

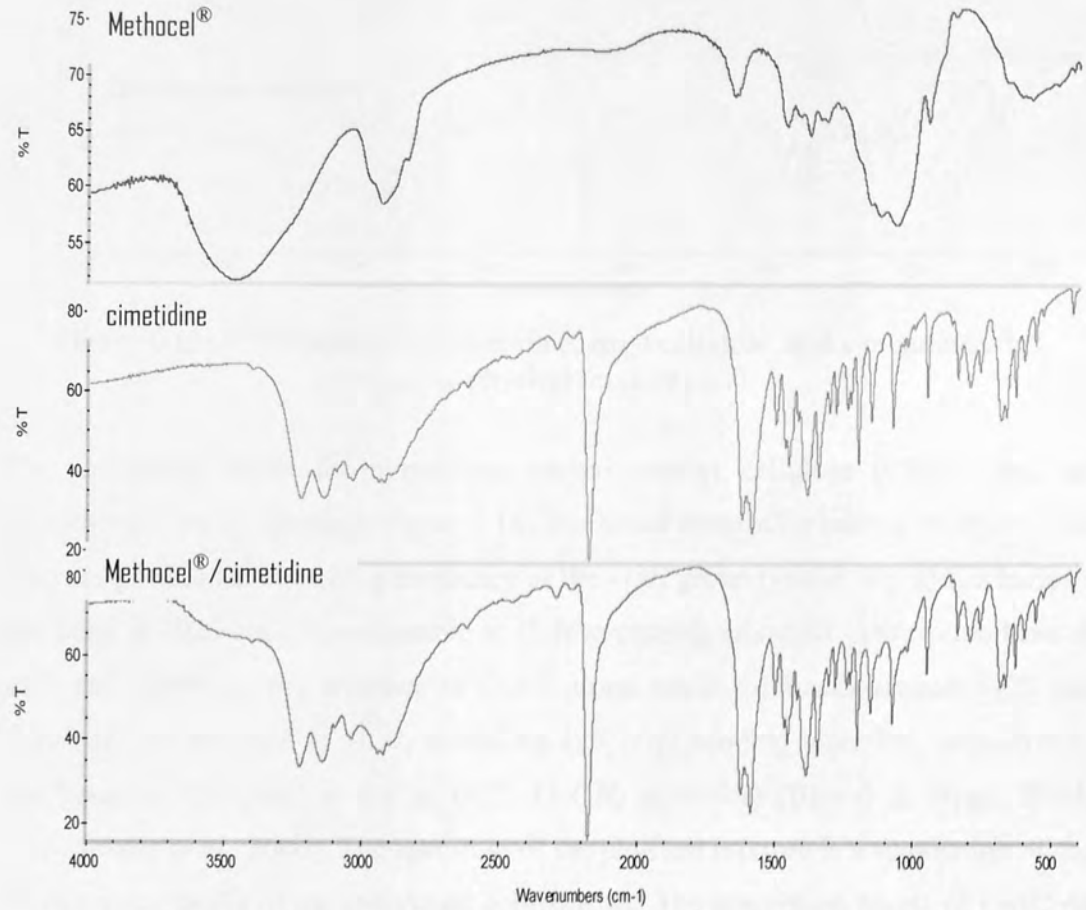


Figure 6.12: FT-IR spectra of cimetidine, methocel®, and cimetidine/Methocel® physical mixture (1:1)

The characteristic absorption peaks of ethyl cellulose were summarised in section 6.3.1. The spectra of the physical mixture of cimetidine and ethyl cellulose (figure 6.13) show only a summation of the characteristic peaks of the individual components of the mixture. There were no alterations in the characteristic peaks of the individual components of the physical mixture. This indicates that no interaction occur between ethyl cellulose and cimetidine.

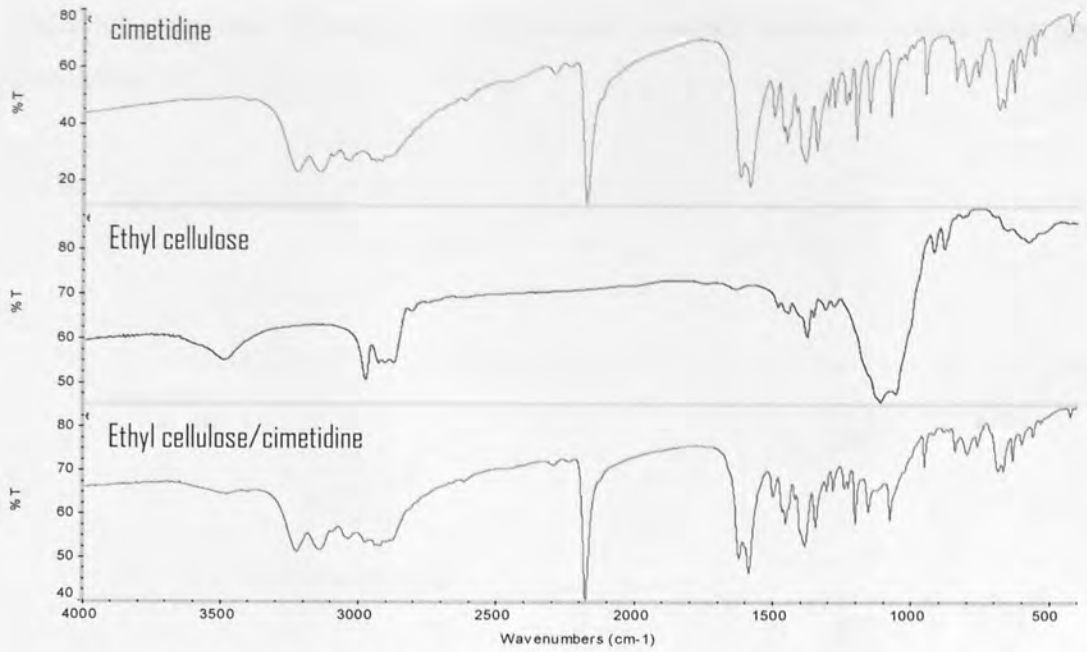


Figure 6.13: FT-IR spectra of cimetidine, ethyl cellulose, and cimetidine/ethyl cellulose physical mixture (1:1)

The absorption bands for cimetidine, carboxymethyl cellulose (CMC), and the physical mixture are shown in figure 6.14. The broad absorption band at 3400 cm^{-1} has been assigned to the stretching frequency of the *–OH* group typical of polysaccharides. The band at 2920 cm^{-1} is attributable to *C–H* stretching vibration. Absorption band at 1605 cm^{-1} confirms the presence of COO^- group while the bands around 1420 and 1320 cm^{-1} are assigned to $–CH_2$ scissoring and *–OH* bending vibration, respectively. The band at 1060 cm^{-1} is due to $OCH–O–CH_2$ stretching (Biswal & Singh, 2004; Pushpamalar *et al.*, 2006). The spectrum of the physical mixture is a summation of the characteristic peaks of the individual components. The absorption bands of CMC do not interfere with the absorption bands of cimetidine and therefore no obvious interaction occur between the drug and polymer.

Grewia polysaccharide gum gives characteristic absorption bands typical of carbohydrates (detailed in section 3.3.6). The spectrum is shown in figure 6.15. Also shown are the spectra of cimetidine and the physical mixture of cimetidine and grewia polysaccharide gum (1:1). The spectrum of the physical mixture was a summation of the peaks of the individual absorption peaks of the components of the physical mixture. Grewia polysaccharide gum did not interfere with characteristic absorption

peaks for cimetidine. Therefore, no interaction occurred between grewia gum and cimetidine.

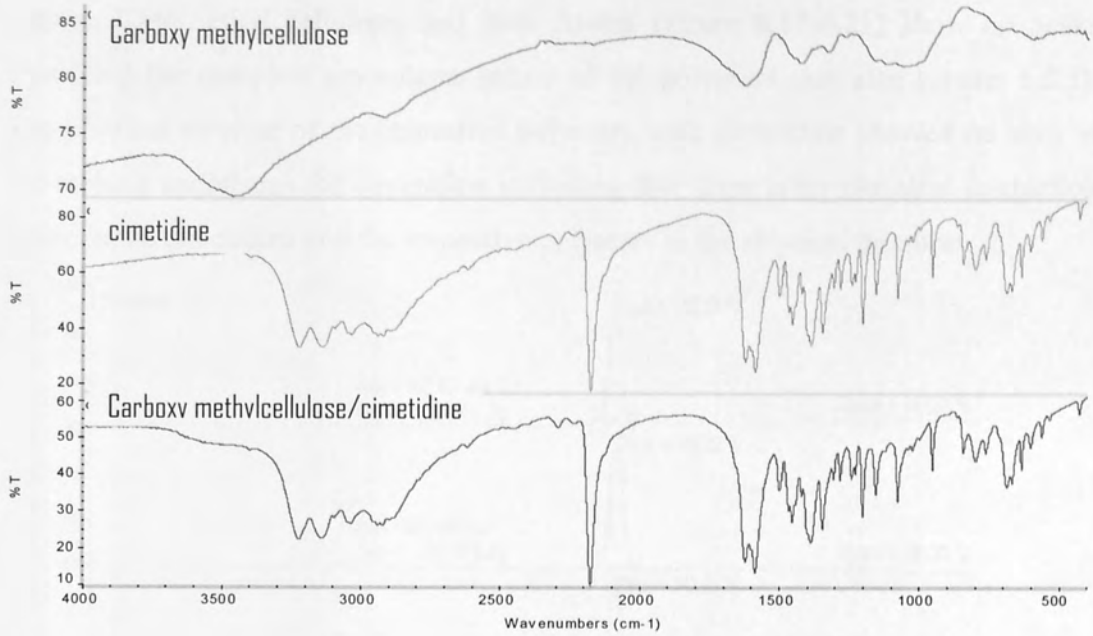


Figure 6.14: FT-IR spectra of cimetidine, CMC, and cimetidine/CMC physical mixture (1:1)

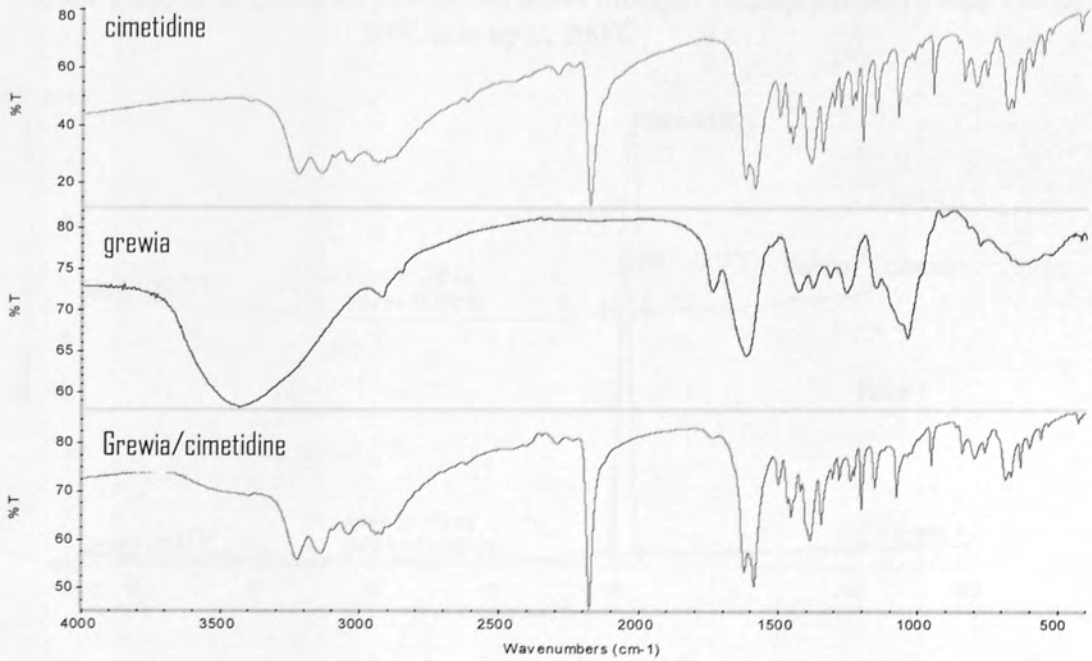


Figure 6.15: FT-IR spectra of cimetidine, grewia gum, and cimetidine/grewia gum physical mixture (1:1)

6.3.2.2 DSC analysis of cimetidine-excipient blends

The DSC trace for cimetidine showed a sharp melting endothermic peak at about 142°C with onset at 140°C as shown in figure 6.16. The DSC traces for Methocel®, grewia, CMC, ethyl cellulose, and gum Arabic (figure 6.17-6.21) show no peaks signifying the complete amorphous nature of the polymers (see also section 6.3.1). The physical mixture of the respective polymers with cimetidine showed no shift in the melting endotherm for cimetidine indicating that there is no chemical interaction between the cimetidine and the respective polymers in the physical mixtures.

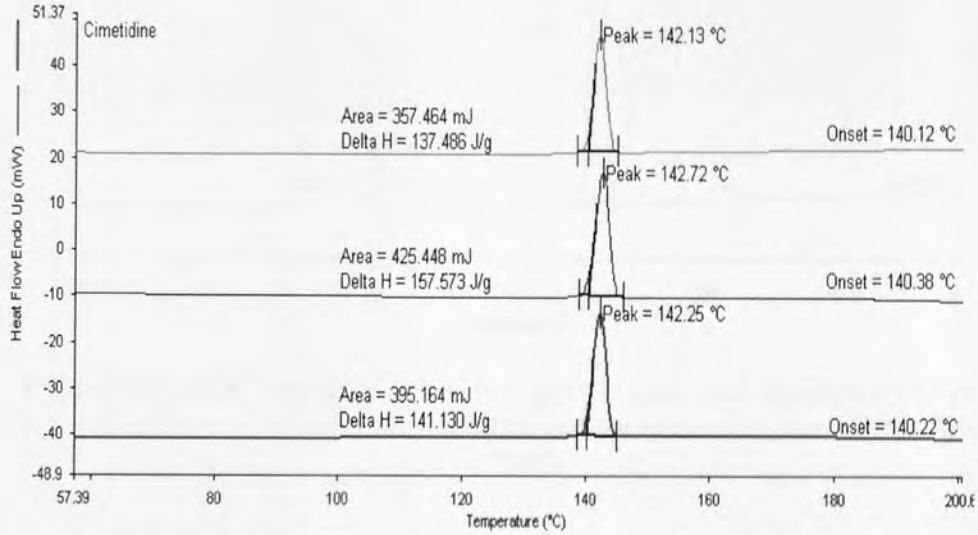


Figure 6.16: DSC traces for cimetidine under nitrogen atmosphere and a scan rate of 10°C/min up to 200°C

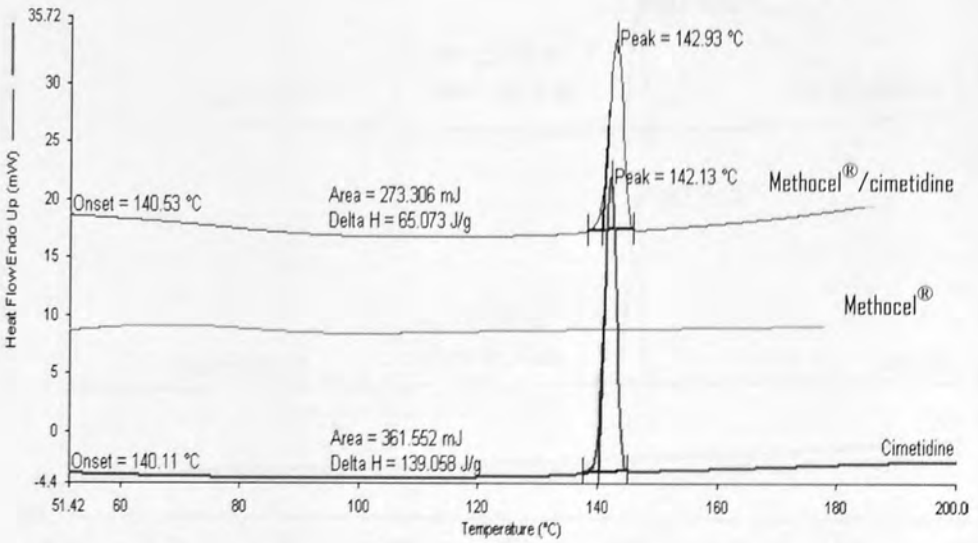


Figure 6.17: DSC traces for cimetidine, Methocel®, and cimetidine/Methocel® physical mixture (1:1) under nitrogen atmosphere and a scan rate of 10°C/min up to 200°C

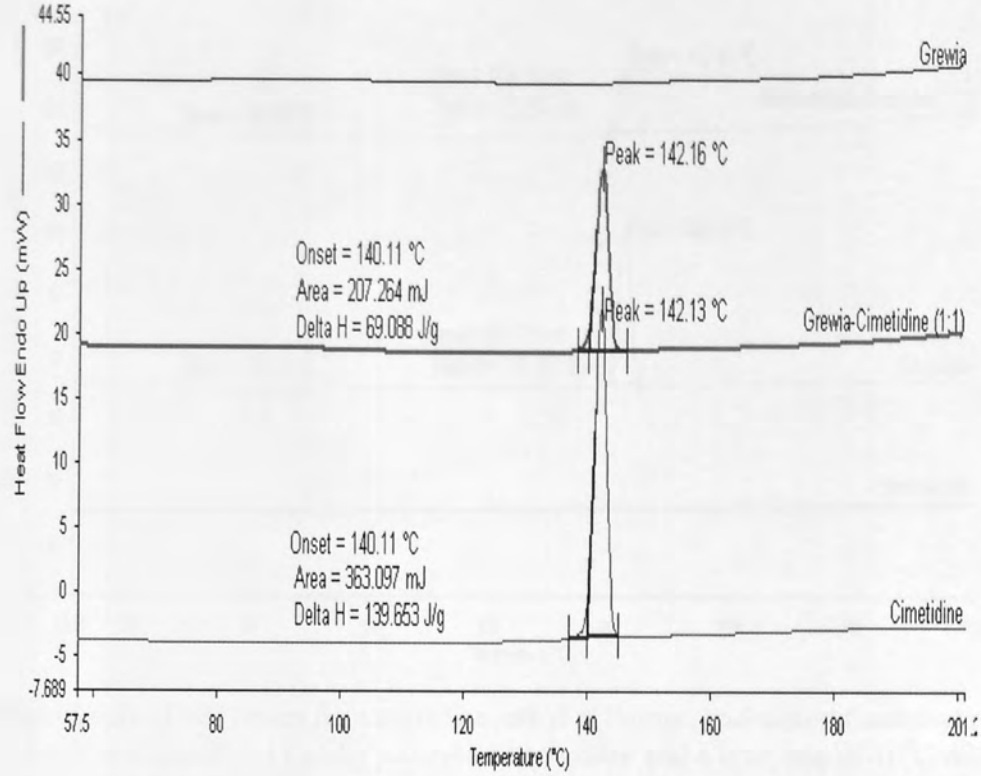


Figure 6.18: DSC traces for cimetidine, grewia gum, and cimetidine/grewia gum physical mixture (1:1) under nitrogen atmosphere and a scan rate of 10°C/min up to 200°C

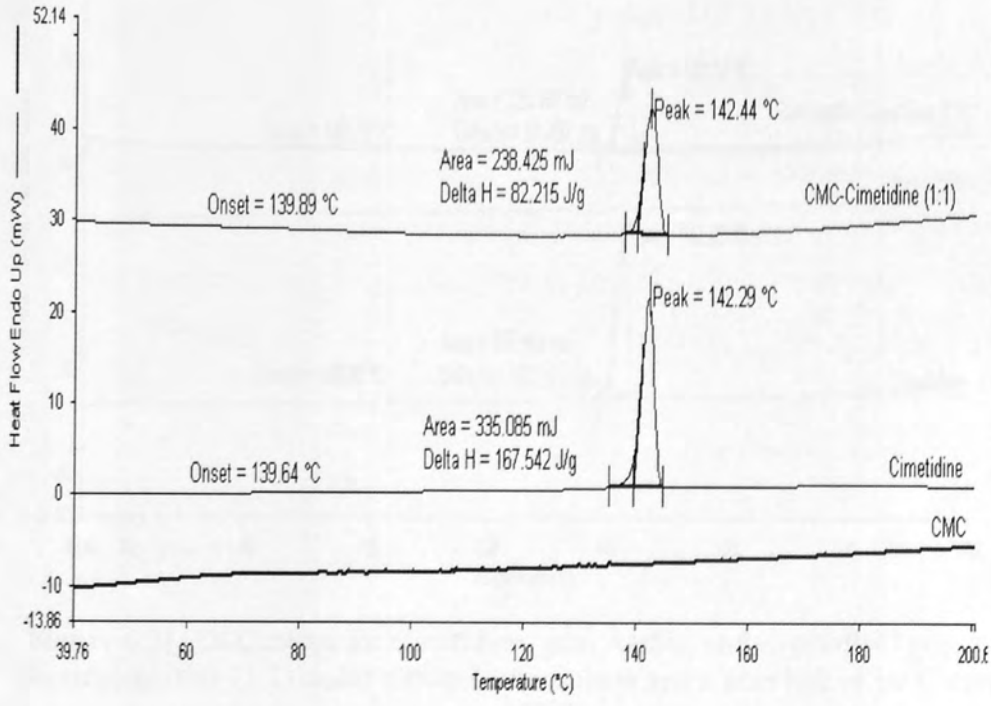


Figure 6.19: DSC traces for cimetidine, CMC, and cimetidine/CMC physical mixture (1:1) under nitrogen atmosphere and a scan rate of 10°C/min up to 200°C

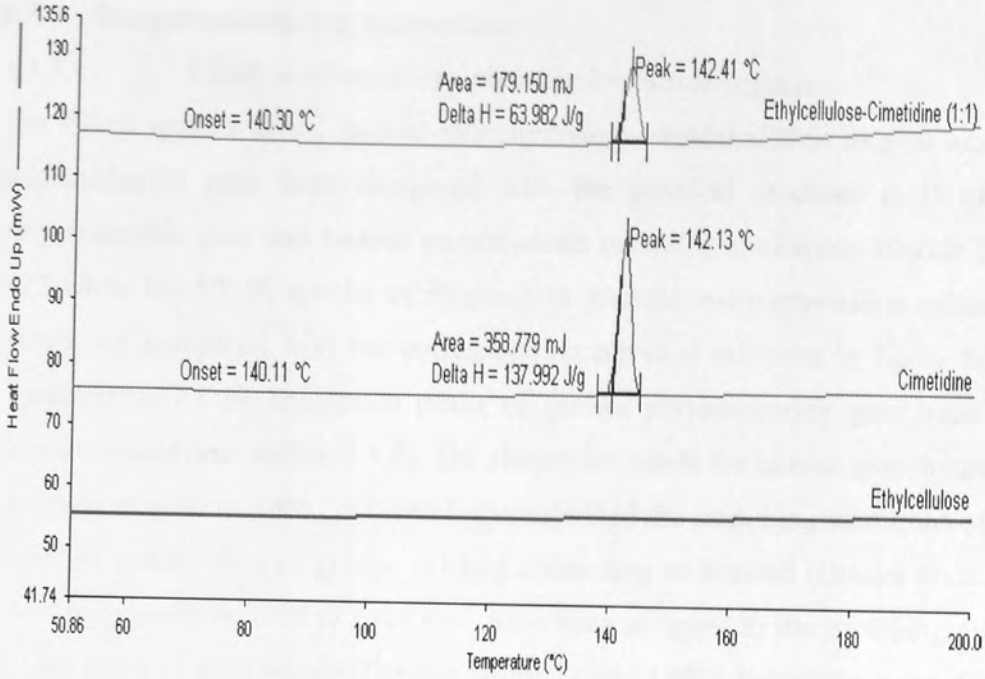


Figure 6.20: DSC traces for cimetidine, ethyl cellulose, and cimetidine/ethyl cellulose physical mixture (1:1) under nitrogen atmosphere and a scan rate of 10°C/min up to 200°C

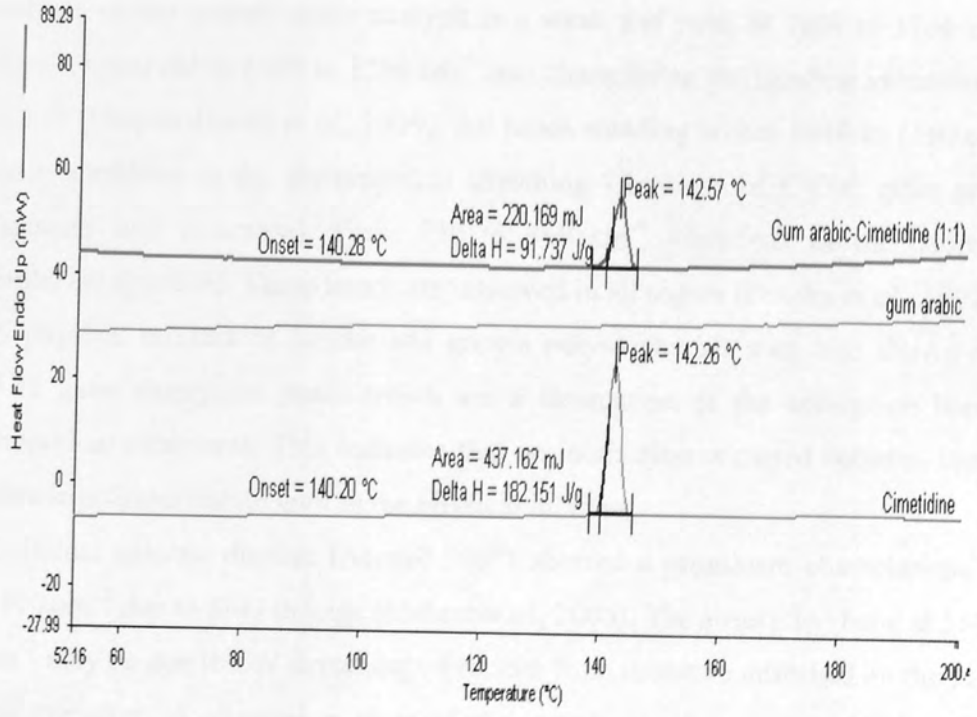


Figure 6.21: DSC traces for cimetidine, gum Arabic, and cimetidine/ gum Arabic physical mixture (1:1) under nitrogen atmosphere and a scan rate of 10°C/min up to 200°C

6.3.3 Excipient-excipient interactions

6.3.3.1 FT-IR analysis of excipient-excipient interactions

The FT-IR spectra of the lactose monohydrate, colloidal silicon dioxide and grewia polysaccharide gum were compared with the physical mixtures (1:1) of grewia polysaccharide gum and lactose monohydrate or colloidal silicone dioxide in figure 6.22 while the FT-IR spectra of magnesium stearate, microcrystalline cellulose and grewia are compared with the corresponding physical mixtures in figure 6.23. The characteristic FT-IR absorption peaks of grewia polysaccharide gum have already been discussed (see section 3.3.6). The absorption bands for lactose monohydrate were observed at 3600 to 3200 cm^{-1} which characterized the stretching vibrations of *C-O-H* bonds of lactose alcohol groups existing either free or bonded (Otsuka *et al.*, 1991). Two sharp bands at 3000 to 2800 cm^{-1} have been assigned to the stretching vibrations of two types of *C-H* bonds (Drapier-Beche *et al.*, 1999): those that were within the constituents of lactose (glucose and galactose units), and those of the methyl alcohol function outside the glucose and galactose units. The stretching vibrations of water from crystallization of *OH* bonds (Otsuka *et al.*, 1991) and of water adsorbed to the surface of the lactose under analysis is a weak and peak at 1600 to 1700 cm^{-1}. The bands, observed at 1500 to 1200 cm^{-1} also characterize the bending vibrations of *C-H* bonds (Drapier-Beche *et al.*, 1999). All bands standing within 1040 to 1160 cm^{-1} have been attributed to the asymmetrical stretching vibrations of *C-O-C* ether unit bonds (glucose and galactose). From 730 to 960 cm^{-1} vibrations of the entire lactose molecule appeared. These bands are observed in all sugars (Otsuka *et al.*, 1991).

A physical mixture of lactose and grewia polysaccharide gum also shown in figure 6.22 gave absorption peaks which are a summation of the absorption bands from individual excipients. This indicates that no interaction occurred between lactose and grewia polysaccharide gum in the physical mixture.

Colloidal silicone dioxide (Aerosil 200®) showed a prominent characteristic peak at 1110 cm^{-1} due to *Si-O* linkage (Maheshwari, 2006). The absorption band at 3600-3200 cm^{-1} may be due to *OH* stretching vibrations from moisture adsorbed on the surface of the excipient. A physical mixture of the excipient with grewia polysaccharide gum gave a resultant absorption band that is a summation of the individual absorption bands of the two excipients. This result also indicates that no interaction occurred

between grewia polysaccharide gum and colloidal silicone dioxide in the physical mixture.

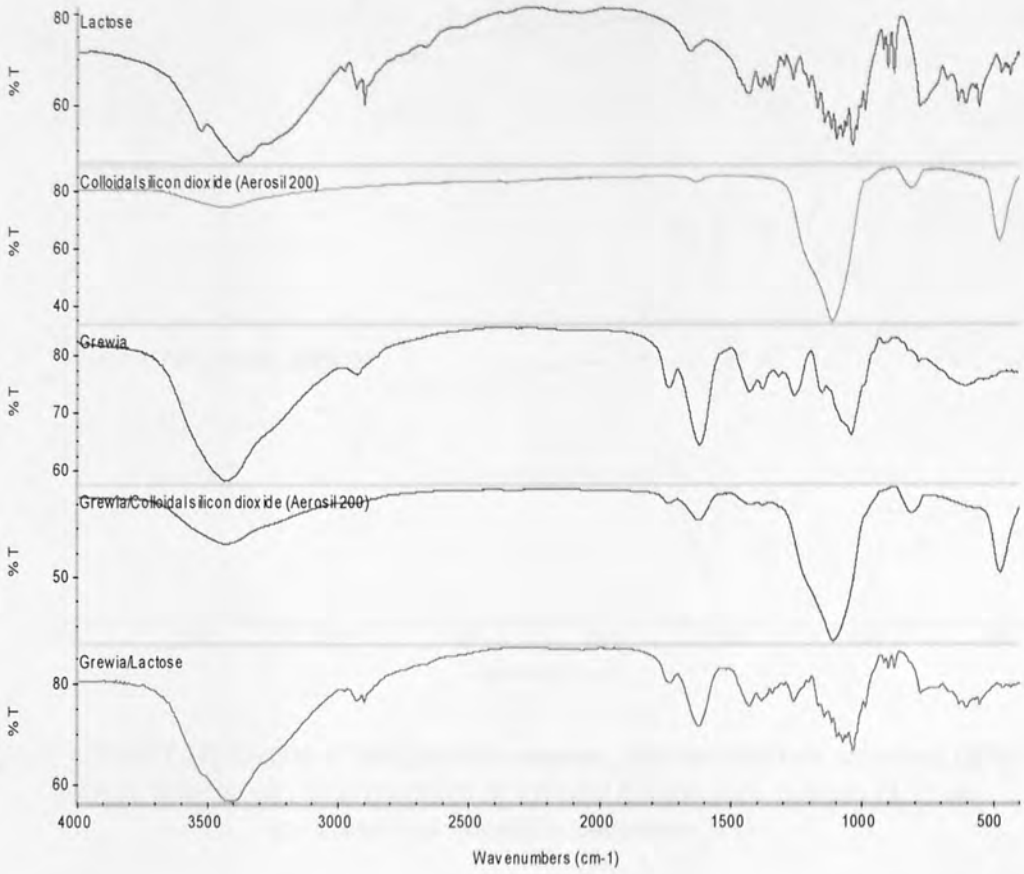


Figure 6.22: FT-IR spectra of lactose, colloidal silicon dioxide, grewia, and the physical mixtures of grewia/colloidal silicon dioxide (1:1) or grewia/lactose (1:1)

The FT-IR spectra of magnesium stearate, microcrystalline cellulose, grewia polysaccharide gum and their physical mixtures (1:1) are shown in figure 6.23. The twin peaks at 1577 cm^{-1} and 1466 cm^{-1} in magnesium stearate are attributed to asymmetric carboxylate (*COO−*) stretching vibration and symmetric carboxylate stretching vibration respectively, while the peaks at 2917 cm^{-1} and 2850 cm^{-1} are attributed to the *C–H* stretching vibration (Ng *et al.*, 2010). The broad band at about 3452 is due to *OH* stretching vibrations of the associated water molecule. The physical mixture of magnesium stearate with grewia polysaccharide gum (1:1) showed the characteristic absorption peaks of magnesium stearate and grewia polysaccharide gum and appears to be a summation of the individual absorption peaks, thus indicating no interactions between the physical mixtures of both excipients.

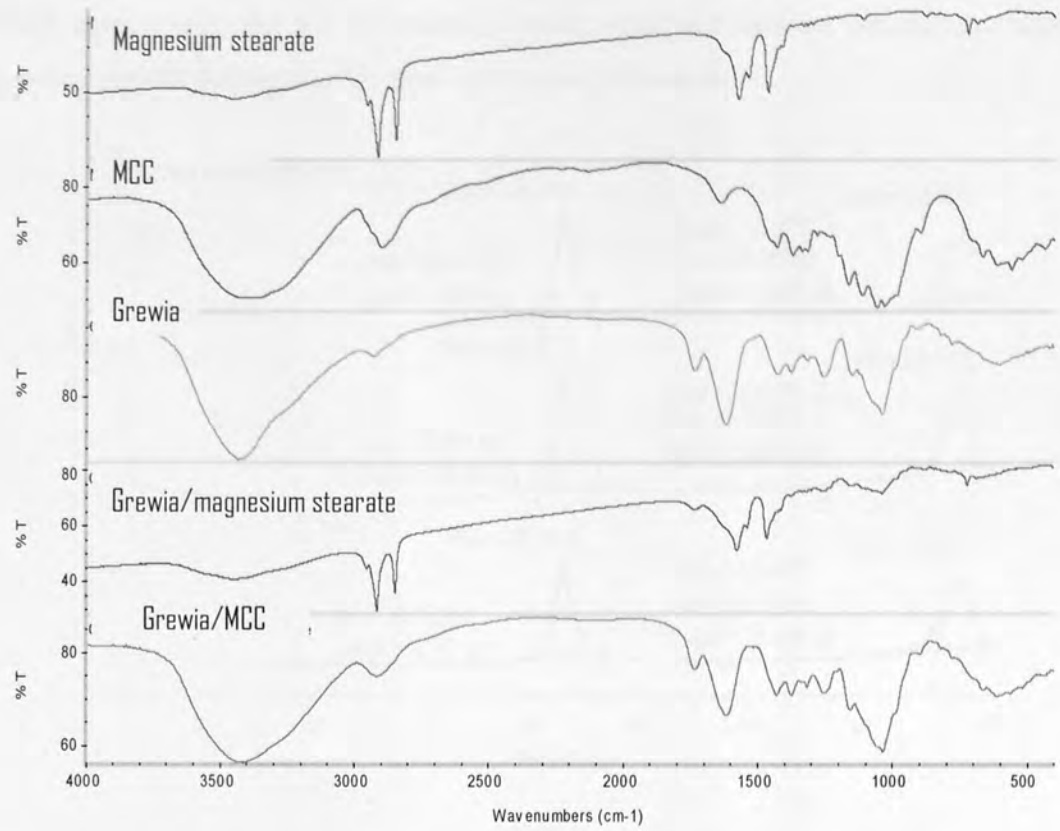


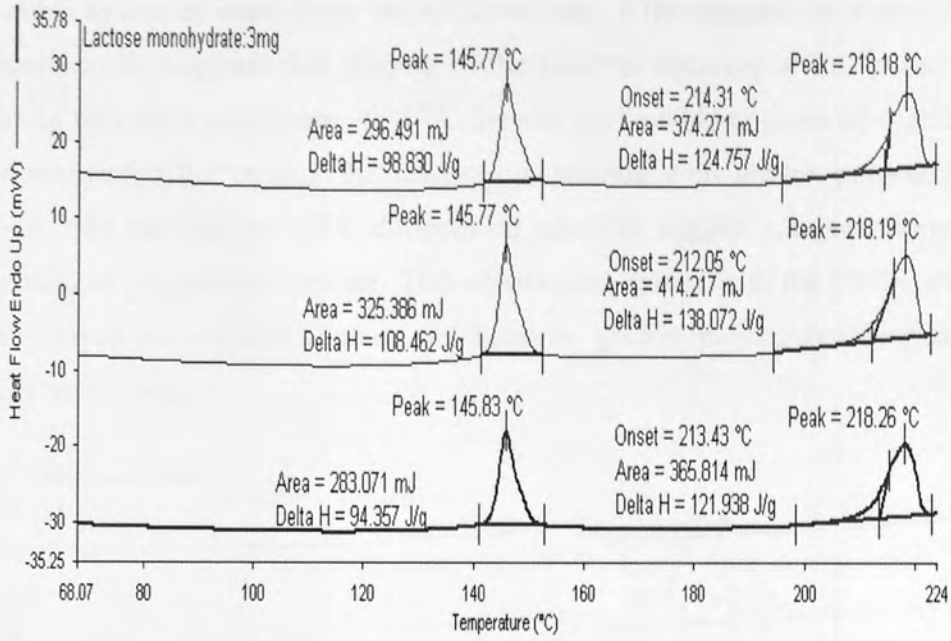
Figure 6.23: FT-IR spectra of magnesium stearate, microcrystalline cellulose (MCC), grewia, and the physical mixtures of grewia/ magnesium stearate (1:1) or grewia/microcrystalline cellulose (1:1)

The pure microcrystalline cellulose spectrum shows several bands characteristic of cellulose structure, 3400 cm^{-1} (hydroxyl (OH) stretching vibrations), 2900 cm^{-1} (*CH* stretching), 1432 cm^{-1} (CH_2 stretching) and 1140–1400 cm^{-1} (*CH*, CH_2 and *C–O* stretching) . The peak at 1640 cm^{-1} is attributed to the presence of water (Spoljaric *et al.*, 2009). Physical mixture of the microcrystalline cellulose with grewia polysaccharide gum (1:1) showed no evidence of interaction. The spectrum of the mixture is only a summation of the absorption bands from the individual components.

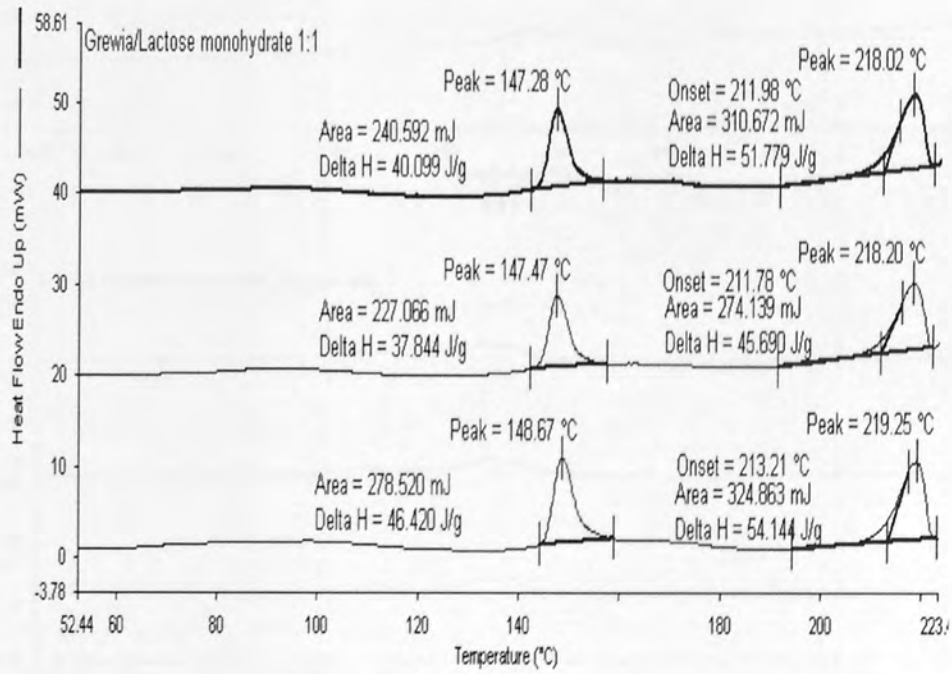
6.3.3.2 DSC analysis of excipient-excipient interactions

The DSC endotherm for lactose monohydrate showed a strong dehydration endotherm with a peak maximum temperature of about 145.7°C, followed by the lactose melting point endotherm with an onset of about 213°C and peak of 218.2°C (figure 6.24*a*). A physical mixture of grewia polysaccharide gum with lactose monohydrate (figure 6.24*b*) showed little effect on the melting point onset and peak temperatures. This

result agrees with the FT-IR analysis which indicated that no interactions occur between grewia polysaccharide gum and lactose monohydrate.



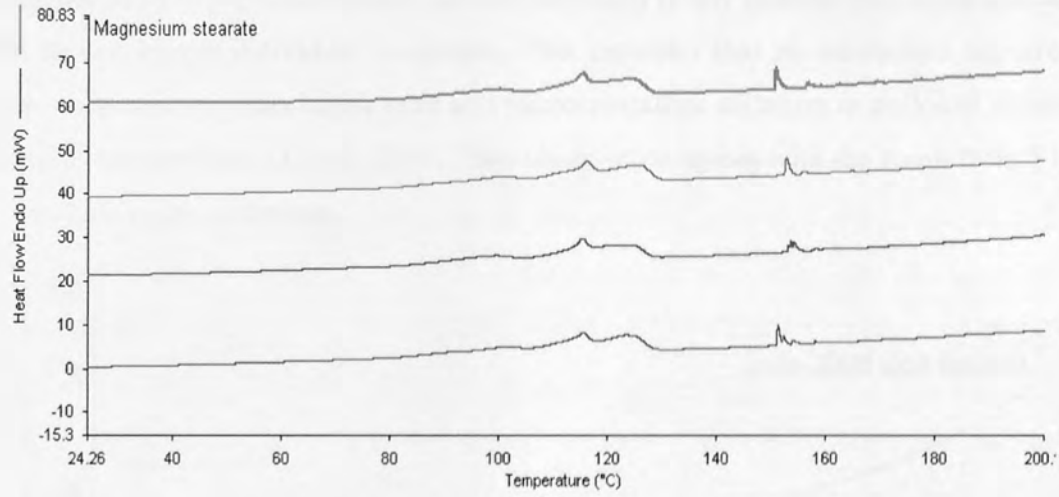
(*a*)



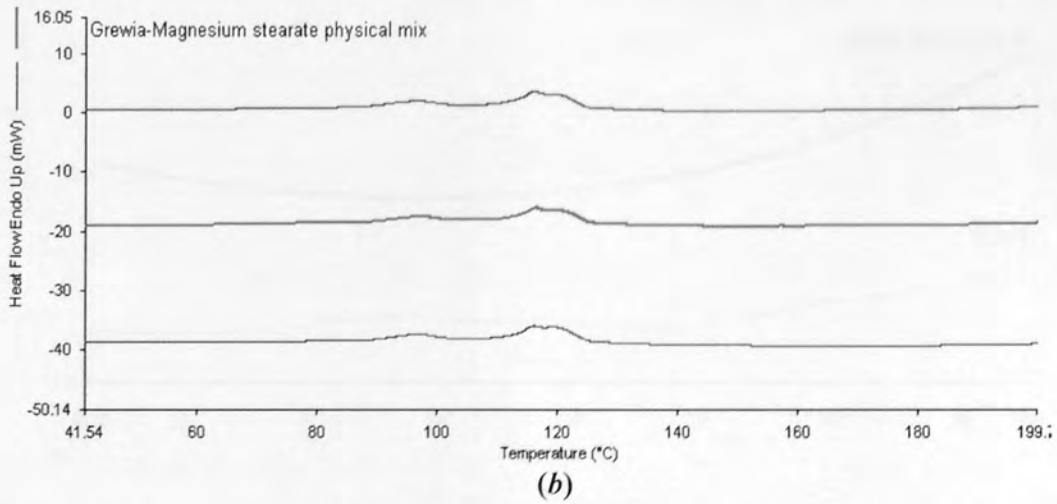
(*b*)

Figure 6.24: DSC traces of (*a*) lactose monohydrate, and (*b*) physical mixture of lactose monohydrate and grewia polysaccharide gum, at 10°C/min up to 225°C under nitrogen atmosphere

The DSC traces of magnesium stearate showed two endothermic events occurring at about 80-110°C and a second at 110°-130°C (figure 6.25*a*). The first event is attributable to loss of water from the hydrated state of the magnesium stearate while the second event suggests that melting of the material occurred at this point. What appears to be a third endotherm at 147°C may be attributable to onset of degradation of the material. After mixing the magnesium stearate with grewia polysaccharide (figure 6.25*b*), the peak at 147°C disappeared and does suggest a suppression of the degradation of magnesium stearate. This observation agrees with the FT-IR analysis which showed no obvious interactions between grewia polysaccharide gum and magnesium stearate.



(*a*)



(*b*)

Figure 6.25: DSC traces of (*a*) magnesium stearate and (*b*) physical mixture of grewia polysaccharide gum with magnesium stearate at 10°C/min under nitrogen atmosphere

It has been reported that anhydrous magnesium stearate produces only one DSC peak at 125.1°C while the dihydrate and trihydrate forms produce two DSC peaks with the lower endotherm appearing between 100.1 and 89.9°C which have been shown to represent the water loss for the hydrated forms and a melting peak at 110°C (Craig, 2006).

The DSC scans of grewia polysaccharide gum and colloidal silicon dioxide or microcrystalline cellulose (figure 6.26 and 6.27 respectively), show no melting point peaks indicating the amorphous nature of these excipients. The observed trace of the physical mixtures of grewia polysaccharide gum with colloidal silicon dioxide or microcrystalline cellulose represent a summation of the data from the individual components of th physical mixture and did not result in any endothermic event outside that shown by the individual excipients. This indicates that no interaction occurred between grewia polysaccharide gum and microcrystalline cellulose or colloidal silicon dioxide (Gaisford and O'Neil, 2006). This observation agrees with the result from FT-IR analysis of the excipients.

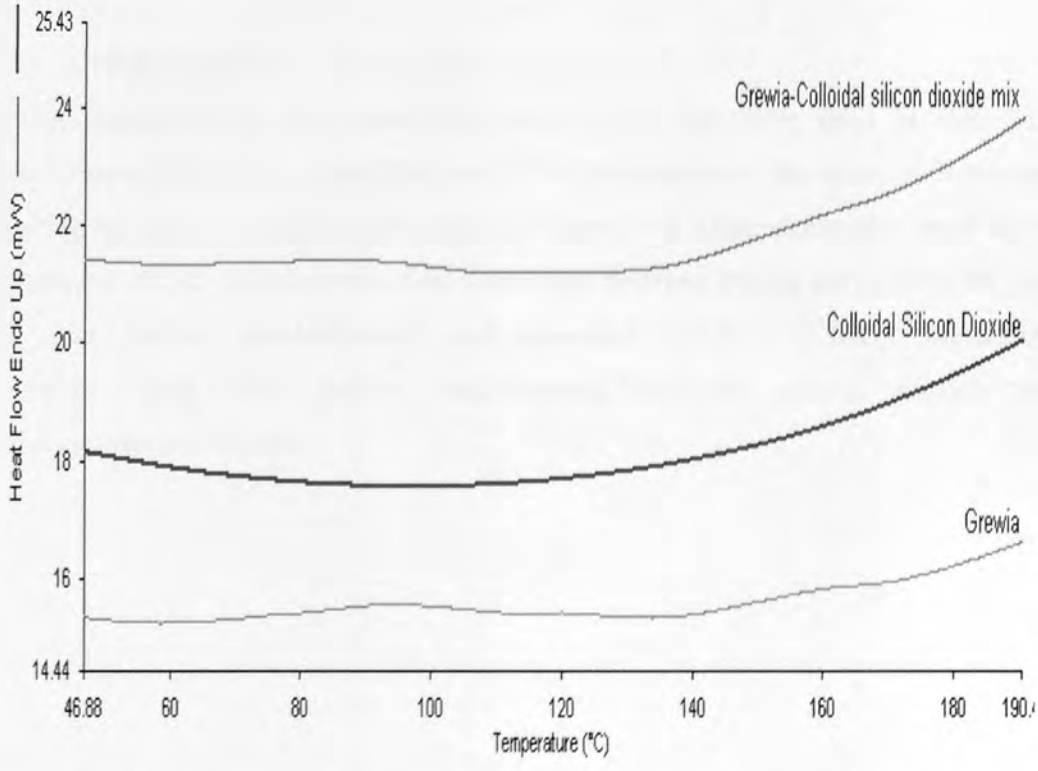


Figure 6.26: DSC traces of grewia polysaccharide gum, colloidal silicon dioxide, and the physical mixture of grewia/colloidal silicon dioxide (1:1) under nitrogen atmosphere and scan rate of 10°C/min up to 200°C

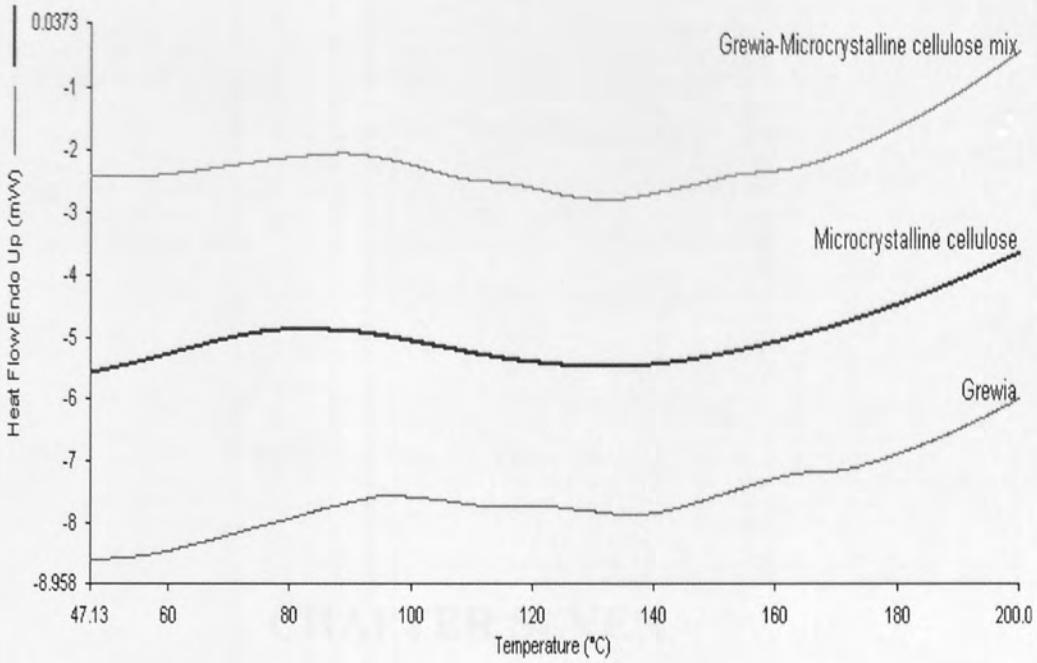


Figure 6.27: DSC traces of grewia polysaccharide gum, microcrystalline cellulose, and the physical mixture of grewia/microcrystalline cellulose (1:1) under nitrogen atmosphere and scan rate of 10°C/min up to 200°C

6.4 CONCLUSION

Grewia polysaccharide gum does not interact with the APIs used in this study (cimetidine or ibuprofen). Both DSC and FT-IR evaluation of the interaction between physical mixtures of grewia polysaccharide gum and other excipients used in the formulation of the tablets revealed no interaction between grewia polysaccharide gum and gum Arabic, ethylcellulose, carboxymethyl cellulose (CMC), Methocel®, Metolose®, guar gum, lactose monohydrate, colloidal silicon dioxide and microcrystalline cellulose.

CHAPTER SEVEN

GREWIA POLYSACCHARIDE GUM MATRIX TABLETS FOR ORAL CONTROLLED RELEASE OF IBUPROFEN AND CIMETIDINE

7.1 INTRODUCTION

Controlled delivery of drugs is intended to deliver the active ingredient at a controlled rate over a desired duration of time. The primary objectives therefore are to ensure safety and to improve efficacy of drugs, as well as to improve patient compliance. A number of approaches have been used to obtain controlled drug release, but a hydrophilic matrix is recognized as the simplest and is the most widely used.

Drug release from hydrophilic matrix tablets results initially from swelling upon ingestion which causes a gel layer to form on the tablet surface. This gel layer retards further ingress of fluid and subsequent drug release. Sujja-areevath *et al.*, (1998) showed that in the case of hydrophilic matrices, swelling and erosion of the polymer occur simultaneously, and both of them contribute to the overall drug-release rate. It is well documented that drug release from hydrophilic matrices shows a typical time-dependent profile, i.e. decreased drug release with time because of increased diffusional path length (Chien 1983; Peppas and Sahlin, 1989). Many controlled-release products are designed on the principle of embedding the drug in a porous matrix. Liquid penetrates the matrix and dissolves the drug, which then diffuses into the exterior liquid (Fessi *et al.*, 1982).

Polysaccharide gums find application in the oral controlled delivery of medicaments. Their ability to hydrate and swell when in aqueous medium makes them a viable platform as hydrophilic matrices for time-dependent release of soluble or insoluble drugs. Hydrophilic matrices from natural polysaccharide gums such as xanthan gum (Talukdar, and Kinget, 1995; Billa and Yuen, 2000; El-Gazayerly, 2003; Fan, 2008), guar gum (Toti and Aminabhavi, 2004; Krishnaiah *et al.*, 2002*a*; Krishnaiah *et al.*, 2002*b*) and karaya gum (Munday and Cox, 2000) have been shown to provide varying degrees of sustained release of medicaments. These natural hydrophilic colloids are still widely used in pharmaceutical dosage forms because of their biocompatibility, low cost and relatively abundant availability (Vendruscolo *et al.*, 2009).

The formulation of a suitable oral drug delivery system for the controlled delivery of highly water soluble drugs is a challenging task to the formulation scientist (Siahi *et al.*, 2005).This is because many highly water-soluble drugs can be readily released from the tablet matrix faster than desired if not properly formulated.

In this study, matrix tablets containing ibuprofen (a poorly soluble drug) and cimetidine (a highly soluble drug), were formulated by wet granulation and direct

compression techniques respectively. Monolithic systems comprising 16%, 32% or 48% of grewia polysaccharide gum were compared with similar hydrophilic monolithic systems of Methocel®, Metolose®, carboxy methylcellulose (CMC), guar gum, gum Arabic (Acacia) or ethyl cellulose, a hydrophobic release retardant. To evaluate synergism between grewia polysaccharide gum and the reference polymers, binary composite matrices of grewia polysaccharide gum and the reference polymers were also prepared and evaluated.

7.2 MATERIALS AND METHODS

Materials used for this study are as detailed in section 6.2.1 except otherwise indicated.

7.2.1 Preparation of ibuprofen granules and tablets

Granules of ibuprofen were formulated using a single polymer as release retardant (monolithic matrices), and by combining different ratios of grewia polysaccharide gum with another polymer standard (binary matrices).

7.2.1.1 Formulation of ibuprofen monolithic granules and tablets

Wet granulation technique (see section 1.11.3.2) was used for the formulation of ibuprofen granules. The batch formula for the formulation of the monolithic matrix systems is shown in table 6.1. All powders were passed through a sieve of 250 μm before wet granulation. Briefly, the amounts of polymer giving 16%, 32% or 48% (w/w of ibuprofen) were mixed with lactose monohydrate and ibuprofen, and wetted with distilled water in a mortar and pestle. The moist mass was passed through a 710 μm sieve to form granules which were dried at 50°C overnight in an oven (Gallenkamp, England).

Table 7.1: Per tablet formula for monolithic matrix tablets of ibuprofen

Ingredients	*I*	*II*	*III*
Ibuprofen (mg)	500	500	500
Polymer (mg)	80	160	240
Lactose monohydrate (mg)	220	140	60
Magnesium stearate (mg)	8	8	8
Colloidal silicon dioxide (mg)	24	24	24

The dried granules were then passed through a 1 mm sieve and stored in air tight containers. After evaluation of granule properties, the granules were mixed with

magnesium stearate and colloidal silicon dioxide before compression of the granules into tablets was carried out using a single station press (Minipress MII RIVA, Germany) to give flat faced tablets of 850 mg (mean weight, to correct for moisture), 13 mm diameter and an average hardness of 70 N. The compressed tablets were stored in air tight containers until evaluation of tablet properties. Similar formulations were prepared using ethyl cellulose, Metolose® and guar gum as polymer standards.

7.2.1.2 Formulation of binary composite granules and tablets of ibuprofen

Granules composed of grewia polysaccharide gum and one other reference standard were formulated according to the batch formula shown in table 7.2.

Table 7.2: Per tablet formula for binary composite matrix tablets of ibuprofen

	I	*II*	*III*	*IV*	*V*	*VI*	*VII*	*VIII*	*IX*
Ibuprofen (mg)	500	500	500	500	500	500	500	500	500
Grewia (mg)	40	40	40	26.7	26.7	26.7	53.3	53.3	53.3
Ethyl cellulose (mg)	40	-	-	53.3	-	-	26.7	-	-
Metolose®(mg)	-	40	-	-	53.3	-	-	26.7	-
Guar (mg)	-	-	40	-	-	53.3	-	-	26.7
Lactose (mg)	220	220	220	220	220	220	220	220	220
Colloidal silicon dioxide (mg)	24	24	24	24	24	24	24	24	24
Magnesium stearate (mg)	8	8	8	8	8	8	8	8	8

The binary composite matrix tablets of ibuprofen containing grewia gum and guar, ethyl cellulose or Metolose® in the ratio 1:1, 1:2 or 2:1 (16% w/w of ibuprofen) were made by wet granulation. The amounts of ibuprofen, lactose monohydrate and polymers were carefully weighed into a mortar. The powders were then moistened with distilled water by mixing inside the mortar with a pestle. Subsequent processing was carried out as outlined above (section 7.2.1.1).

7.2.2 Preparation of cimetidine tablets

Monolithic and binary composite matrix tablets of cimetidine were prepared by direct compression. The monolithic matrix tablets of cimetidine were made using 40% (w/w cimetidine) of ethyl cellulose, Metolose®, Methocel®, CMC, gum Arabic (reference polymers), or grewia polysaccharide gum (test polymer) as polymer matrix according to the batch formula shown in table 7.3. Binary composite matrix formulations were made using the test and polymer standards in a ratio of 1:1, equivalent to 40% w/w polymer matrix according to the batch formula shown in table 7.4.

Table 7.3: Per tablet formula for directly compressed monolithic matrix tablets of cimetidine

Ingredients	*Amounts*
Cimetidine (mg)	250
Polymer (mg)	100
Microcrystalline cellulose (mg)	125
Magnesium stearate (mg)	4.75
Colloidal silicon dioxide (mg)	14.25

All the powders were passed through a sieve of 250μm before direct compression. The amounts of ingredients as shown in the tables were accurately weighed into a mortar and manually blended using a pestle. The powders were characterised prior to compression in a single station press (Minipress MII RIVA, Germany) to give flat faced tablets of 520 mg mean weight (due to moisture correction), 13 mm diameter and an average crushing strength of 80 N. The compressed tablets were stored in air tight containers prior to evaluation. The final tablet weight was determined by the setting of the press to achieve the desired average tablet crushing strength of 80 N.

Table 7.4: Per tablet formula for directly compressed binary composite matrix tablets of cimetidine

Ingredients	*I*	*II*	*III*	*IV*	*V*
Cimetidine (mg)	250	250	250	250	250
Grewia (mg)	50	50	50	50	50
Ethylcellulose (mg)	50	-	-	-	-
Metolose® (mg)	-	50	-	-	-
CMC (mg)	-	-	50	-	-
Methocel® (mg)	-	-	-	50	-
Gum Arabic (mg)	-	-	-	-	50
Microcrystalline cellulose	125	125	125	125	125
Colloidal silicon dioxide (mg)	14.25	14.25	14.25	14.25	14.25
Magnesium stearate (mg)	4.75	4.75	4.75	4.75	4.75

7.2.3 Volume correction

To account for the sample of drug lost and the sample dilution during dissolution studies, M_t was calculated from the following equation:

$$M_t[n] = \frac{V_r C[n] + V_s \sum_{m-1}^{n-1} C[m]}{1000} \qquad \text{--------- (Equation 7.1)}$$

Where $M_t[n]$ is the current, cumulative mass of drug released at time t, $C[n]$ is the current concentration in the dissolution medium (mg/L), $\sum C[m]$ is the summed total

of the previous measured concentrations in mg/L {m=1-(n-1)}, V_r is the volume of the dissolution medium (ml), and V_s is the volume of sample removed for analysis (ml).

Example: *Mt* for ibuprofen at $t = 90$ minutes, when, $V_r = 900$ ml, $C[n] = 0.051622$ mg/ml (51.622 mg/L), Vs = 4 ml, $\sum C[m] = 0.081121$ mg/ml (81.121 mg/L).

$$Mt = \frac{(900 \times 51.622) + (4 \times 81.121)}{1000} = 46.78 \text{ mg}$$

7.2.4 Stability of the API

Representative formulations were selected for stability testing, according to the USP (1999) guidelines. The formulations were kept in a stability chamber (Firlabo, U.K.) at 25°C and relative humidity of 60%. Samples were stored open and withdrawn at 0, 15, 30, 45 and 60 days for evaluation of appearance and drug content. Ibuprofen matrices containing 48% w/w of polymer were chosen as these would be the most extreme in terms of highest polymer:drug ratio.

7.3 RESULTS AND DISCUSSION

7.3.1 Granules and powder properties

The powder properties of blends of ingredients for direct compression of cimetidine tablets and the granules of ibuprofen were evaluated (refer to section 2.20).

7.3.1.1 Properties of ibuprofen granules

The physical properties of granules of both monolithic and binary composite systems were evaluated and results are presented in table 7.5.

The moisture content of granules from grewia monolithic and binary combinations is relatively high. Grewia polysaccharide gum has a high moisture sorption capacity, thus accounting for the high content. The angle of repose was between 27 and 33° for all the batches of granules indicating "fair flow" of granules (Staniforth, 2002).

The angle of repose represents an indirect method of quantifying powder flowability due to its relation to inter-particulate cohesion. It provides a reliable index of powder flow. The value of the angle of repose will be high if the powder is cohesive and low if the powder is non-cohesive. With increasing departure from the spherical, the angle of repose increases while bulk density and flowability increases. Carr (1965 & 1970) and Raymus (1985) suggested that angles of repose below 30° indicate good flowability, 30°- 45° some cohesiveness, 45°-55° true cohesiveness, and >55° sluggish or very high cohesiveness and very limited flowability. Some cohesiveness in the

granules may be attributable to the high percentage of fines < 250 μm (see section 7.3.2). In the determination of powder flowability, the angle of repose is preferred over Hausner ratio because the latter is easier to measure and involves more powder movement such as often occurs in powder processing (Geldart *et al.*, 2006).

Table 7.5: Some physical properties of ibuprofen granules from monolithic and binary composite matrices (n=3, mean ± s.d.)

Formulation	*Moisture content (%)*	*Angle of repose (°)*	*Bulk density (g/ml)*	*Tapped density (g/ml)*	*Hausner ratio*
Grewia 16%	11.2±0.2	32.1±1.4	0.33 ±0.01	0.51 ±0.01	1.56 ± 0.03
Grewia 32%	9.8±1.5	28.8±1.3	0.27 ±0.01	0.39 ±0.01	1.45 ± 0.02
Grewia 48%	8.9±0.8	30.0±1.2	0.29 ±0.01	0.39 ±0.01	1.36 ± 0.02
Guar 16%	6.8±0.8	32.4±0.4	0.30 ±0.01	0.46 ±0.01	1.53 ± 0.04
Guar 32%	6.6±1.1	31.2±2.8	0.26 ±0.02	0.39 ±0.03	1.51 ± 0.02
Guar 48%	7.7±0.8	33.0±1.8	0.28 ±0.02	0.43 ±0.02	1.55 ± 0.03
Metolose® 16%	7.2±1.1	30.4±1.2	0.25 ±0.01	0.35 ±0.01	1.40 ± 0.01
Metolose® 32%	8.1±0.8	30.7±0.5	0.23 ±0.01	0.34 ±0.02	1.46 ± 0.01
Metolose® 48%	7.4±1.1	33.2±0.5	0.25±0.00	0.39 ±0.00	1.55 ± 0.01
Ethyl cellulose 16%	6.2±1.0	29.3±1.0	0.31 ±0.00	0.40±0.01	1.31 ± 0.01
Ethyl cellulose 32%	6.7±0.2	28.1±2.1	0.28 ±0.00	0.36 ±0.00	1.28 ± 0.02
Ethyl cellulose 48%	6.2±0.7	29.4±1.5	0.24 ± 0.00	0.31 ±0.00	1.29 ± 0.01
Grewia/Guar (1:1)	11.7±0.5	29.5±1.7	0.32 ±0.00	0.50 ± 0.00	1.57 ± 0.01
Grewia/ Metolose® (1:1)	10.3±1.4	29.6±1.2	0.33 ±0.01	0.51 ±0.01	1.57 ± 0.03
Grewia/Ethyl cellulose (1:1)	10.5±1.7	30.2±0.4	0.28 ±0.01	0.38 ±0.00	1.37 ± 0.02
Grewia/Guar (1:2)	8.4±2.1	30.7±1.0	0.34 ±0.02	0.54 ±0.00	1.60 ± 0.10
Grewia/ Metolose® (1:2)	10.1±0.3	28.4±0.4	0.28 ±0.00	0.43 ±0.01	1.54 ± 0.01
Grewia/Ethyl cellulose (1:2)	10.2±1.8	27.7±0.2	0.27 ±0.01	0.42 ±0.01	1.60 ± 0.04
Grewia/Guar (2:1)	11.0±1.7	27.8±0.8	0.29 ±0.02	0.48 ±0.02	1.63 ± 0.03
Grewia/ Metolose®(2:1)	9.8±2.1	29.8±1.8	0.29 ±0.00	0.45 ±0.01	1.55 ± 0.02
Grewia/Ethylcellulose (2:1)	9.1±2.0	30.1±0.5	0.28 ±0.01	0.44 ±0.01	1.53 ± 0.03

The Hausner quotient (ratio) is related to the inter-particle friction and as such can be used to predict powder flow properties (also see section 1.11.4.4). Hausner showed that powders with low inter-particle friction, such as coarse spheres had ratios of approximately 1.2 whereas more cohesive, less free flowing powders such as flakes have Hausner ratios greater than 1.6. From this result, it can be seen that not all the granules formed have Hausner ratio of < 1.6 and indicate that such granules may have high interparticulate friction and thus may not flow readily requiring vigorous agitation or shaking to flow through the feed of the tableting machine. The Hausner ratio which is the ratio of the tapped and bulk densities (also see section 1.11.4.4) is

not an intrinsic property of the powder and may vary depending on factors such as the diameter of the cylinder, number of times the powder is tapped to achieve tapped density, mass of material used in the test, and rotation of sample during tapping.

7.3.1.2 Properties of cimetidine powder blends

The moisture content and the flow parameters of the cimetidine-containing powder blends are presented in table 7.6. The results show that all powder blends have moisture contents of between 6.80 and 8.62%. The moisture content of tablets is very important because when tablets lost moisture on aging or during storage, they may become friable and consequently lead to a decrease in tablet strength. However, in the preparation of pharmaceutical tablets, it is generally accepted that a small proportion of moisture is present.

The bulk density of a powdered material is dependent on packing of particles and it changes as the powder consolidates (Staniforth, 2002). It is a measure of the particles' packing characteristics. An increase in the tapped or consolidated density is of an advantage in tableting because of reduced volume of fill (Sanghvi *et al.*, 1993). Good flow rate and complete filling of die cavity will ensure both weight uniformity and content uniformity of compressed tablets (Jones and Pilpel, 1966; James, 2002).

Table 7.6: Some properties of powder blends for cimetidine matrix tablets

Formulation	*Moisture content (%)*	*Angle of repose (°)*	*Bulk density (g/ml)*	*Tapped density (g/ml)*	*Hausner ratio*
Grewia	8.5±0.5	35.2±1.3	0.19±0.00	0.30±0.01	1.55±0.07
Gum Arabic	8.6±0.6	30.1±1.4	0.50±0.00	0.65±0.02	1.29±0.04
Metolose®	7.7±1.4	31.6±1.0	0.33±0.00	0.51±0.01	1.55±0.05
Ethyl cellulose	7.1±0.4	32.7±1.3	0.28±0.00	0.43±0.01	1.53±0.01
Methocel®	7.4±0.5	32.2±1.1	0.32±0.00	0.41±0.01	1.30±0.01
CMC	7.7±0.8	30.3±1.7	0.62±0.01	0.73±0.02	1.18±0.04
Grewia/Gum Arabic (1:1)	7.6±0.5	30.0±0.7	0.33±0.01	0.42±0.01	1.28±0.06
Grewia/ Metolose® (1:1)	8.0±0.6	30.7±0.5	0.22±0.00	0.34±0.02	1.55±0.09
Grewia/Ethyl cellulose (1:1)	8.0±0.8	31.7±1.2	0.19±0.00	0.28±0.00	1.44±0.03
Grewia/Methocel® (1:1)	6.8±1.0	30.3±0.8	0.22±0.01	0.33±0.00	1.54±0.04
Grewia/CMC (1:1)	7.6±0.8	30.5±0.7	0.33±0.01	0.44±0.01	1.35±0.03

The powder blends for all the cimetidine tablet formulations showed an angle of repose of between 30 and 35°. Generally, powders with an angle of repose greater than 50° will not flow satisfactorily and are highly cohesive, while powders with an angle

of repose below 25° will flow well but are not readily available for solid dosage form production. The upper limit of this angle, i.e. 42° is considered a good working range for most pharmaceutical powders and granules (Jones and Pilpel, 1966).

Values of Hausner ratio less than 1.25 indicate good flow, while greater than 1.6 indicates poor flow behaviour (James, 2002). All the powders have Hausner ratio of less than 1.6. This indicates that the powder blends will not present problems of flow through the feed of the tablet press

7.3.2 Size analysis of ibuprofen granules

Results of sieve size analysis of the monolithic and binary composite granules of ibuprofen are presented in figures 7.1 and 7.2 below:

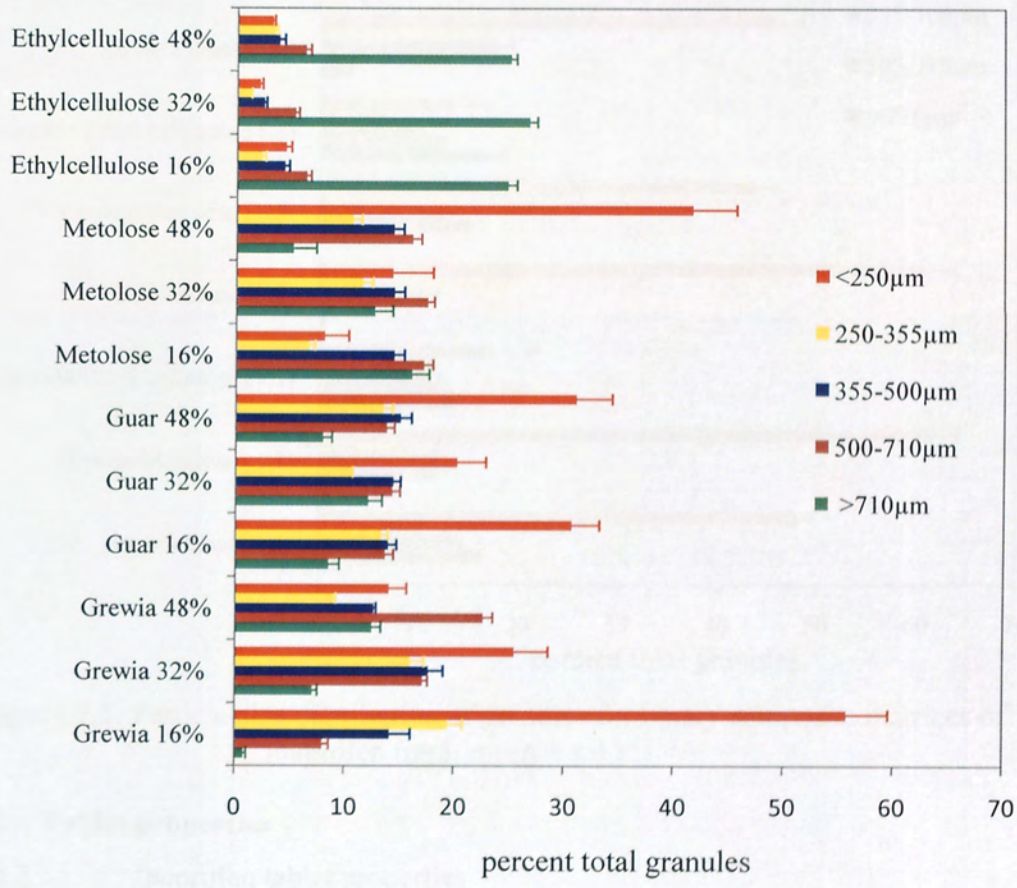


Figure 7.1: Particle-size distribution of granules for monolithic tablets of ibuprofen (n=3, mean±s.d.)

The particle-size distribution of the granules show that monolithic granules of grewia (16, 32%), guar (16, 32, 48%), and Metolose® (48%) predominantly consist of smaller particle sizes of < 250µm. Inter-particulate attraction tends to be higher in fine

particles and are therefore more cohesive and less free flowing. This is supported by data for angle of repose where for example the ethyl cellulose-containing matrices (16%, 32% or 48% w/v) gave angle of repose values of < 30° (see table 6.5 and figure 6.1). Granules made of binary composites all predominantly consisted of particles of < 250 µm, except granules consisting of grewia/ethyl cellulose (1:2 and 1:1). For the purpose of this study, the granule sizes of <250 µm were not used for tableting in an attempt to improve the flow through the tableting press.

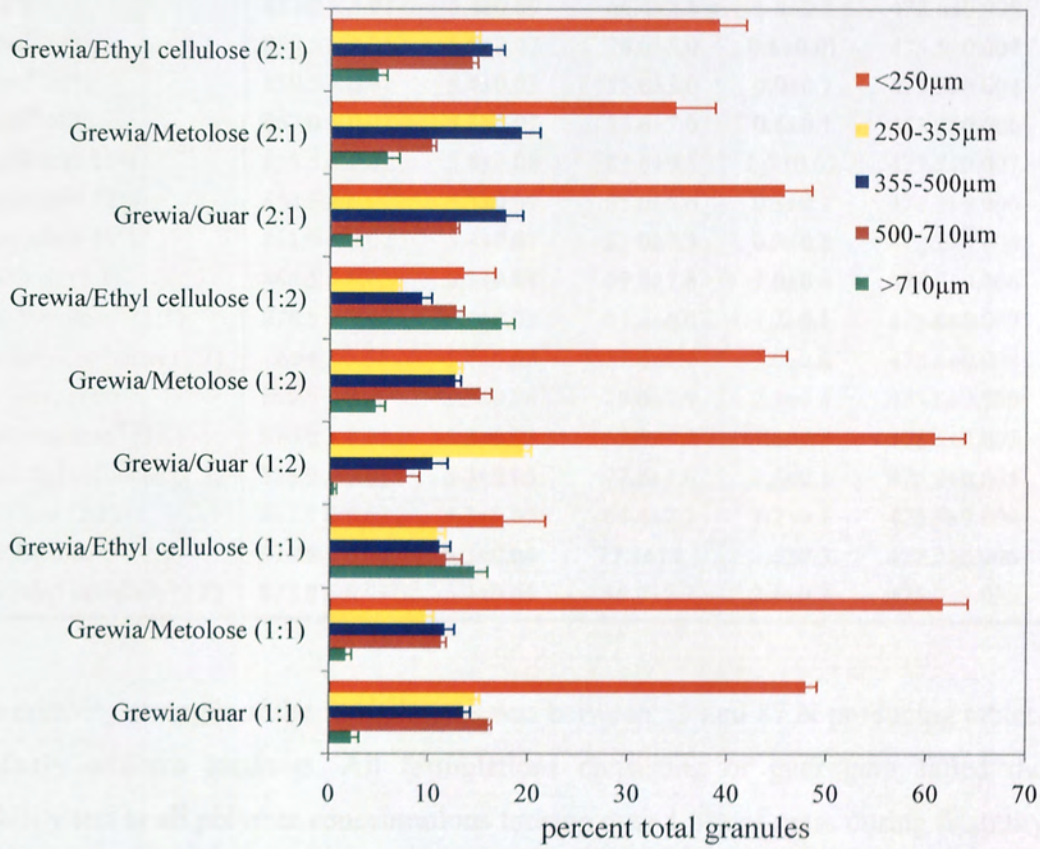


Figure 7.2: Particle-size distribution of granules for binary composite matrices of ibuprofen (n=3, mean ± s.d.)

7.3.3 Tablet properties

7.3.3.1 Ibuprofen tablet properties

The physical properties of the monolithic and binary composite matrices of ibuprofen were evaluated (see section 2.2.1) and the results are shown in table 7.7.

According to the B. P. (2010) and other texts, not more than two tablets of individual weights should deviate from the average weight or mean by 5% for tablet weighing 250mg or more. All formulations passed the uniformity of weight test with no single tablet deviating from the mean (n = 20) weight by >5%.

Table 7.7: Properties of ibuprofen monolithic and binary composite matrices (n = 3, mean ± s.d. except where stated otherwise).

Formulation	*Weight (mg) (n=20)*	*Thickness (mm)(n=10)*	*Crushing strength(N)*	*Friability (%)*	*Drug content (mg)*
Grewia 16%	911.5 ± 0.01	5.6±0.07	58.8±6.9	1.1±0.03	480.3±0.005
Grewia 32%	892.5 ± 0.01	5.5±0.04	59.8±3.8	0.9±0.1	480.9±0.007
Grewia 48%	896.0 ± 0.01	5.4±0.04	69.2±6.1	0.8±0.1	482.1±0.004
Guar 16%	859.5 ± 0.01	5.3±0.05	57.4±4.4	2.0±0.8	474.6±0.004
Guar 32%	867.5 ± 0.01	5.2±0.09	62.4±6.4	1.6±0.5	475.2±0.006
Guar 48%	857.0 ± 0.01	5.4±0.09	66.4±2.6	1.8±0.1	472.6±0.005
Metolose® 16%	838.5 ± 0.01	5.3±0.07	76.0±5.0	0.8±0.01	475.5±0.004
Metolose® 32%	859.5 ± 0.01	5.4±0.05	55.6±3.0	0.9±0.2	475.5±0.004
Metolose® 48%	867.0 ± 0.01	5.3±0.07	83.6±7.0	0.6±0.1	471.2±0.006
Ethyl cellulose 16%	859.5 ± 0.01	5.4±0.08	82.6±9.5	0.7±0.02	477.2±0.007
Ethyl cellulose 32%	854.5 ± 0.01	5.3±0.09	85.0±6.6	0.3±0.1	470.2±0.006
Ethyl cellulose 48%	861.0 ± 0.02	5.4±0.07	87.0±7.3	0.3±0.1	472.2±0.004
Grewia/Guar (1:1)	863.5 ± 0.02	5.3±0.04	69.8±7.8	1.9±0.4	475.7±0.006
Grewia/ Metolose® (1:1)	879.5 ± 0.02	5.4±0.03	81.2±6.6	1.2±0.1	475.6±0.007
Grewia/Ethyl cellulose (1:1)	860.0 ±0.01	5.4±0.05	87.0±5.1	7.0±0.8	475.6±0.008
Grewia/Guar (1:2)	860.5 ± 0.01	5.3±0.06	79.6±7.9	2.5±0.4	475.6±0.009
Grewia/ Metolose® (1:2)	870.0 ± 0.01	5.3±0.03	62.6±4.3	1.8±0.5	475.6±0.005
Grewia/Ethyl cellulose (1:2)	848.0 ± 0.01	5.3±0.05	77.8±7.6	2.6±0.1	475.9±0.005
Grewia/Guar (2:1)	867.5 ± 0.02	5.3±0.05	64.4±2.3	1.2±0.1	475.9±0.006
Grewia/ Metolose® (2:1)	871.5 ± 0.01	5.3±0.04	77.4±14.1	1.6±0.3	472.2±0.006
Grewia/Ethyl cellulose (2:1)	872.0 ± 0.01	5.3±0.05	56.2±2.7	2.4±0.2	475.7±0.005

The crushing strength of the formulations was between 55 and 87 N producing tablets of fairly uniform hardness. All formulations consisting of guar gum failed the friability test at all polymer concentrations loosing over 1.0% of mass during friability testing. Monolithic matrix tablets of grewia (16%) and all binary composite tablets also failed friability test. The highest friability value of 6.97 ± 0.83 % was demonstrated by formulations containing grewia/ethyl cellulose (1:1). This may be due to the tablet defect of capping which was observed with the binary composite as shown in figure 7.3. There are several causes of capping in tablets which include insufficient binder, too much fines in the granules, poor formulation and bad processing, high speed of compression, but more specific to this case may be air entrapment during compression and the hydrophobic nature of ethyl cellulose. The hydrophobic nature of ethyl cellulose also means that insufficient moisture is available to help particles lock together.

The disintegration time test showed that all monolithic and binary composite matrix tablets were non-disintegrating for >120 minutes apart from guar gum monolithic matrices and grewia/guar (1:2) binary composite matrices which disintegrated in <15 minutes. This may be attributable to poor compressibility which results in tablets of relatively low crushing strength, in addition to the rapid hydration of guar gum. When tablets disintegrate rapidly to expose the API to dissolution, desirable sustained release of medicament is compromised. Guar gum has good disintegrant properties and has been used as disintegrant in pharmaceuticals (Krishnaiah *et al.*, 2002) and accounts for the rapid distegaration of guar-containing matrices.

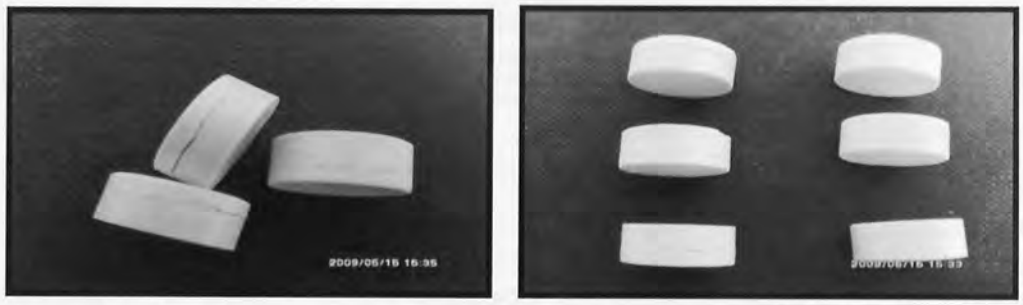


Figure 7.3: Tablet defect seen on binary composite matrices of grewia/ethyl cellulose (1:1 and 1:2)

7.3.3.2 Cimetidine tablet properties

The properties of the directly compressed cimetidine tablet formulations are shown in table 7.8.

The thickness of a tablet is often directly related to tablet hardness and can be used as an initial control of this parameter. During the routine manufacture of tablets, changes in thickness can often indicate problems in flow through the feeder or faulty deposition of powder in the die. Tablets which are too thin, are liable to break easily and those which are too thick, may give difficulty in swallowing. Manufacturers set limits on the thickness of the tablets of various products in order to assure trouble-free packaging. Tablets that are of inconsistent thickness block the channels of counting machinery. When the tablets are thicker than normal, the container may be inadequate to hold the required count.

The mean weights for all the directly compressed cimetidine formulations are also shown in table 7.8. All the tablets passed the test for weight uniformity. If there is variation in the addition and weighing of tablet excipients, it could result in weight

variation of the tablets. Variations in tablet thickness and diameter could also result in variation in weight of tablets. If the diameter and thickness are both defined, the volume and weight of tablets are obviously fixed.

The content uniformity test is used to ensure that every tablet contains the amount of drug substance intended with little variation among tablets within a batch. The monographs set acceptable limits of variation within a batch. The drug content and content uniformity of a batch will depend to a large extent on variations in tablet weight, thickness or diameter. Tablet monographs with a content uniformity requirement do not have weight variation requirements.

Table 7.8: Properties of cimetidine matrix tablets (n = 3, mean ± s.d.) except where stated otherwise.

Formulation	*Weight (mg) (n=20)*	*Thickness (mm) (n=10)*	*Mechanical strength (N)*	*Friability (%)*	*Drug content (mg)*
Grewia	531.0±0.01	3.6±0.04	84.2±1.5	0.7±0.3	253.1±0.004
Gum Arabic	565.0±0.01	3.7±0.13	72.2±2.3	7.6±7.5	269.2±0.003
Metolose®	541.0±0.01	3.6±0.05	81.0±3.7	0.8±0.1	256.3±0.002
Ethyl cellulose	544.0±0.01	3.7±0.05	91.0±1.9	0.7±0.3	249.7±0.002
Methocel®	569.5±0.01	3.7±0.04	74.6±2.8	0.8±0.2	247.8±0.003
CMC	520.5±0.02	3.7±0.04	66.2±3.6	1.9±0.6	250.3±0.002
Grewia/Gum arabic (1:1)	520.5±0.01	3.4±0.05	84.2±2.9	0.6±0.4	252.5±0.002
Grewia/ Metolose® (1:1)	520.5±0.01	3.6±0.03	72.8±5.1	0.6±0.6	251.7±0.002
Grewia/Ethyl cellulose (1:1)	517.0±0.02	3.6±0.05	88.6±8.2	0.6±0.6	253.5±0.003
Grewia/Methocel®(1:1)	534.0±0.01	3.4±0.05	81.4±5.9	0.5±0.5	249.2±0.005
Grewia/CMC (1:1)	518.5±0.01	3.5±0.05	74.6±2.7	0.5±0.5	251.4±0.002

The mean mechanical strength of the formulations ranged between 66.2 and 91.0 N. Hardness is associated with other properties such as density and porosity, all of which affect the disintegration behaviour. Hardness generally shows a tendency to increase with normal storage of tablets. The degree of hardness of tablets depends on its physical size and shape together with the characteristics of the components that go into the formulation and the pressure applied during compression. If the tablet is too soft, it may not produce sustained release action or withstand the necessary multiple shocks occurring during handling, shipping and dispensing. The cimetidine tablets possess reasonable hardness to withstand multiple shocks associated with shipment, handling and dispensing.

Formulations containing gum Arabic or CMC failed the friability test. In the case of the cimetidine formulations containing gum Arabic, tablet friability was very high (7.58±7.53 %). This can be explained to be as a result of the capping and lamination observed after compression of the formulations containing gum Arabic (figure 7.4) or chipping in the case of formulations containing CMC as polymer matrix. A number of reasons have been advanced for capping, lamination and chipping; insufficiency in binder cohesiveness, presence of entrapped air, presence of excess fines of powders in the case of granules, pressure and speed of compression, moist and soft granulation, worn and imperfect dies, and punches (Cooper and Gunns, 1965).

Figure 7.4: Capping and lamination on gum Arabic matrix tablets of cimetidine

Although disintegration tests are not usually a recommendation for sustained release formulations, it was studied to ascertain that the tablets are non-disintegrating under the conditions required. The results showed that all the matrix formulations did not disintegrate within 120 minutes. The concentration of the binder (in this case, the polymer matrix) is a factor that affects the disintegration of tablets. If the binder concentration is high, it will increase the hardness of the tablets and consequently the disintegration time is increased. Matrix formulations are intended to give sustained release of medicaments and usually contain high concentrations of binder with no disintegrants. Disintegrants are added to oppose the efficiency of the tablet binder and the physical forces that act under compression to form the mechanical body of the tablet. The stronger the effect of the binder, the more efficient must be the action of the disintegrant or the tablet may not release its active ingredients at the proper time and place (Gunsel and Kanig, 1976). The resultant effect is a sustained release action.

Table 7.8 also summarises the drug content of the matrix formulations. All formulations pass the content uniformity test as stipulated in the B.P. (2010).

Variations in the percentage of medicaments might occur for various reasons (Cooper and Gunn, 1965) which include: limits of accuracy in preparing granules, variations in the weight of the tablets, permitted variation in the purity of the drug and errors of 'random sampling'. The official limits are wider if fewer than 20 tablets are used in the tests. These variations are however allowed when the stated limits are between 90 and 110 percent (Cooper and Gunns, 1965).

7.3.4 *In vitro* release studies

7.3.4.1 *In vitro* release of ibuprofen from tablets

Effect of polymer type on ibuprofen release from monolithic matrices

The release of ibuprofen from the matrix was studied in 900 ml of phosphate buffer (pH 7.2) using the USP II (paddle method) equipped with a 40 mesh sinker. The dissolution test was performed at 100 rpm and the temperature was 37 ± 1°C (Also see section 2.17) over 24 hour period and the results are presented. Figures 7.5-7.7 represent the drug release profile for ibuprofen from monolithic matrix tablets containing 16, 32 or 48% w/w of grewia, guar, Metolose® or ethyl cellulose.

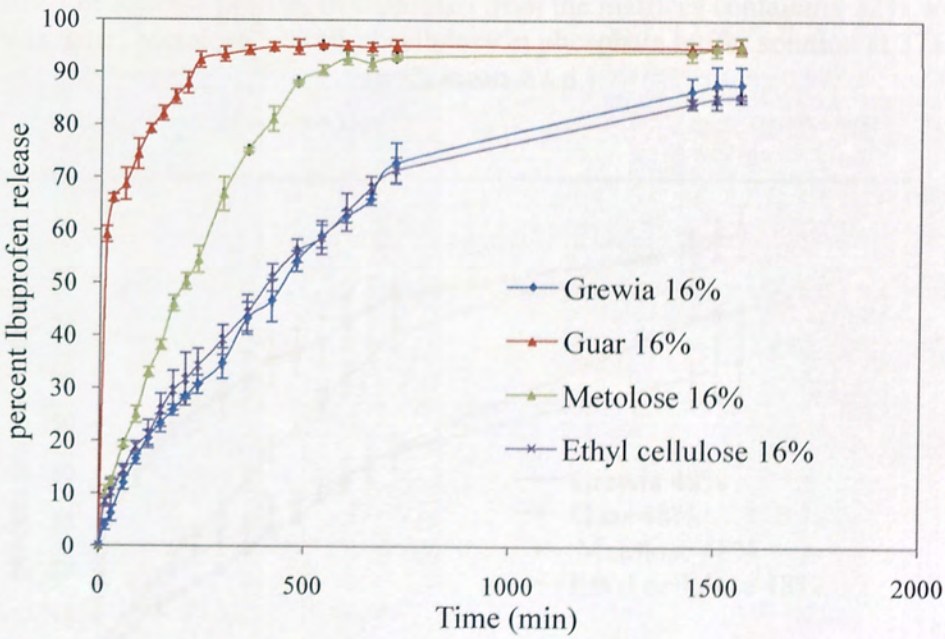


Figure 7.5: Release profiles of ibuprofen from the matrices containing 16% w/w of grewia, guar, Metolose® or ethyl cellulose in phosphate buffer solution at 37± 1°C (n=3, mean ± s.d.)

As figures 7.5-7.7 indicate, there was an initial burst effect seen with guar gum matrices which is absent from grewia, Metolose® and ethyl cellulose matrices. Table

7.9 shows the time for 50% drug released (t_{50}) and the drug released after 24 hours for the monolithic matrices.

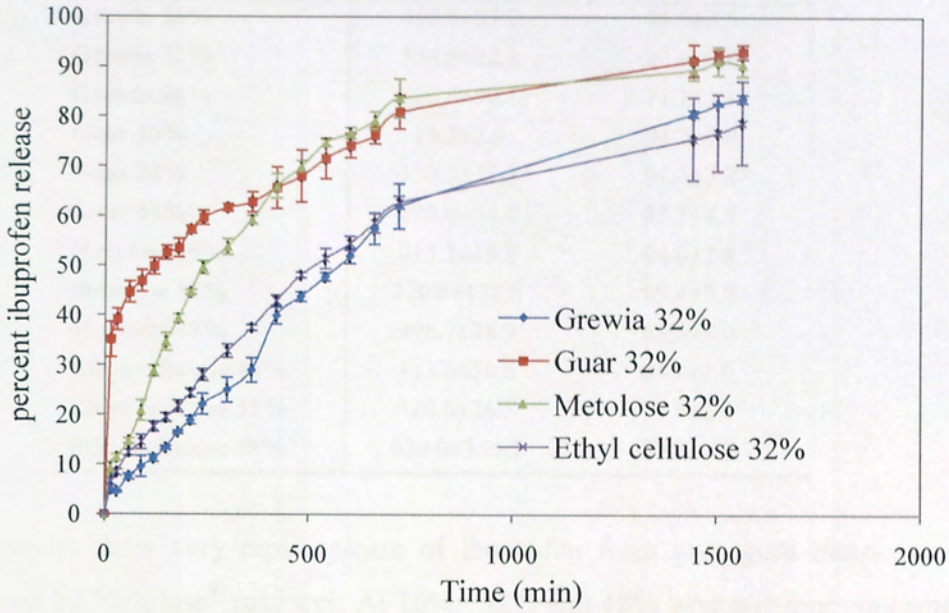


Figure 7.6: Release profiles of ibuprofen from the matrices containing 32% w/w of grewia, guar, Metolose®, or ethyl cellulose in phosphate buffer solution at 37± 1°C (n=3, mean ± s.d.)

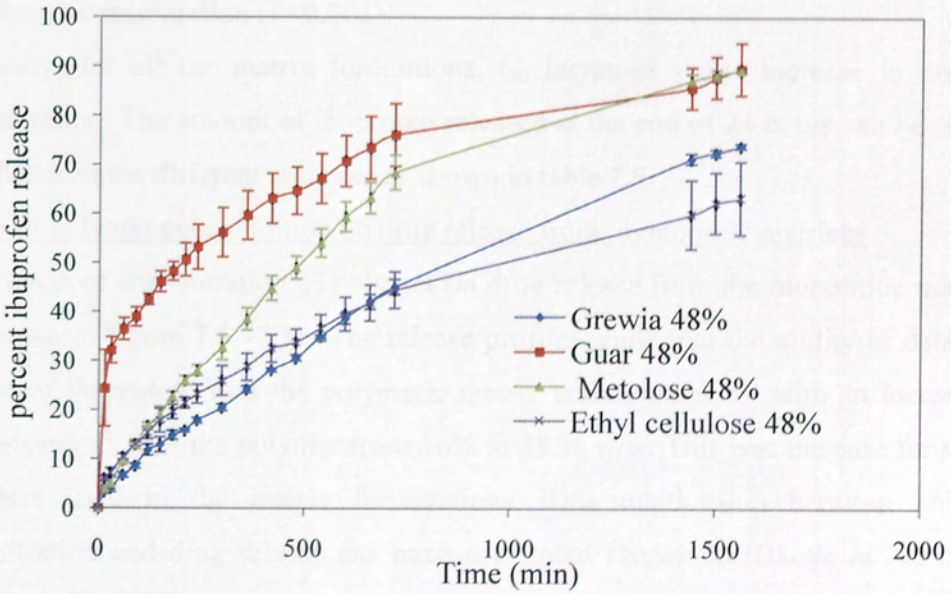


Figure 7.7: Release profiles of ibuprofen from the matrices containing 48% w/w of grewia, guar, Metolose®, or ethyl cellulose in phosphate buffer solution at 37± 1°C (n=3, ± s.d.)

Table 7.9: Time for 50% ibuprofen release (t_{50}) and % ibuprofen release from monolithic matrices after 24 hours in phosphate buffer (pH 7.2), (n=3, mean±s.d.)

Formulation	*t_{50} (min)*	*% release (24h)*
Grewia 16%	440.0±21.2	86.6±2.5
Grewia 32%	574.6±22.1	80.6±0.8
Grewia 48%	806.6±90.1	71.1±1.1
Guar 16%	13.3±2.9	94.7±1.0
Guar 32%	130.0±17.3	91.3±3.2
Guar 48%	220.0±34.6	85.5±4.2
Metolose 16%	213.3±15.3	94.0±1.8
Metolose 32%	320.0±138.6	89.4±3.9
Metolose 48%	496.7±28.9	68.3±2.0
Ethyl cellulose 16%	413.3±30.6	84.2±1.0
Ethyl cellulose 32%	520.0±26.5	75.6±8.3
Ethyl cellulose 48%	920.0±346.3	59.8±7.0

The results show very rapid release of ibuprofen from guar gum matrices closely followed by Metolose® matrices. At 16%, 32% and 48% w/w polymer concentration, there was no significant diference ($P>0.05$) in the t_{50} between grewia polysaccharide gum and ethyl cellulose, both matrices providing a more sustained release of ibuprofen than Metolose® ($P<0.001$) at 48% w/w polymer concentration or guar gum at all levels of polymer concentration ($P<0.001$).

Generally, for all the matrix formultions, t_{50} increases with increase in polymer concentration. The amount of ibuprofen released at the end of 24 hours can be seen to vary between the different matrices as shown in table 7.9.

Effect of polymer concentration on drug release from monolithic matrices

The effects of concentration of polymer on drug release from the monolithic matrices are shown in figure 7.8 -7.11. The release profiles show that the ability to delay the release of ibuprofen from the polymeric matrix tablets increases with an increase in the concentration of the polymer from 16% to 48 % w/w. This was the case for all the polymers used in the matrix formulations. This relationship between polymer concentration and drug release has been confirmed elsewhere (Okoye *et al.*, 2009). The very good disintegrant property of guar gum (Krishnaiah *et al.*, 2002*a*) coupled with the relatively lower crushing strength of guar-containing matrices and high friability values account for the fast disintegration of the tablets. This will in turn result in fast dissolution rates. Consequently, increasing the concentration of guar

from 32% to 48% w/w did not result in any significant increase in the sustained release of the API (table 7.9).

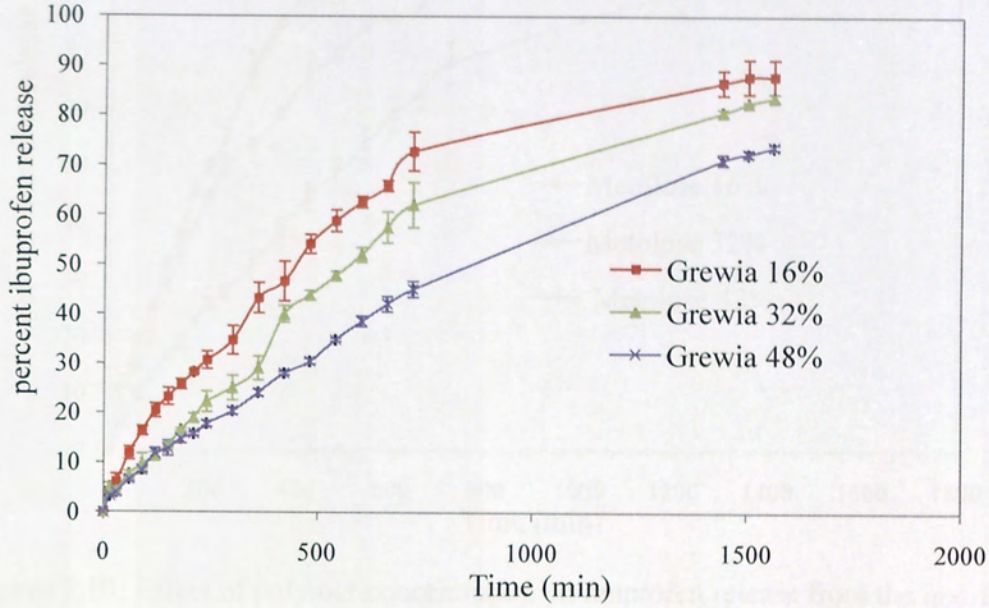


Figure 7.8: Effect of polymer concentration on ibuprofen release from the matrices containing 16, 32 or 48% w/w of grewia polysaccharide gum in phosphate buffer solution at 37± 1°C (n=3, mean ± s.d..)

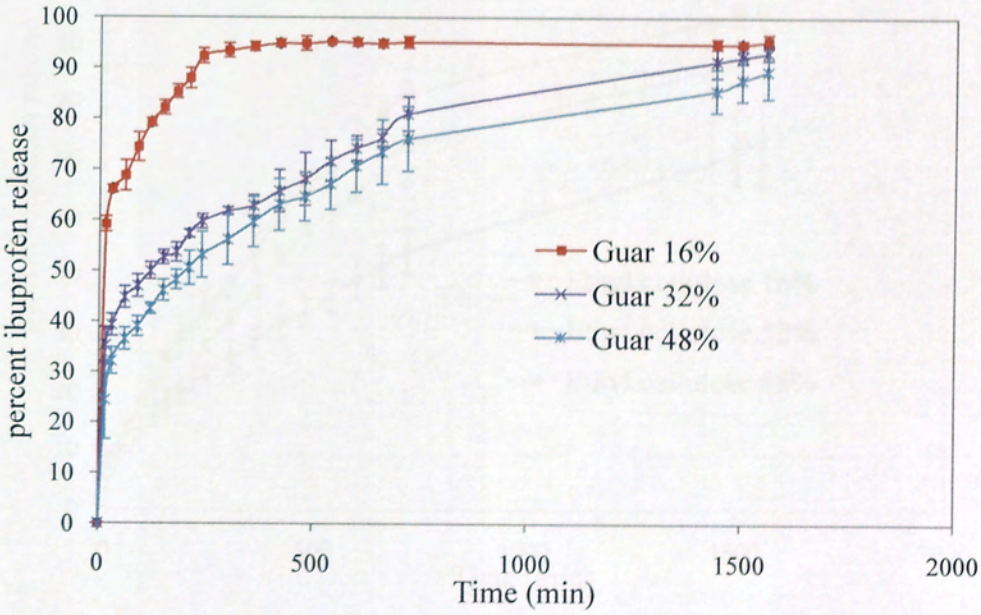


Figure 7.9: Effect of polymer concentration on ibuprofen release from the matrices containing 16, 32 or 48% w/w of guar gum in phosphate buffer solution at 37± 1°C (n=3, mean ± s.d.)

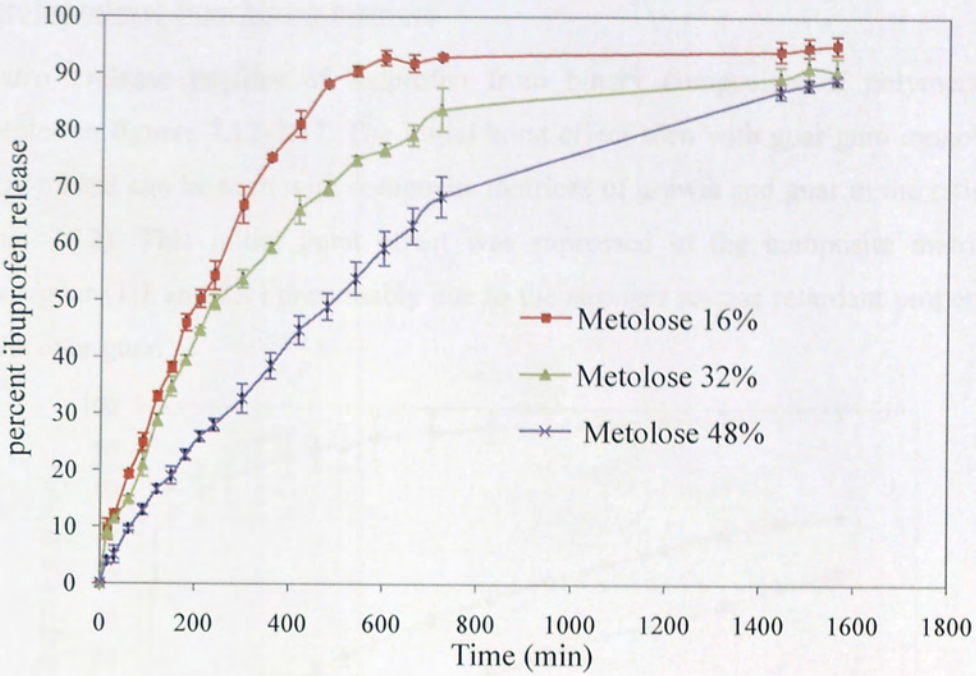


Figure 7.10: Effect of polymer concentration on ibuprofen release from the matrices containing 16, 32 or 48% w/w of Metolose® in phosphate buffer solution at 37± 1°C (n=3, mean ± s.d.)

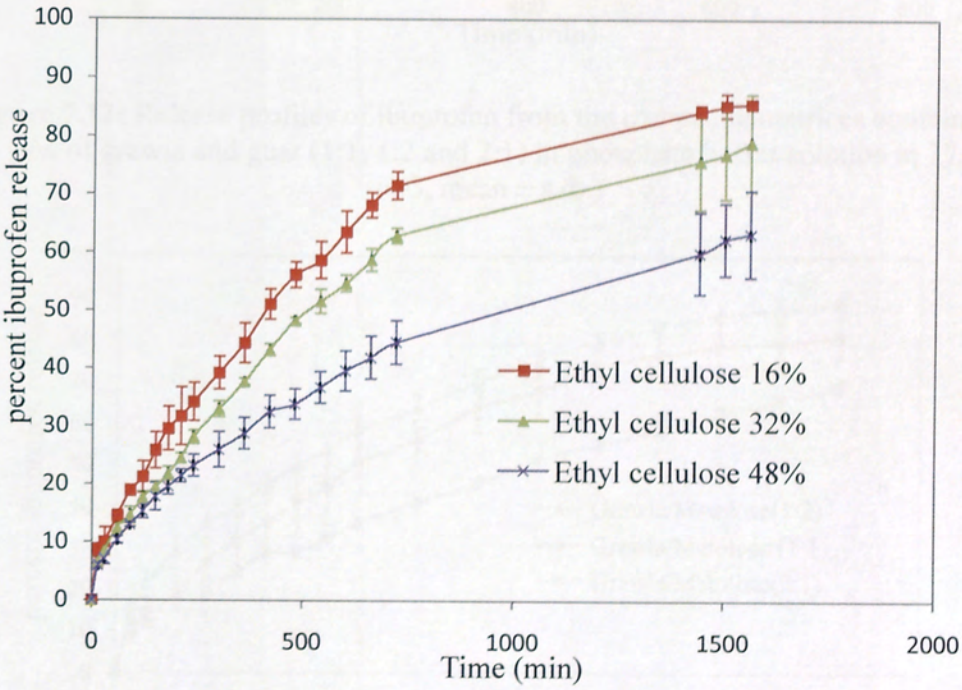


Figure 7.11: Effect of polymer concentration on ibuprofen release from the matrices containing 16, 32 or 48% w/w of ethyl cellulose in phosphate buffer solution at 37± 1°C (n=3, mean ± s.d.)

Ibuprofen release from binary matrices

In vitro release profiles of ibuprofen from binary composites of polymers are presented in figures 7.12-7.17. The initial burst effect seen with guar gum monolithic matrix tablets can be seen with composite matrices of grewia and guar in the ratio 1:2 (figure 7.12). This initial burst effect was supressed in the composite matrix of grewia/guar (1:1 and 2:1) presumably due to the stronger release retardant property of grewia over guar.

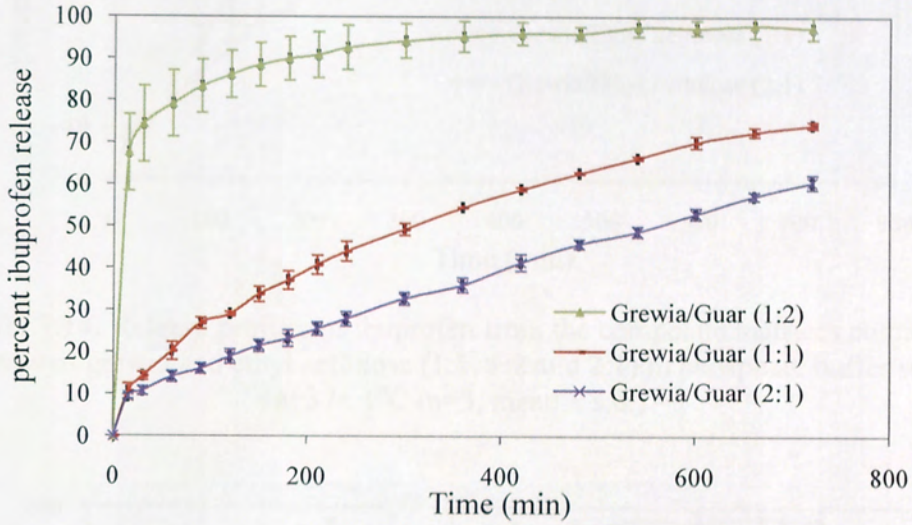


Figure 7.12: Release profiles of ibuprofen from the composite matrices containing 16% w/w of grewia and guar (1:1, 1:2 and 2:1) in phosphate buffer solution at 37± 1°C (n=3, mean ± s.d..)

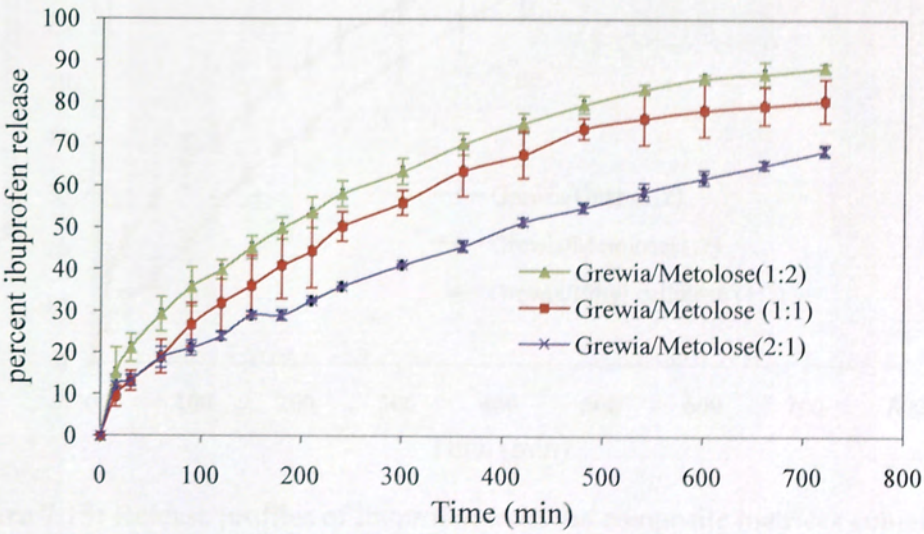


Figure 7.13: Release profiles of ibuprofen from the composite matrices containing 16% w/w grewia and Metolose® (1:1, 1:2 and 2:1) in phosphate buffer solution at 37± 1°C (n=3, mean ± s.d..)

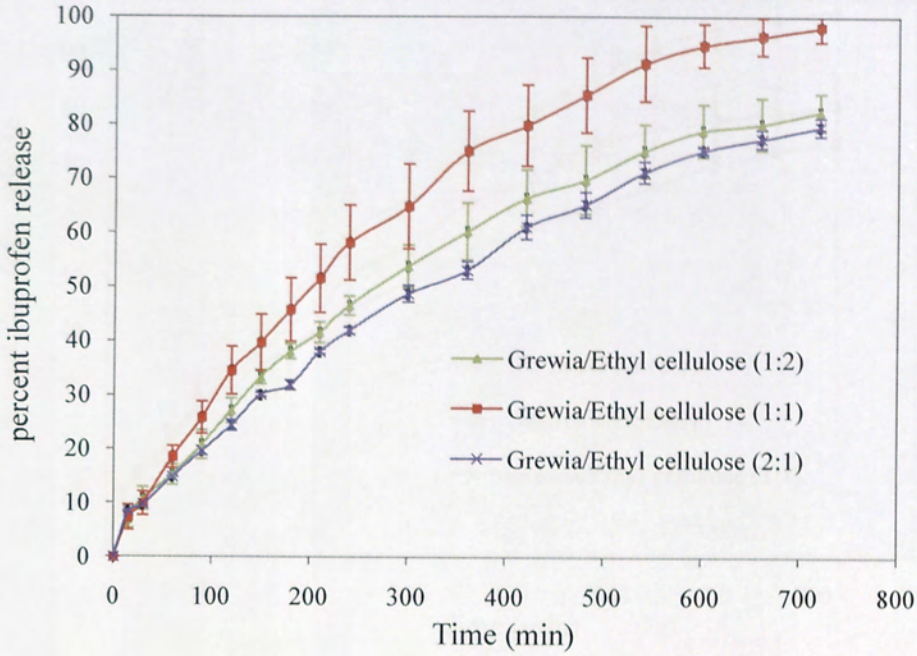


Figure 7.14: Release profiles of ibuprofen from the composite matrices containing 16% w/w of grewia and ethyl cellulose (1:1, 1:2 and 2:1) in phosphate buffer solution at 37± 1°C (n=3, mean ± s.d.)

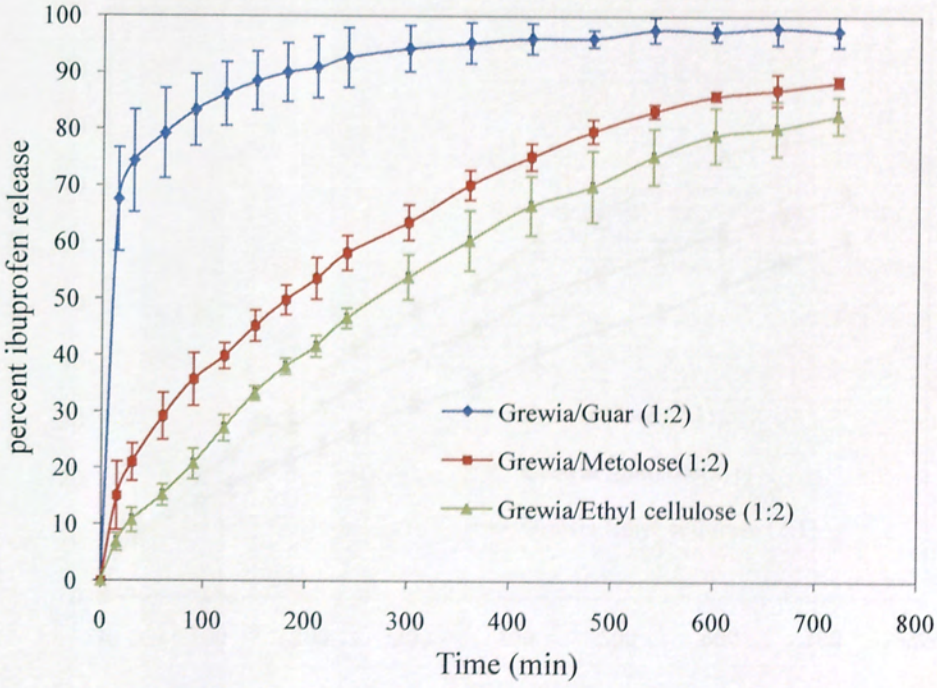


Figure 7.15: Release profiles of ibuprofen from the composite matrices containing 16% w/w of grewia and guar, Metolose®, or ethyl cellulose (1:2) in phosphate buffer solution at 37± 1°C (n=3, mean ± s.d.)

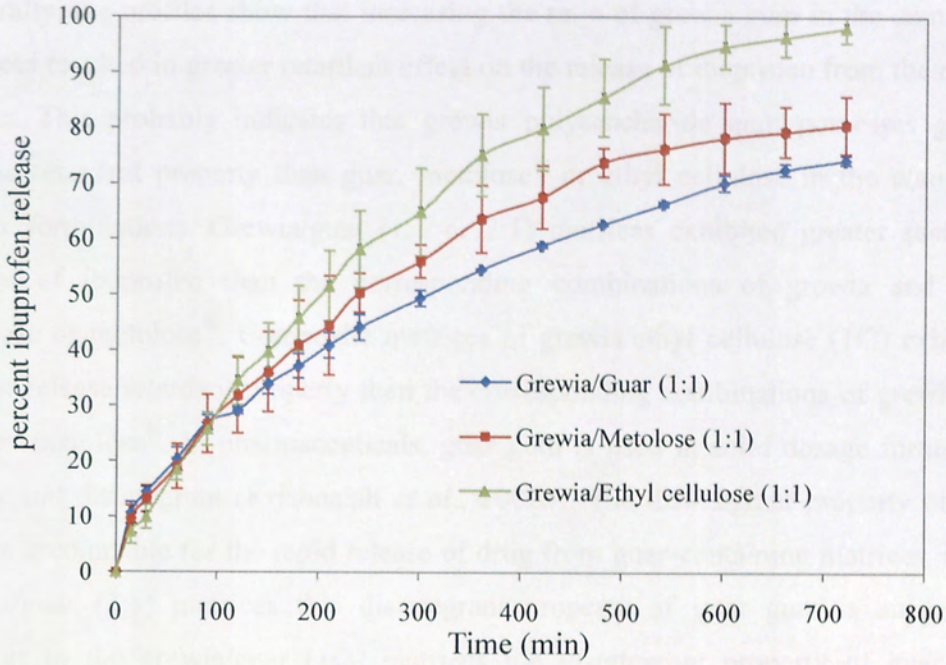


Figure 7.16: Release profiles of ibuprofen from the composite matrices containing 16% w/w of grewia and guar, Metolose®, or ethyl cellulose (1:1) in phosphate buffer solution at 37± 1°C (n=3, mean ± s.d.)

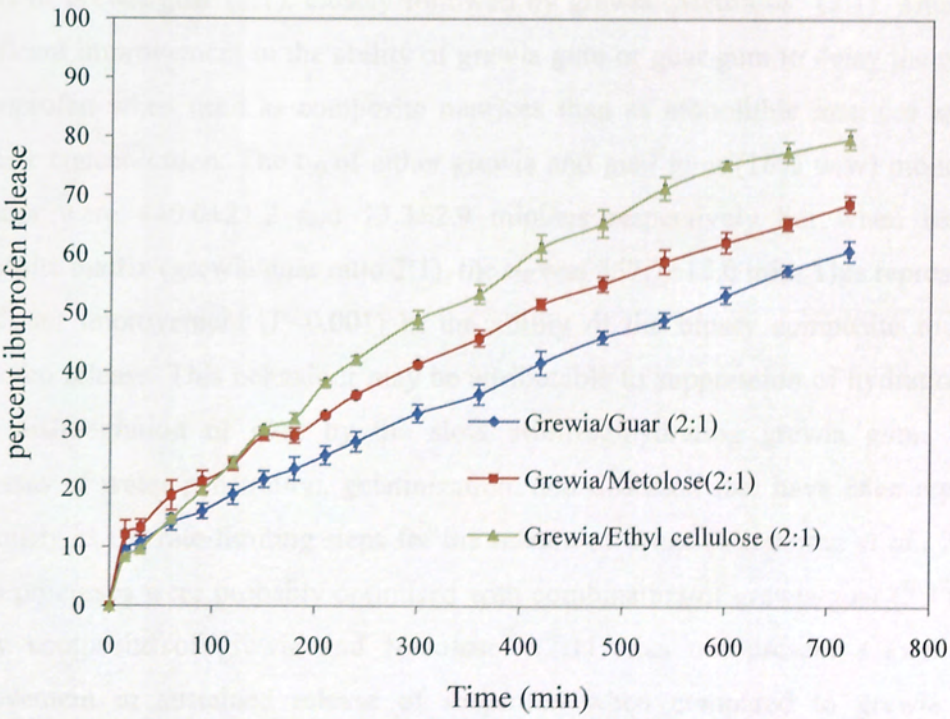


Figure 7.17: Release profiles of ibuprofen from the composite matrices containing 16% w/w of grewia and guar, Metolose®, or ethyl cellulose (2:1) in phosphate buffer solution at 37± 1°C (n=3, mean ± s.d.)

Generally, the profiles show that increasing the ratio of grewia gum in the composite matrices resulted in greater retardant effect on the release of ibuprofen from the matrix tablets. This probably indicates that grewia polysaccharide gum possesses greater release retardant property than guar, metolose® or ethyl cellulose in the composite matrix formulations. Grewia/guar (1:1 or 2:1) matrices exhibited greater sustained release of ibuprofen than the corresponding combinations of grewia and ethyl cellulose or metolose®. Composite matrices of grewia/ethyl cellulose (1:2) exhibited greater release retardant property than the corresponding combinations of grewia and guar or metolose®. In pharmaceuticals, guar gum is used in solid dosage forms as a binder and disintegrant (Krishnaiah *et al.*, 2002*a*). The disintegrant property of guar gum is accountable for the rapid release of drug from guar-containing matrices. In the grewia/guar (2:1) matrices this disintegrant property of guar gum is suppressed whereas in the grewia/guar (1:2) matrices the disintegrant property of guar gum predominates with consequent rapid release of drug.

The time for 50% ibuprofen release from composite matrices is shown in table 7.10. The result shows that delay in release of ibuprofen was highest for composite matrix tablets of grewia/guar (2:1), closely followed by grewia/ Metolose® (2:1). There is a significant improvement in the ability of grewia gum or guar gum to delay the release of ibuprofen when used as composite matrices than as monolithic matrices at 16% polymer concentration. The t_{50} of either grewia and guar gum (16% w/w) monolithic matrices were 440.0±21.2 and 13.3±2.9 minutes respectively but when used as composite matrix (grewia/guar ratio 2:1), the t_{50} was 557.7±18.0 min. This represents a significant improvement ($P<0.001$) in the ability of the binary composite to delay ibuprofen release. This behaviour may be attributable to suppression of hydration and rapid disintegration of guar by the slow swelling/hydrating grewia gum. Three processes of water penetration, gelatinization, and diffusion rate have been reported previously as the rate-limiting steps for the release of drugs (Varshosaz *et al.*, 2006). These processes were probably optimised with combination of grewia/guar (2:1). The binary composite of grewia and Metolose® (2:1) does not present a significant improvement in sustained release of ibuprofen when compared to grewia 16% monolithic matrices ($P>0.05$). This was however significant when compared with guar 16% monolithic matrix tablets ($P<0.001$). All other binary composites of the polymer

matrix tablets of ibuprofen gave t_{50} values which are less than the corresponding monolithic polymer (16%) matrix tablets.

Table 7.10: Time for 50% ibuprofen release (t_{50}) and % ibuprofen release from binary matrices after 12 hours in phosphate buffer (pH 7.2), (n=3, mean±s.d.)

Formulation	t_{50} (min)	*% release (12 h)*
Grewia/Guar (1:2)	13.3±1.5	97.3±2.8
Grewia/Guar (1:1)	306.0±15.1	74.3±0.8
Grewia/Guar (2:1)	557.7±18.0	60.6±1.8
Grewia/Metolose (1:2)	185.7±23.1	88.5±0.9
Grewia/Metolose (1:1)	235.3±35.6	80.6±5.1
Grewia/Metolose (2:1)	405.0±8.7	68.6±1.4
Grewia/Ethyl cellulose (1:2)	273.3±25.2	82.5±3.3
Grewia/Ethyl cellulose (1:1)	206.7±35.1	98.0±2.7
Grewia/Ethyl cellulose (2:1)	316.0±14.2	79.6±1.7

The amount of ibuprofen released after 12 hours is shown in table 7.10. Approximately 100% ibuprofen release was seen with grewia/guar composites (1:2) and grewia/ethyl cellulose composites (1:1). Relatively varying amounts of ibuprofen were released by the different binary composite matrix tablets at the end of 12 hours of ibuprofen release.

It has been reported that gastric mean residence time of a controlled release ibuprofen formulation is in excess of 9 ± 3 hours and is dependent upon the meal size (Washington *et al.*, 2000). This sugest that with a large meal the ibuprofen matrix tablets will release a higher percentage of the drug in the stomach. However, it will be expected that all the matrix formulations of ibuprofen may not release all of the drug before reaching the large intestine as small intestinal transit is around 4 hours irrespective of physical state, size or presence of food (Washington *et al.*, 2000). Studies have suggested that if matrix tablets are designed to release their contents over 12 hours then colonic absorption of the drug is necessary and the drug must not be degraded by colonic bacteria (Washington *et al.*, 2000).

7.3.4.2 *In vitro* release of cimetidine from tablets

The profiles of drug release from the monolithic matrices and binary composite matrices of cimetidine are shown in figure 7.18 and 7.19 respectively. The release profiles show a more sustained release effect with monolithic matrices of ethyl

cellulose. The monolithic matrices of CMC showed the least delay in release of cimetidine. An initial burst effect could be seen for Methocel®, Metolose®, CMC and gum Arabic matrices. The initial burst effect has been attributed to the time taken for the formation of an efficient gel layer (Colombo *et al.*, 2000).

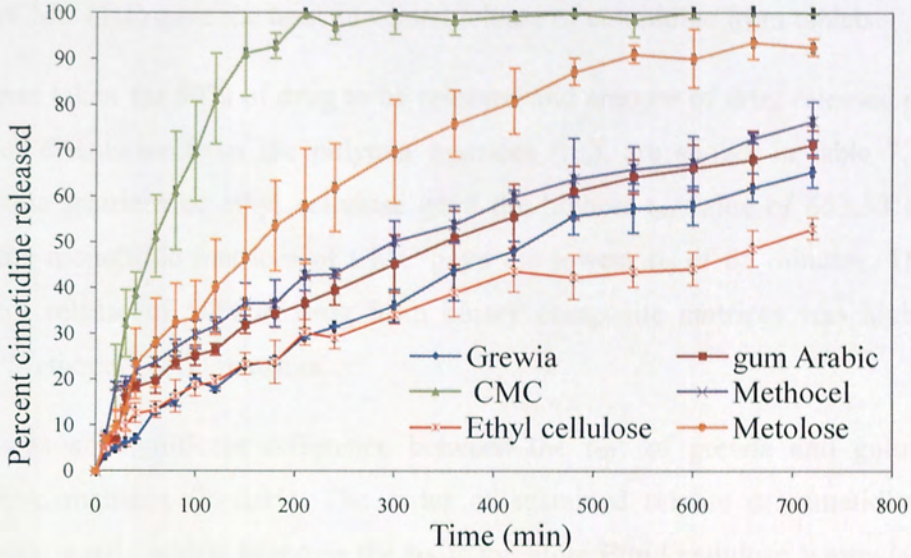


Figure 7.18: Release profiles of cimetidine from the monolithic matrices containing 40% w/w of grewia, gum arabic, CMC, Methocel®, Metolose® or ethylcellulose in phosphate buffer (pH 7.2) solution at 37± 1°C (n=3, mean ± s.d.)

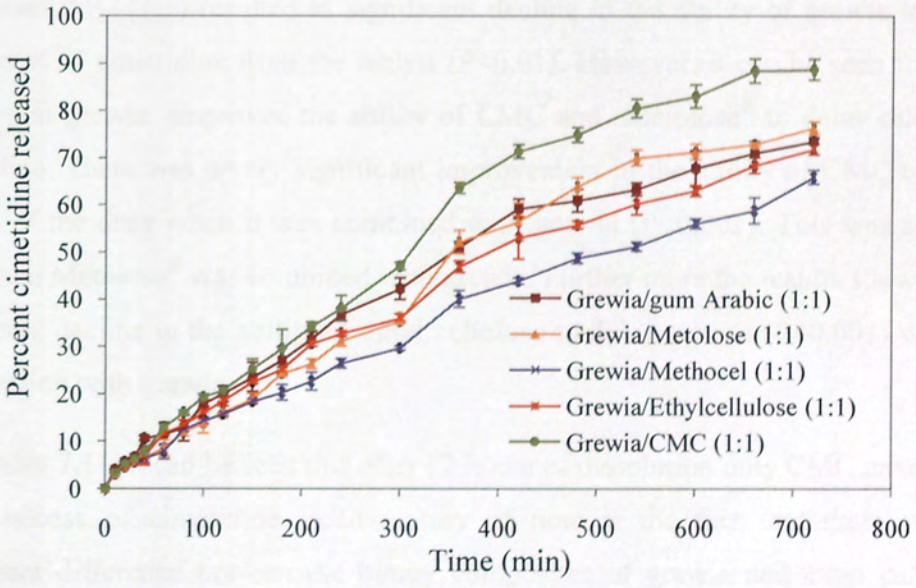


Figure 7.19: Release profiles of cimetidine from the binary composite matrices containing 40% w/w of grewia/gum Arabic (1:1), grewia/CMC (1:1), grewia/Methocel® (1:1), grewia/ Metolose® (1:1), or grewia/ethyl cellulose (1:1) in phosphate buffer (pH 7.2) solution at 37± 1°C (n=3, mean ± s.d.)

No initial burst effect was observed any of the binary matrix formulations but drug release was typically zero order for the first 399 minutes of drug release. The binary composites of grewia/Methocel® (1:1) showed a more sustained release of drug compared with the other binary composite matrices. The binary composite matrices of grewia/CMC (1:1) gave the least sustained release of cimetidine from tablets.

The times taken for 50% of drug to be released and amount of drug released after 12 hours of dissolution.from the polymer matrices (t_{50}), are shown in table 7.11 The monolithic matrices of ethyl cellulose gave the highest t_{50} value of 663.33 minutes while the monolithic matrices of CMC gave the lowest t_{50} of 65 minutes. The time taken for release of 50% of drug from binary composite matrices was highest for grewia/Methocel® (1:1) matrices.

There was no significant difference between the t_{50} of grewia and gum arabic monolithic matrices ($P>0.05$). The order of sustained release of cimetidine from monolithic matrix tablets based on the t_{50} is therefore Ethyl cellulose > grewia = gum arabic > Methocel® > Metolose® > CMC.

Combination of the reference polymers with grewia caused no significant improvement in the ability of grewia to delay release ($P>0.05$) rather binary composite of grewia/CMC (1:1) resulted in significant decline in the ability of grewia to delay the release of cimetidine from the tablets ($P<0.01$). However, it can be seen from the results that grewia improved the ability of CMC and Metolose® to delay release of cimetidine. There was a very significant improvement in the ability of CMC to delay release of the drug when it was combined with grewia ($P<0.001$). This was also the case when Metolose® was combined with grewia. Further more the results show a very significant decline in the ability of ethyl cellulose to delay release ($P<0.001$) when in combination with grewia.

From table 7.11, it can be seen that after 12 hours of dissolution only CMC gave about 100% release of cimetidine. Also worthy of note is the fact that there was no significant difference beween the binary composites of grewia and ethyl cellulose, gum arabic and Metolose® in their ability to delay release of cimetidine from tablets. However, the results show that binary composite matrices of grewia/Methocel® were

significantly superior to the other composite matrices ($P<0.05$) in their ability to sustain the release of cimetidine from tablets.

Table 7.11: Time for 50% cimetidine release (t_{50}) and % cimetidine release from monolithic or binary matrices after 12 hours in phosphate buffer (pH 7.2), (n=3, mean±s.d.)

Formulation	*t_{50}(min)*	*% release (12h)*
Grewia	465.0±69.5	65.4±3.6
gum Arabic	356.7±67.5	71.3±2.9
CMC	65.0±20.0	100.0±0.4
Methocel®	310.0±43.6	76.4±4.1
Ethyl cellulose	663.3±30.6	53.0±2.4
Metolose®	170.0±50.0	92.4±1.9
Grewia/gum Arabic(1:1)	356.7±5.8	73.5±0.7
Grewia/CMC (1:1)	316.0±2.0	88.7±2.2
Grewia/Methocel® (1:1)	521.7±20.2	65.9±1.5
Grewia/ethyl cellulose (1:1)	398.3±28.4	71.8±1.1
Grewia/Metolose® (1:1)	355.0±5.0	76.2±1.3

7.3.5 Swelling and erosion studies

7.3.5.1 Swelling and erosion of ibuprofen matrix tablets

Swelling and erosion studies (section 2.18) were carried out on the monolithic matrix tablets of ibuprofen containing 16%, 32% or 48 % w/w of grewia, guar, Metolose® or ethyl cellulose. The relationships between water uptake *versus* time and erosion *versus* time for the monolithic matrices are shown in figures 7.20-7.22 and 7.23-7.25 respectively.

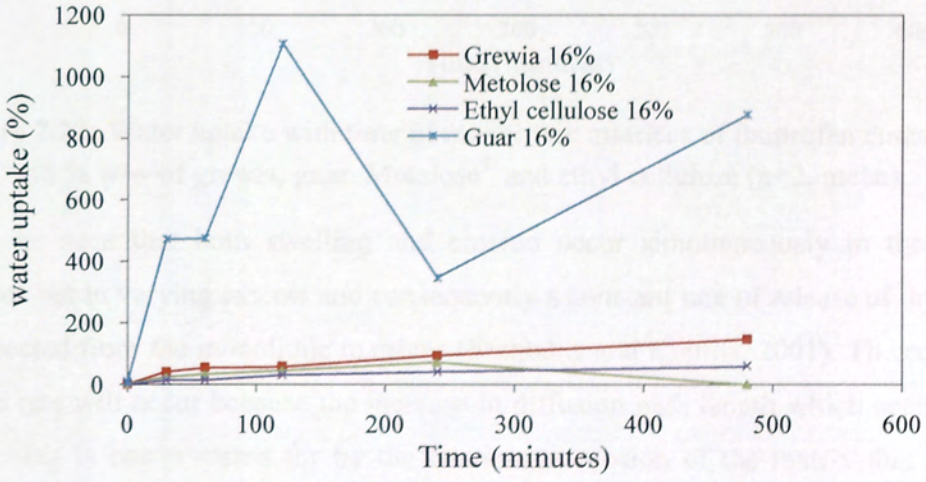


Figure 7.20: Water uptake with time of monolithic matrices of ibuprofen containing 16 % w/w of grewia, guar, Metolose® and ethyl cellulose (n=2, mean).

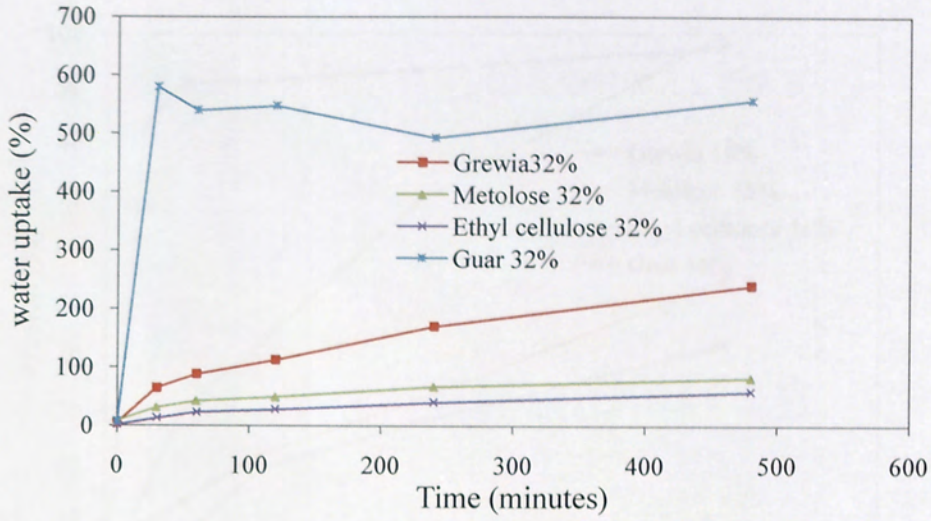


Figure 7.21: Water uptake with time of monolithic matrices of ibuprofen containing 32 % w/w of grewia, guar, Metolose® and ethyl cellulose (n=2, mean).

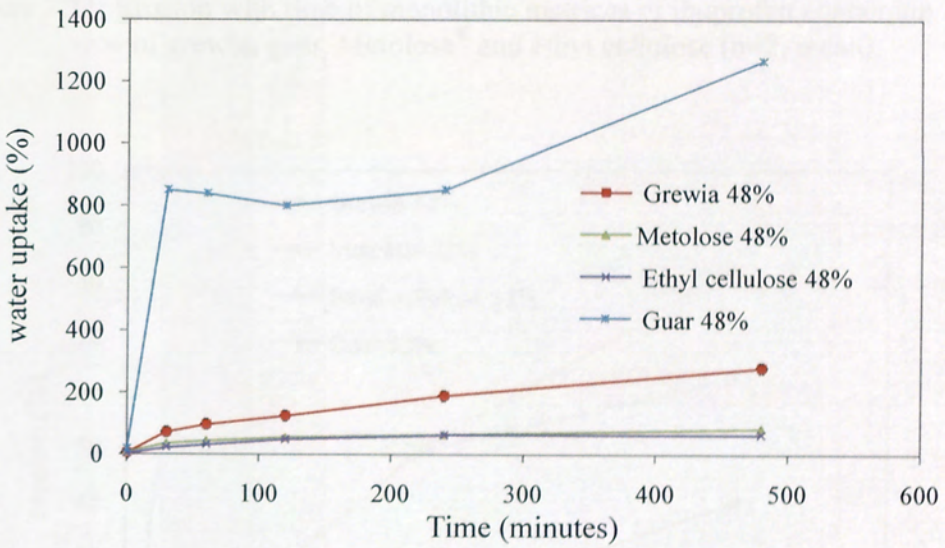


Figure 7.22: Water uptake with time of monolithic matrices of ibuprofen containing 48 % w/w of grewia, guar, Metolose® and ethyl cellulose (n=2, mean).

It can be seen that both swelling and erosion occur simultaneously in the tablet matrices but to varying extents and consequently a constant rate of release of drug can be expected from the monolithic matrices (Efentakis and Koutlis, 2001). The constant release rate will occur because the increase in diffusion path length which occurs due to swelling is compensated for by the continuous erosion of the matrix that occurs simultaneously (Mockel and Lippold, 1993).

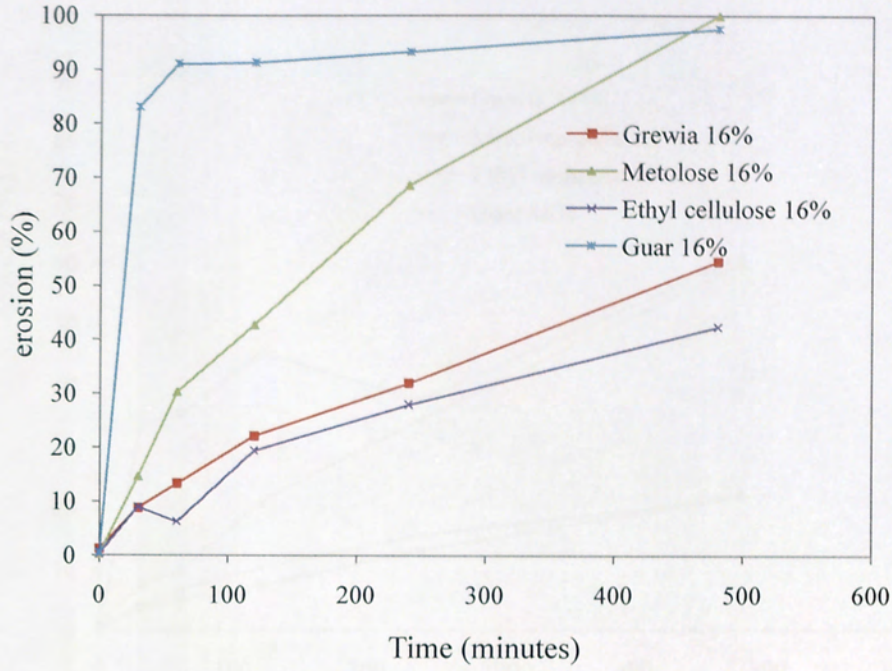


Figure 7.23: Erosion with time of monolithic matrices of ibuprofen containing 16 % w/w of grewia, guar, Metolose® and ethyl cellulose (n=2, mean).

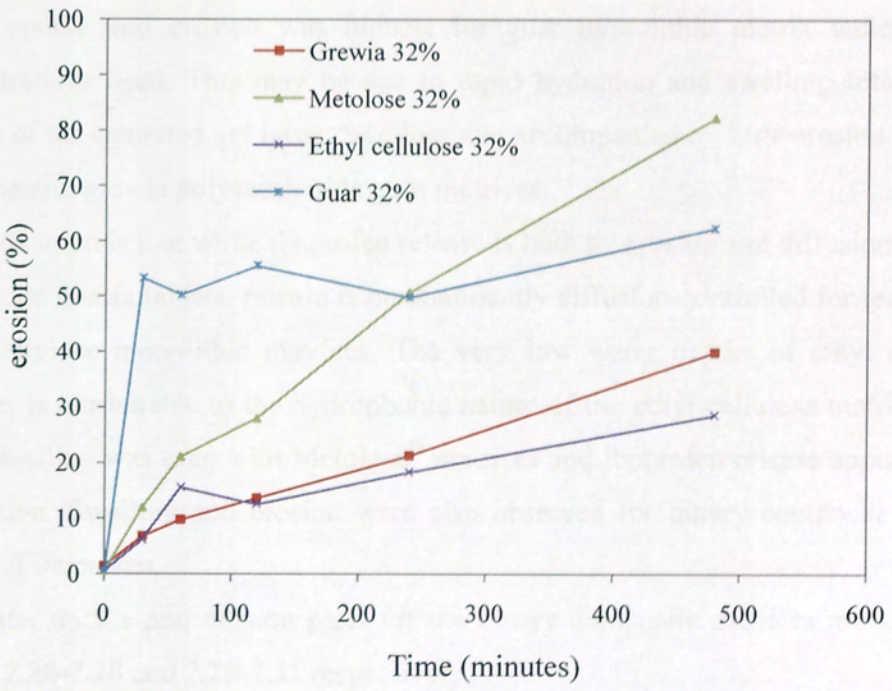


Figure 7.24: Erosion with time of monolithic matrices of ibuprofen containing 32 % w/w of grewia, guar, Metolose® and ethyl cellulose (n=2, mean).

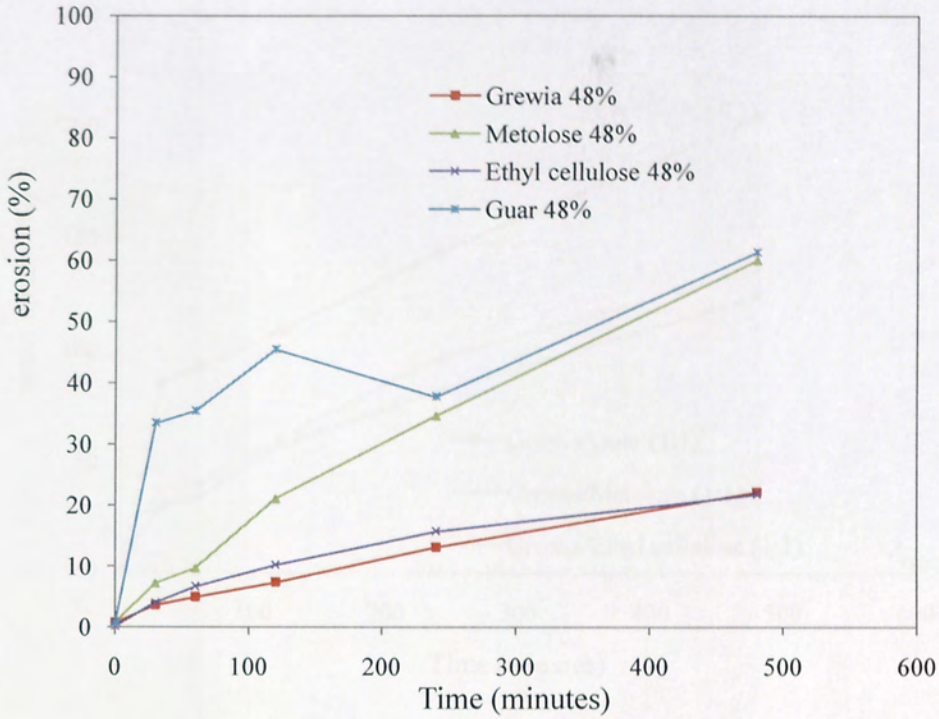


Figure 7.25: Erosion with time of monolithic matrices of ibuprofen containing 48 % w/w of grewia, guar, Metolose® and ethyl cellulose (n=2, mean).

Water uptake and erosion was highest for guar monolithic matrix tablets at all concentrations used. This may be due to rapid hydration and swelling followed by erosion of the saturated gel layer. Swelling was accompanied by little erosion for ethyl cellulose and grewia polysaccharide gum matrices.

The indication is that while ibuprofen release is both by erosion and diffusion for guar monolithic matrix tablets, release is predominantly diffusion-controlled for grewia and ethyl cellulose monolithic matrices. The very low water uptake of ethyl cellulose matrices is attributable to the hydrophobic nature of the ethyl cellulose matrices. Not much swelling was seen with Metolose® matrices and ibuprofen release appears to be by erosion. Swelling and erosion were also observed for binary composite matrix tablets of ibuprofen.

The water uptake and erosion plots for the binary composite matrices are shown in figures 7.26-7.28 and 7.29-7.31 respectively.

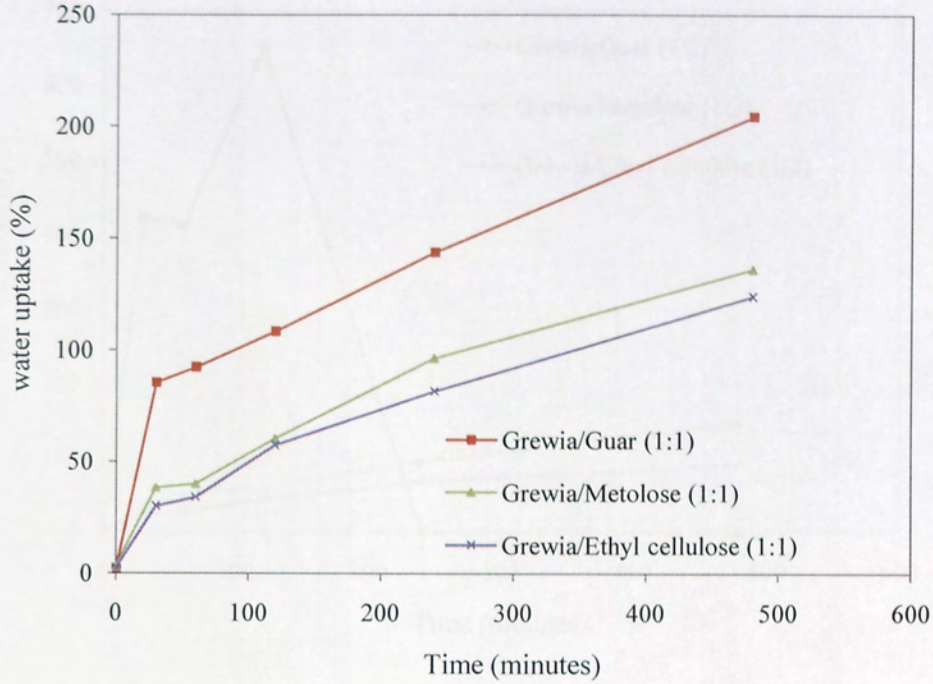


Figure 7.26: water uptake with time of binary composite matrices of ibuprofen containing grewia, and guar, Metolose® or ethyl cellulose in the ratio 1:1 (n=2, mean).

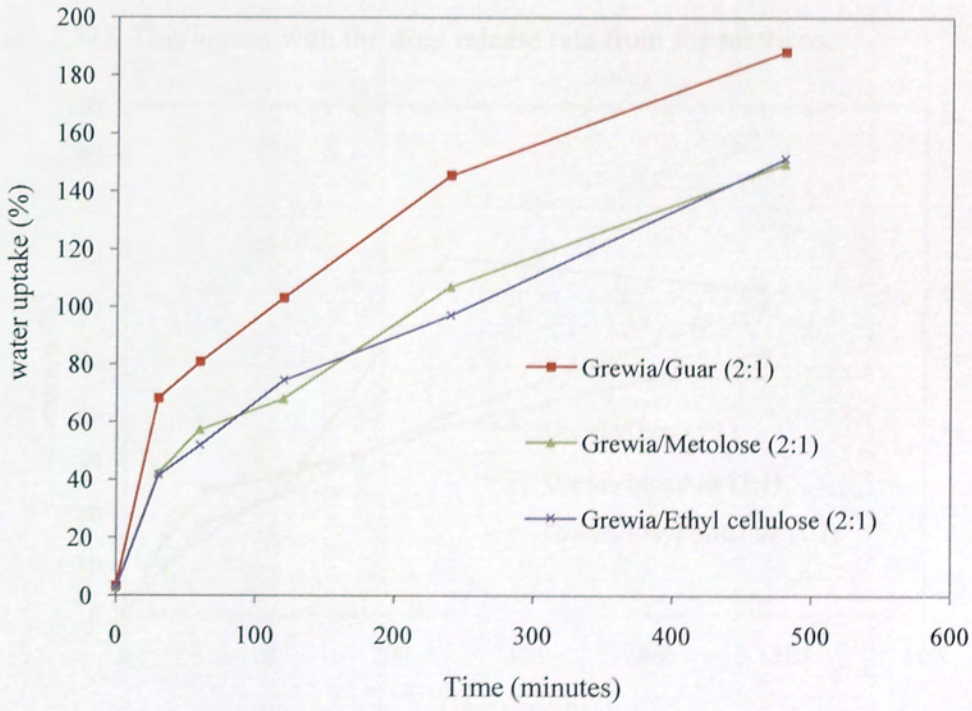


Figure 7.27: water uptake with time of binary composite matrices of ibuprofen containing grewia, and guar, Metolose® or ethyl cellulose in the ratio 2:1 (n=2, mean).

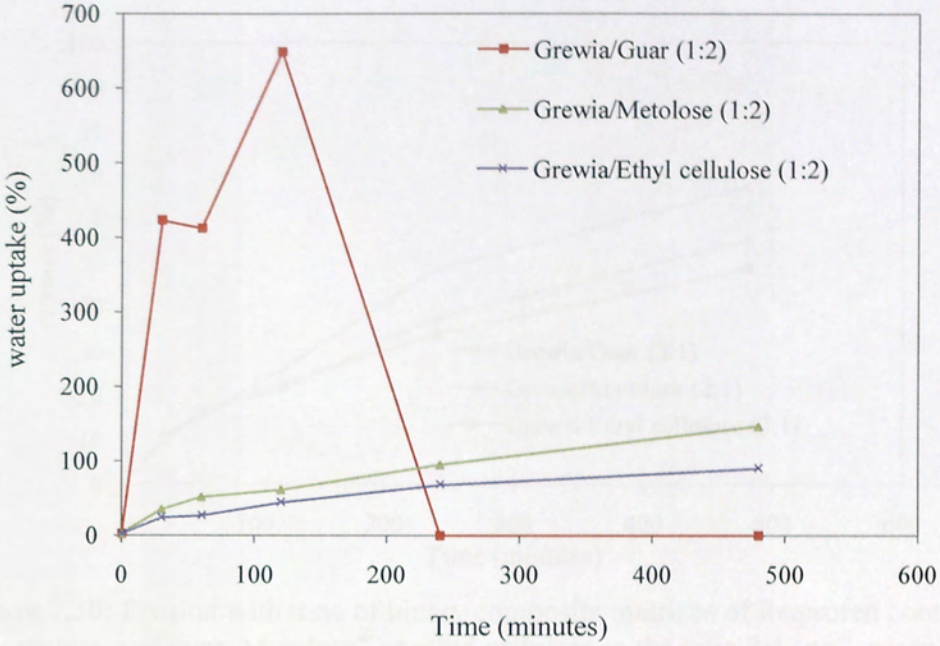


Figure 7.28: water uptake with time of binary composite matrices of ibuprofen containing grewia, and guar, Metolose® or ethyl cellulose in the ratio 1:2 (n=2, mean).

The results show that erosion and water uptake occur simultaneously for all batches. The complete erosion of grewia/guar (1:2) tablet matrices occurred within 2 hours (figure 7.31). This agrees with the drug release rate from the matrices.

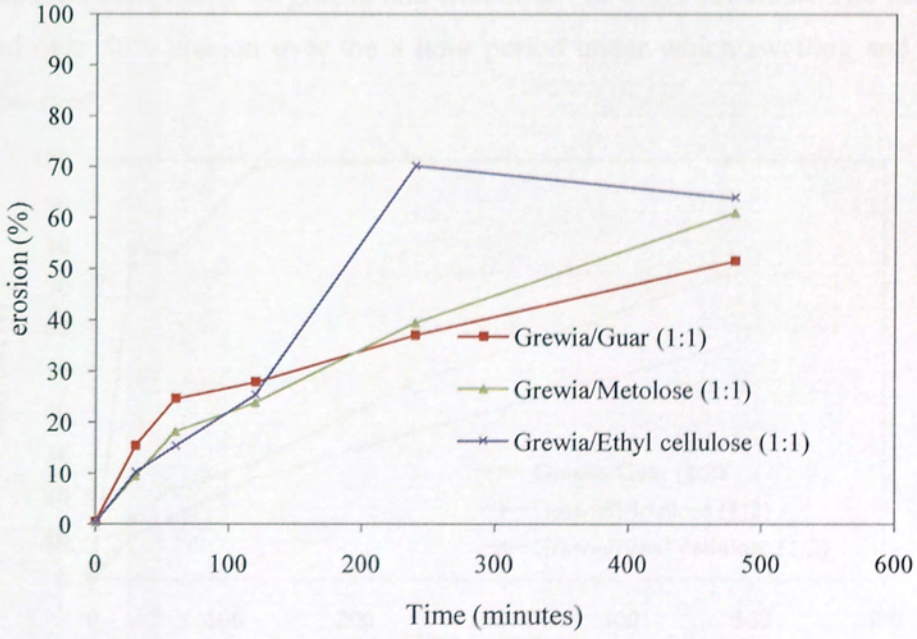


Figure 7.29: Erosion with time of binary composite matrices of ibuprofen containing grewia, and guar, Metolose® or ethyl cellulose in the ratio 1:1 (n=2, mean).

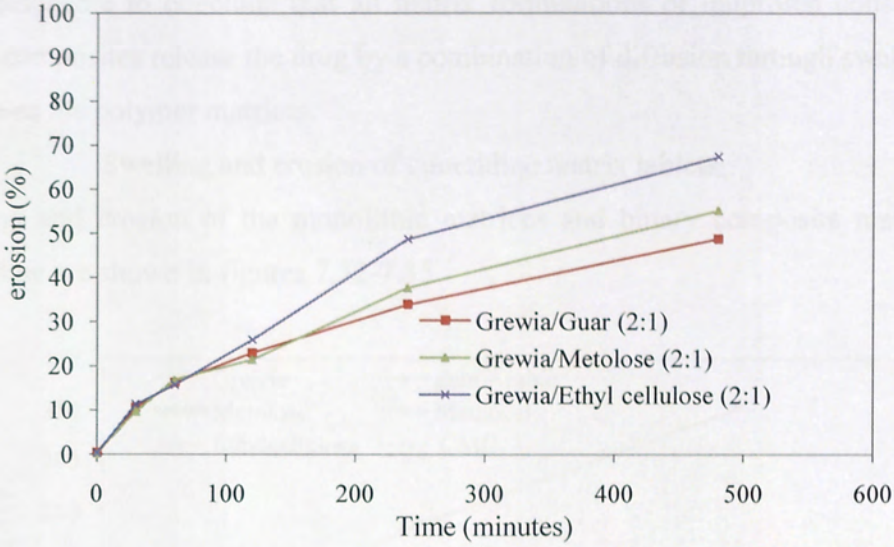


Figure 7.30: Erosion with time of binary composite matrices of ibuprofen containing grewia, and guar, Metolose® or ethyl cellulose in the ratio 2:1 (n=2, mean).

The matrix tablets of ibuprofen comprising of binary composites of grewia:guar (1:1 and 2:1) gave the highest degree of swelling as indicated by water uptake. At a ratio of grewia/guar 1:2, the high and rapid swelling dropped after 2 hours of swelling and erosion. This is an indication that at this ratio, erosion of the matrix tablets far outweighs water uptake. There was over 100% water uptake by composite matrix formulations comprising of grewia and Metolose® or ethyl cellulose. The same also showed over 50% erosion over the 8 hour period under which swelling and erosion was observed.

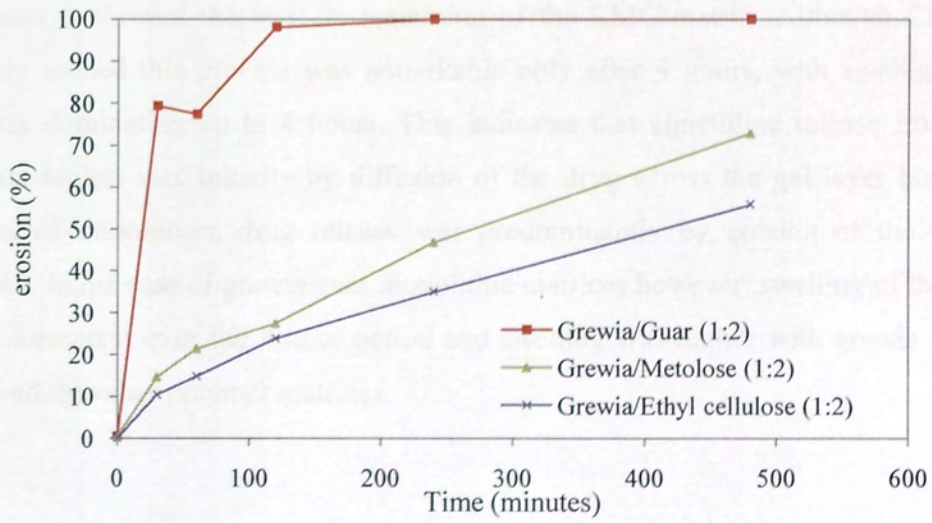


Figure 7.31: Erosion with time of binary composite matrices of ibuprofen containing grewia and guar, Metolose® or ethyl cellulose in the ratio 1:2 (n=2, mean).

It is reasonable to conclude that all matrix formulations of ibuprofen consisting of binary composites release the drug by a combination of diffusion through swelling and erosion of the polymer matrices.

7.3.5.2 Swelling and erosion of cimetidine matrix tablets

Swelling and erosion of the monolithic matrices and binary composite matrices of cimetidine are shown in figures 7.32-7.35

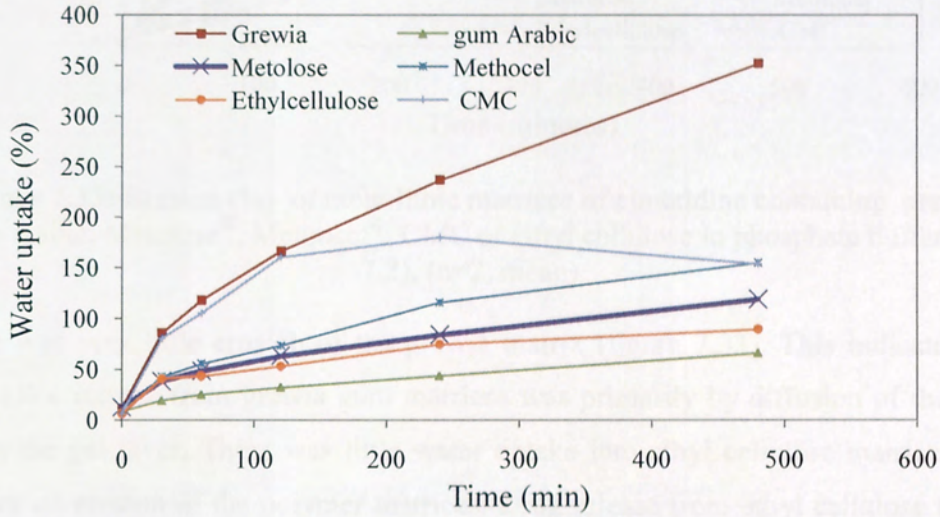


Figure 7.32: Water uptake of monolithic matrices of cimetidine containing grewia, gum arabic, Metolose®, Methocel®, CMC or ethyl cellulose in phosphate buffer (pH 7.2), (n=2, mean).

The results show erosion of monolithic matrices of CMC was highest and at the end of 8 hours it showed the least % remaining of the CMC matrix. Although CMC was highly eroded this process was remarkable only after 4 hours, with swelling of the matrix dominating up to 4 hours. This indicates that cimetidine release from CMC matrix tablets was initially by diffusion of the drug across the gel layer but after 4 hours of dissolution, drug release was predominantly by erosion of the polymer matrix. In the case of grewia gum monolithic matrices however, swelling of the tablets was consistent over the 8 hour period and swelling was higher with grewia matrices than all the other polymer matrices.

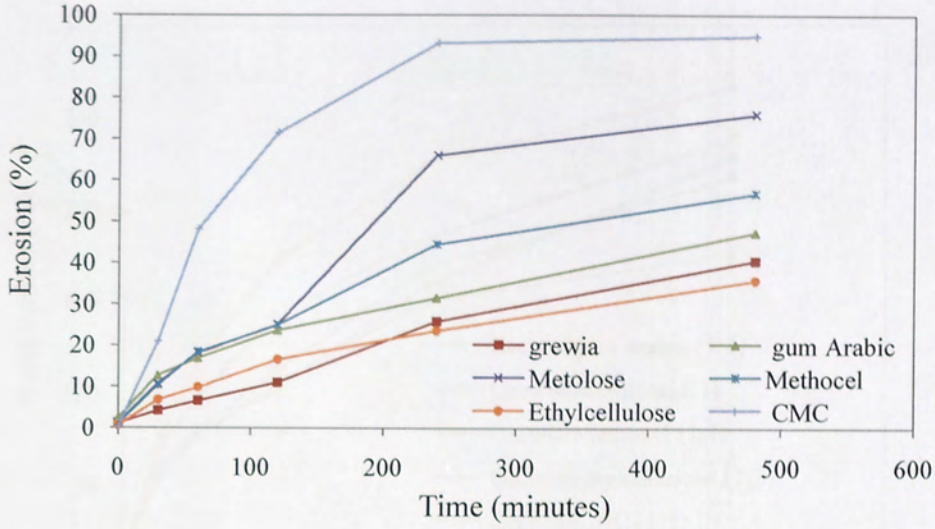


Figure 7.33: Erosion (%) of monolithic matrices of cimetidine containing grewia, gum arabic, Metolose®, Methocel®, CMC or ethyl cellulose in phosphate buffer (pH 7.2), (n=2, mean).

There was very little erosion of the grewia matrix (figure 7.33). This indicates that cimetidine release from grewia gum matrices was primarily by diffusion of the drug across the gel layer. There was little water uptake into ethyl cellulose matrices, and little or no erosion of the polymer matrices. Drug release from ethyl cellulose matrix tablets therefore did not occur by erosion. The percolation theory explains that the release of a water soluble drug from a water insoluble or hydrophobic polymer matrix occurs by dissolution of the active ingredient through capillaries composed of interconnecting drug particle clusters and the pore network (Leuenberger *et al.*, 1987; Holman and Leuenberger, 1988; Crowley *et al.*, 2004). As drug release continues, the interconnecting clusters increase the pore network through which interior drug clusters can diffuse. The gum Arabic and Metolose® matrices exhibit a combination of both swelling and erosion (7.32-7.33).

The binary composite matrix tablets all swell and erode to release the drug from the polymer matrices (figure 7.34-7.35). Diffusion of the drug across gel matrices therefore played a significant role in the release of the drug across the polymer matrices.

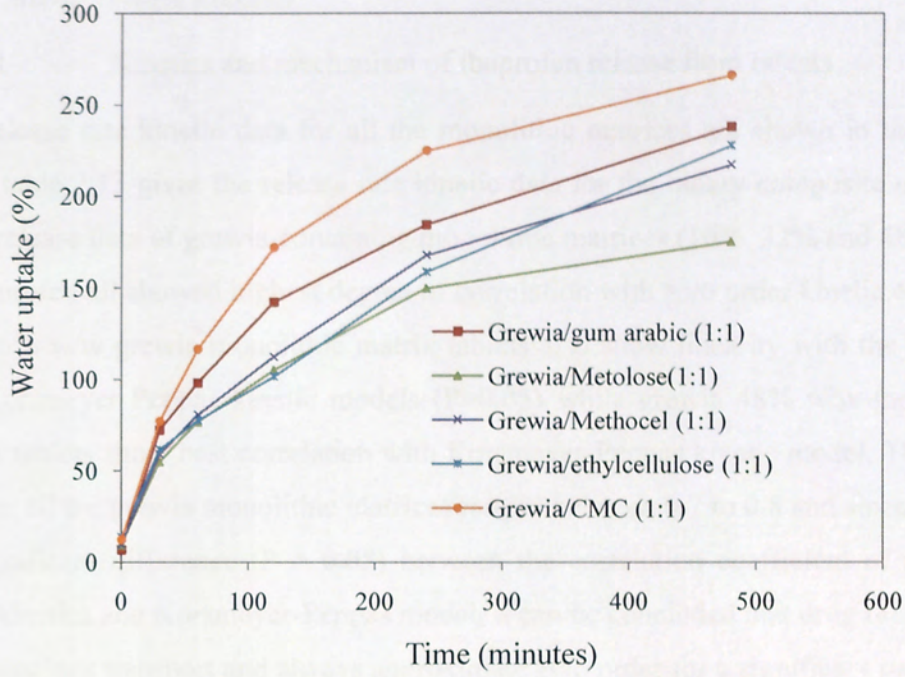


Figure 7.34: Water uptake of binary composite matrices of grewia, and gum arabic, Metolose®, Methocel®, CMC or ethyl cellulose (ratio 1:1) in phosphate buffer (pH7.2), (n=2, mean).

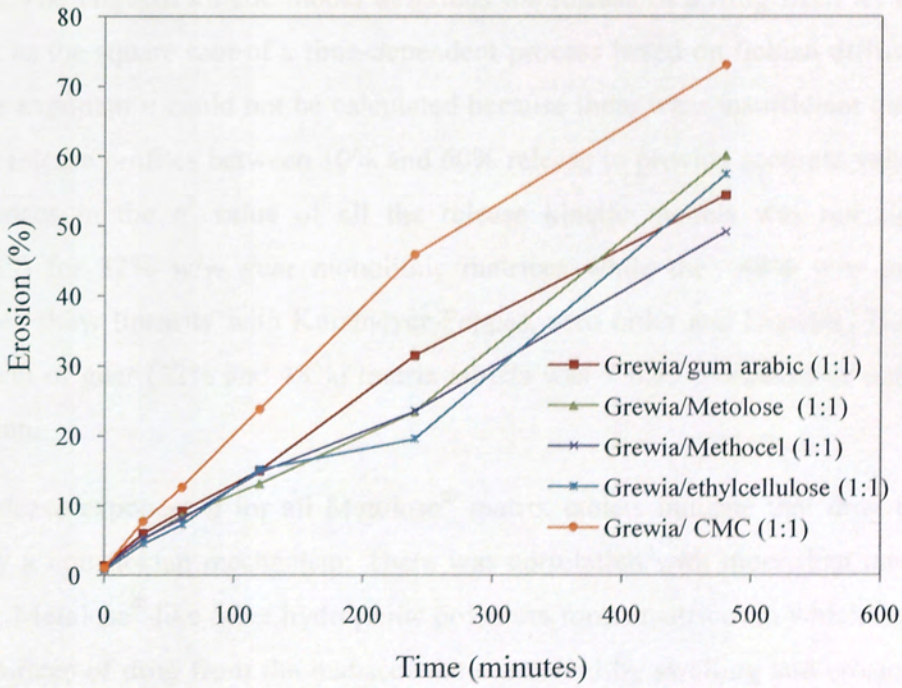


Figure 7.35: Erosion (%) of binary composite matrices of grewia, and gum arabic, Metolose®, Methocel®, CMC or ethyl cellulose (ratio 1:1) in phosphate buffer (pH7.2), (n=2, mean).

7.3.6 Drug release kinetics

7.3.6.1 Kinetics and mechanism of ibuprofen release from tablets

The release rate kinetic data for all the monolithic matrices are shown in table 7.12 while table 7.13 gives the release rate kinetic data for the binary composite matrices. Drug release data of grewia-containing monolithic matrices (16%, 32% and 48% w/w) of ibuprofen all showed highest degree of correlation with zero order kinetic equation. The 16% w/w grewia monolithic matrix tablets also show linearity with the Higuchi and Korsmeyer Peppas kinetic models ($P>0.05$) while grewia 48% w/w monolithic matrix tablets show best correlation with Korsmeyer-Peppas kinetic model. The value of n for all the grewia monolithic matrices ranged between 0.7 to 0.8 and since there is no significant difference ($P > 0.05$) between the correlation coefficient of the zero order kinetics and Korsmeyer-Peppas model, it can be concluded that drug release was by anomalous transport and always approximate zero order for a significant part of the total release time (Varshosaz *et al.*, 2006).

The 16% w/w guar monolithic matrix tablets show linearity with the Higuchi kinetic model. The Higuchi kinetic model describes the release of a drug from an insoluble matrix as the square root of a time-dependent process based on fickian diffusion. The release exponent n could not be calculated because there were insufficient data points on the release profiles between 10% and 60% release to provide accurate values. The differences in the r^2 value of all the release kinetic models was not significant ($P>0.05$) for 32% w/w guar monolithic matrices while the 48% w/w guar gum matrices show linearity with Korsmeyer-Peppas, zero order and Higuchi. The release exponent of guar (32% and 48%) matrix tablets was < 0.45 indicative of non-fickian diffusion.

The release exponent n for all Metolose® matrix tablets indicate that drug transport was by a non-fickian mechanism. There was correlation with more than one release model. Metolose® like other hydrophilic polymers forms matrices in which the overall release rates of drug from the matrices are controlled by swelling and erosion which occur simultaneously (Sujja-areevath *et al.*, 1998; Gohel *et al.*, 2000). Drug release from ethyl cellulose monolithic matrix tablets also showed linearity with more than one kinetic model. The release exponent indicate that drug transport was by anomalous or non-fickian mechanism.

Table 7.12: The release kinetics of monolithic matrix tablets of ibuprofen (n=3, mean ± s.d.)

Formulation	*Diffusion exponent (n)*	*Korsmeyer-Peppas (r^2)*	*Zero order (r^2)*	*First-order(r^2)*	*Higuchi (r^2)*	*Similarity factor (f_2)*
Grewia 16%	0.8±0.08	0.99±0.002	0.98±0.004	0.782±0.03	0.98±0.012	-
Grewia 32%	0.7±0.04	0.95±0.02	0.99±0.008	0.886±0.05	0.94±0.01	-
Grewia 48%	0.7±0.03	0.99±0.001	0.99±0.002	0.85±0.01	0.97±0.004	-
Guar 16%	N/A	N/A	0.68±0.03	0.64±0.04	0.84±0.020	16.2±0.4
Guar 32%	0.2±0.04	0.96±0.02	0.93±0.05	0.87±0.08	0.98±0.02	26.1±0.6
Guar 48%	0.3±0.07	0.97±0.02	0.93±0.02	0.82±0.07	0.98±0.014	25.9±1.5
Metolose® 16%	0.7±0.06	0.99±0.01	0.92±0.01	0.76±0.02	0.98±0.004	32.8±0.3
Metolose® 32%	0.7±0.04	0.98±0.001	0.94±0.02	0.77±0.03	0.99±0.004	34.2±0.9
Metolose® 48%	0.8±0.03	0.99±0.002	0.99±0.001	0.82±0.03	0.98±0.003	42.9±2.5
Ethyl cellulose 16%	0.6±0.05	0.98±0.01	0.99±0.01	0.85±0.02	0.99±0.01	70.4±4.1
Ethyl cellulose 32%	0.6±0.03	0.98±0.003	0.99±0.001	0.88±0.003	0.98±0.003	61.9±4.6
Ethyl cellulose 48%	0.6±0.03	0.99±0.004	0.98±0.003	0.84±0.004	0.99±0.001	61.3±5.9

The release kinetic parameters of the binary composite matrices are shown in table 7.13. The results show that release of drug from all the binary composite matrices show linearity with a number of kinetic models. The kinetic exponents were all indicative of non-fickian or anomalous transport. The release exponent *n* could not be calculated for the grewia/guar (1:2) binary matrices because there were insufficient data points on the release profiles between 10% and 60% release to provide accurate values.

Table 7.13: Release kinetics of binary composite matrix tablets of ibuprofen (n=3, mean ± s.d.)

Formulation	*Diffussional exponent, n*	*Korsmeyer-Peppas (r^2)*	*Zero order (r^2)*	*First order (r^2)*	*Higuchi (r^2)*	*Similarity factor (f_2)*
Grewia 16%	0.8±0.08	0.99±0.002	0.98±0.004	0.78±0.03	0.98±0.012	-
Grewia/Guar (1:1)	0.5±0.04	0.99±0.002	0.95±0.007	0.79±0.02	0.99±0.002	51.4±2.3
Grewia/Guar (1:2)	N/A	N/A	0.69±0.086	0.75±0.04	0.78±0.096	14.5±1.9
Grewia/Guar (2:1)	0.5±0.02	0.96±0.007	0.99±0.002	0.92±0.01	0.98±0.005	61.1±2.1
Grewia/ Metolose® (1:1)	0.6±0.05	0.98±0.007	0.92±0.015	0.75±0.04	0.98±0.005	42.7±6.0
Grewia/ Metolose® (1:2)	0.5±0.07	0.98±0.011	0.92±0.015	0.76±0.05	0.99±0.005	33.9±1.1
Grewia/ Metolose® (2:1)	0.5±0.04	0.98±0.022	0.98±0.002	0.88±0.02	0.99±0.005	65.5±1.7
Grewia/Ethyl cellulose (1:1)	0.7±0.06	0.98±0.004	0.94±0.025	0.73±0.06	0.99±0.005	32.7±4.2
Grewia/Ethyl cellulose (1:2)	0.7±0.04	0.99±0.007	0.95±0.003	0.76±0.04	0.99±0.008	45.8±4.1
Grewia/Ethyl cellulose (2:1)	0.7±0.01	0.99±0.001	0.97±0.003	0.82±0.002	0.99±0.002	52.6±1.9

7.3.6.2 Kinetics and mechanism of cimetidine release from tablets

The kinetics of drug release from the monolithic and binary composite tablets were plotted according to the various kinetic law equations and the regression coefficients and the release exponent based on the Korsmeyer-Peppas model are shown in table 7.14.

Table 7.14: Kinetic parameters of cimetidine matrix tablets (n=3, mean ± s.d.)

Formulation	*Release exponent (n)*	*Korsmeyer-Peppas (r^2)*	*Zero-order kinetic (r^2)*	*First-order kinetic (r^2)*	*Higuchi model (r^2)*	*Similarity factor (f_2)*
Grewia	0.7±0.04	0.99±0.002	0.96±0.01	0.60±0.02	0.98±0.01	-
Metolose®	0.7±0.03	0.96±0.015	0.88±0.08	0.46±0.07	0.97±0.03	32.0±4.1
CMC	1.1±0.39	0.89±0.051	0.55±0.06	0.28±0.02	0.76±0.1	15.3±0.3
gum Arabic	0.6±0.02	0.96±0.003	0.93±0.004	0.53±0.09	0.99±0.002	54.4±11.9
Methocel®	0.5±0.02	0.95±0.023	0.92±0.014	0.41±0.01	0.99±0.002	46.9±5.8
Ethyl cellulose	0.9±0.01	0.91±0.036	0.91±0.03	0.49±0.05	0.98±0.01	57.0±8.6
Grewia/ Metolose® (1:1)	0.8±0.02	0.98±0.014	0.98±0.002	0.69±0.02	0.95±0.001	58.3±7.8
Grewia/CMC (1:1)	0.8±0.02	0.99±0.008	0.97±0.002	0.65±0.01	0.96±0.01	42.7±5.0
Grewia/gum Arabic (1:1)	0.7±0.01	0.98±0.022	0.97±0.003	0.62±0.02	0.97±0.01	61.6±8.2
Grewia/Methocel® (1:1)	0.7±0.01	0.99±0.014	0.99±0.002	0.66±0.02	0.96±0.01	64.8±5.8
grewia/ethyl cellulose (1:1)	0.8±0.01	0.99±0.010	0.98±0.006	0.63±0.01	0.98±0.001	71.0±10.1

The grewia monolithic matrices show linearity with the Higuchi and Korsmeyer-Peppas model ($P>0.05$). The release exponent, n is indicative of non-fickian or anomalous transport. All other monolithic matrices also reveal a non-fickian transport of drug. This indicates that the release of cimetidine from the monolithic matrices of grewia gum is diffusion controlled and approximates zero order release. But the relative contributions of both swelling and erosion to cimetidine release in the grewia gum matrices produced a release exponent n of 0.7, indicative of anomalous or non-fickian drug release mechanism. Methocel® matrices show a fickian release mechanism with best fit to the Higuchi release model. Varshosaz *et al.*, (2006) reported similar finding when working with Methocel® releasing the water soluble drug tramadol hydrochloride. Methocel® contains a large proportion hydroxypropyl groups and produce strongly viscous gels forming a barrier across which the drug diffuses (Varshosaz *et al.*, 2006). This result correlates with the erosion data as Methocel® matrices show relatively lower erosion of the matrices indicating that release of drug from the Methocel® matrices was predominantly diffusion controlled.

The grewia/Methocel® binary matrices show linearity with zero order release kinetics. The release exponent indicates anomalous behaviour. It can be inferred that cimetidine release was by anomalous transport and always approximates zero order for a significant part of the total release time (Varshosaz *et al.*, 2006). Cimetidine transport from the binary matrices is also attributable to relative contributions of drug diffusion, polymer relaxation and matrix erosion (Colombo *et al.*, 2000; Varshosaz *et al.*, 2006; Sriamornsak *et al.*, 2007).

7.3.6.3 Similarity of dissolution profiles (Similarity factor, f_2)

Similarity of dissolution profiles of ibuprofen matrices

The similarity factor for the monolithic matrices and binary composite matrices are also shown in table 7.12 and 7.13 respectively. Ethyl cellulose-containing matrices of ibuprofen gave the highest f_2 of 70.4, 61.9 and 61.3 showing greater similarity with 16%, 32% and 48% w/w grewia-containing ibuprofen matrices respectively. The guar gum monolithic matrices were the least similar formulations to grewia monolithic matrices at all concentrations of polymer used. The hydrophilic matrices control drug release by swelling and diffusion across the gel layer. Ethyl cellulose has water repelling properties and unlike the hydrophilic polymers such as Metolose®, its hydrophobic nature repels water from penetrating the tablet matrix and as a result retards drug release from the tablet matrix (Katikaneni, 1995).

The binary composite matrices with the highest f_2 were grewia/guar (2:1) and grewia/ Metolose® (2:1) indicating the highest similarity to grewia 16% monolithic matrices. This reflects the dominant effect of grewia at these ratios.

Similarity of dissolution profiles for cimetidine matrices

The similarity factor (f_2) was used to compare drug release from the polymer matrices. The test polymer was the grewia (40% w/w) monolithic matrix formulation. The results are also shown in table 7.14. No formulation was identical with the grewia monolithic matrix tablets. The least degree of similarity exists between the profiles of grewia and CMC monolithic matrices (f_2 =15.31), while the highest similarity is seen between the profiles of grewia monolithic matrices and the binary matrices of grewia/ethyl cellulose (1:1) with f_2 of 70.95. The rank order of similarity of monolithic polymer matrices to grewia monolithic matrix tablets is ethyl cellulose > gum Arabic > Methocel® > Metolose® > CMC.

7.3.7 Stability of cimetidine and ibuprofen matrix tablet formulations

Both the cimetidine and ibuprofen matrix tablet batches did not show any change in appearance even after 60 days of exposure at room temperature and 60% relative humidity.

The effect of exposure to room temperature (60% relative humidity) for 60 days on the drug content of the ibuprofen matrices is shown in table 7.15. The variation in drug content for all the batches was however insignificant ($P > 0.05$) and indicate that the ibuprofen matrices will be stable (maintaining acceptable drug levels) under adverse storage conditions.

Table 7.15: Effect of temperature and relative humidity on drug content (%) stability of ibuprofen tablets containing 48% polymer (n=5, mean ± s.d.)

Formulation	*Day 0*	*Day 15*	*Day 30*	*Day 45*	*Day 60*
Grewia 48%	96.5±1.1	96.1±0.7	95.8±0.6	95.8±1.7	95.8±1.1
Metolose® 48%	94.1±0.7	94.1±1.3	94.0±0.6	93.9±1.5	93.7±1.2
Guar 48%	93.4±0.8	93.2±1.1	93.1±1.2	92.5±1.2	92.0±1.1
Ethyl cellulose 48%	94.4±1.1	94.1±1.0	94.2±1.3	94.5±1.1	94.0±1.1

The result of stability testing on the cimetidine tablet matrices under the same conditions are shown in table 7.16. For all batches, there was no significant difference ($P > 0.05$) in the drug content of matrix tablets at the end of the 60 days. The important point here is that grewia matrices for controlled release of cimetidine or ibuprofen demonstrated good product stability over the duration of the study.

Table 7.16: Effect of temperature and relative humidity on drug content (%) stability of cimetidine matrices (n=5, mean ± s.d.)

Formulation	*Day 0*	*Day 15*	*Day 30*	*Day 45*	*Day 60*
Grewia	101.1±2.0	99.3±2.1	99.5±1.3	98.8±1.1	98.5±0.6
gum arabic	107.9±1.2	106.9±1.3	106.4±1.4	106.3±1.0	106.4±1.2
Metolose®	102.8±0.8	101.9±0.9	101.4±1.2	101.4±1.3	101.3±1.7
Ethyl cellulose	100.1±1.2	99.2±2.0	98.1±2.4	100.1±1.2	99.0±1.1
Methocel®	99.2±1.7	98.2±2.8	98.0±2.9	97.7±2.9	98.8±1.6
CMC	100.6±1.6	100.4±1.5	99.7±1.3	99.4±1.3	99.1±1.5

The significance of this result to grewia polysaccharide gum is that the gum may not interfere with the stability of APIs when used as an excipient in tablet formulation.

7.4 CONCLUSION

The results from this study indicate that grewia polysaccharide gum can provide sustained release of ibuprofen from matrix tablets for up to 24 hours. The drug release was by anomalous diffusion but always approximately zero order for the most part of the release. The release profiles bear greatest similarity to ethyl cellulose. The current study reveals no synergism between grewia gum and guar, Metolose® or ethyl cellulose as matrices for the sustained release of ibuprofen. However, at 16% w/w the results indicate synergism between grewia and guar gum in the ratio 2:1.

The study also demonstrates that grewia polysaccharide gum, at 40% w/w, was capable of prolonging the release of cimetidine from matrix tablets for up to 12 hours. The strong sustained-release potential of grewia polysaccharide gum was superior to hydrophilic matrices of Methocel®, CMC, Metolose® and gum Arabic. The release of drug from the grewia polysaccharide gum matrices showed best correlation with Korsmeyer-Peppas and Higuchi kinetic models. Synergism was observed between grewia and Methocel® in the ratio of 1:1 for sustained release of cimetidine from tablets in which release kinetics tended towards a zero order release.

Grewia polysaccharide gum may provide a viable alternative to its natural counterparts like guar gum and gum Arabic in the sustained release formulation of solid dosage forms. Its relatively free availability, low cost and non-toxicity compared with the semi-synthetic and synthetic counterparts means that Methocel®, CMC, Metolose® and the hydrophobic release retardant ethyl cellulose, may not be preferable to grewia polysaccharide gum when sustained release of medicaments is indicated.

CHAPTER EIGHT

COMPARATIVE EVALUATION OF THE *IN VITRO* MUCOADHESIVE PERFORMANCE OF GREWIA POLYSACCHARIDE GUM GEL, GRANULES AND COMPACTS

8.1 INTRODUCTION

Mucus has been described as a complex viscous adherent secretion synthesised by specialized goblet cells lining all organs of the body exposed to the external environment (Andrews *et al.*, 2009). It comprises water (>95%) and mucins, a high molecular weight (2-14 x 10^6 g/mol) glycoprotein. Other components which make up less than 1% of mucus include proteins, lipids and mucopolysaccharides. The rheological property of mucus is attributable to the mucin glycoproteins which form highly entangled network of macromolecules that associate with one another through non-covalent bonds. It is this ability of mucin to associate that account for its interaction with mucoadhesive systems (see section 1.17.2).

Several approaches have been used for the *in vitro* evaluation of the interaction between mucin and mucoadhesive systems and for measuring of the mucoadhesive potential of candidate delivery platforms. One of such approaches is to determine the adhesive strength between polymer and the attached substrate. This can be determined by measuring the force required to detach one entity from the other through the application of an external force in the form of a shearing, tensile or peeling force. A number of these techniques have been reported (Smart *et al.*, 1984; Leung and Robinson, 1988; Ranga Rao and Buri, 1989).

Tobyn *et al.*, (1995), validated a new technique for mucoadhesion testing using the TA.XT2 texture analyzer and porcine stomach tissue. Wong *et al.*, (1988) worked on the effect of various instrumental parameters on candidate mucoadhesive polymers and indicated that variables such as contact force, contact time and the speed of removal of the probe from the mucosal tissue can influence the mucoadhesive performance of a system.

In this study different techniques were employed to evaluate the mucoadhesive performance of grewia polysaccharide gum systems:

1. Adherence of polymer granules to pig oesophageal mucosa
2. Interaction of polymer gels with mucin
3. Adherence of polymer compacts to a mucin covered probe.

The mucoadhesive performance of these systems were compared to similar systems using carbopol 971P (CBP 971P), hydroxypropyl methylcellulose (HPMC), carboxymethyl cellulose (CMC) and guar gum as standards.

8.2 MATERIALS AND METHODS

Materials used for this study include carbopol 971P (Noveon) and mucin from porcine stomach type II (Sigma, UK). All other materials used are as outlined in section 6.2. Grewia polysaccharide gum was extracted and air-dried according to the method in section 2.3.

8.2.1 Preparation of polymer granules

Granules of the polymers were prepared by moistening a 5 g quantity of polymer with distilled water. The damp mass was then forced through a 710 μm sieve to form granules which were dried at 50°C overnight in an oven (Gallenkamp, England). Acetone was used as granulating fluid for carbopol 971P. The dried granules were then transferred into the arrangement of sieve sizes 710, 500, 355 and 250 μm respectively. The sieves were arranged such that the largest size (710 μm) is on top and the smallest (250 μm) sitting on top of the collector. Only granule sizes of >500 μm were used for the mucoadhesive experiment.

8.2.2 Preparation of polymer compacts

A quantity of 300 mg of the polymer powder was compressed on an infrared (IR) press by the application of 2 tonne load for 2 minutes. The compact diameter was 13 mm and providing a potential surface contact area of 1.33 cm^2. The same surface (bottom) of the compressed polymer discs was used in all mucoadhesive experiments given the possibility of different surface porosities between the top and bottom surfaces.

8.2.3 Preparation of polymer gels, polymer/mucin gels and mucin gels

3.0% w/w gels of carbopol 971P, HPMC, CMC, guar gum and grewia polysaccharide gum were prepared by dispersing the powder of the polymer in distilled water by means of paddle stirrer mixer for 10 minutes. The gel samples were left to hydrate for 24 hours and then centrifuged at 3000 rpm for 10 minutes to remove air bubbles. Also gel mixtures containing polymer/mucin were prepared by weighing 10 g of mucin into 100 ml of 3.0% w/w dispersions of polymer and allowing hydration for 12 hours. All samples containing mucin were stored at 4°C until use. Mucin gel was prepared by dispersing mucin powder in distilled water to give 10 % or 30% w/w dispersion. Before evaluation of mucoadhesion, the viscosity of the gels was measured using a Brookfield viscometer (DV-1+version 5, Brookfield Engineering Labs, USA).

8.2.4 Isolation and preparation of pig oesophagus

The oesophageal mucosa was isolated from freshly slaughtered pigs from a slaughter house in Stoke-on-Trent, UK and stored at -80°C until required. Before use the refrigerated tissues were allowed to thaw at room temperature.

8.2.5 Determination of mucoadhesive performance of polymer granules in 0.1 N HCl

The mucoadhesive properties of the granules were determined according to the method designed by Ranga Rao and Buri (1989) reported in Tao *et al.*, (2009) with modifications. A photograph of the equipment used for the study is shown in figure 8.1. The set up consist of a water bath set to equilibrate the chamber at 37°C, a plate for mounting the tissue mucosa and a pump delivering hydration medium at a controlled rate. Pig oesophagus as isolated (see section 8.2.4) was used as biological substrate. A section of the tissue measuring 2 cm by 5 cm was mounted on a plate at an angle of 45°, flushed with 0.1 N HCl and equilibrated at 37°C for 5 minutes. Thereafter 20 particles of granules were uniformly scattered on the surface of the tissue and allowed to hydrate for 1 minute after which the tissue was flushed with 0.1N HCl for 2 minutes at a constant flow rate of about 10 ml/minute. The granules remaining on the surface of tissue were counted and the percentages of the granules remaining adhered to the mucosa calculated.

Figure 8.1: Set-up for mucoadhesive test according to Ranga Rao and Buri (1989) with modification

8.2.6 Texture analysis of polymer gels

Mucoadhesive studies were done on the polymer gels and compacts according to the methods of Tamburic and Craig (1997) with slight modification, using software controlled penetrometre, TA.XTPlus texture analyzer (Stable MicroSystems, UK) equipped with a 5 kg load cell, 10 mm plastic cylindrical probe. A picture of the texture analyzer used is shown in figure 8.2. The resistance to penetration and withdrawal of the probe was measured at a pre-test speed of 2 mm/s, test speed of 1 mm/s, post test speed of 10 mm/s and acquisition rate of 100 points/s. The penetration depth of probe into the gel was fixed at 5 mm. Trigger type was set to auto-0.01g while tare mode was set to auto with the option of return to start. The probe was covered with a thin layer of 30% mucin gel and then brought into contact with the polymer gel and held for 60 s. The gel sample was placed in a wide mouth (100 ml) glass jar. The surface of gel exposed to the probe was carefully flattened before each test.

Figure 8.2: The TA.XTPlus texture analyzer (Stable MicroSystems, UK)

8.2.7 Tensile analysis of polymer compacts

The polymer compacts were fixed to the lower platform of the texture analyzer with a contact adhesive. The compact was first wetted with 0.1 ml of distilled water using a syringe onto the centre surface of the compact, quickly spread evenly over the whole surface of the compact and left to hydrate for 1 min before measurement. A contact

force of 0.5 N was applied for 60 s upon contact of the surface of the compact with the mucin-covered probe. The probe was a 20 mm aluminium cylinder. Other settings for the detachment force measurements were pretest speed -1 mm/s, test speed -0.5 mm/s, post test speed -10 mm/s and an acquisition rate - 500 points/s.

8.3 RESULTS AND DISCUSSION

8.3.1 Mucoadhesive performance of polymer granules

The percentages of granules still adhering to the oesophageal tissue after flushing with 0.1 N HCl for 2 min at a constant flow rate of about 10 ml/min are shown in figure 8.3.

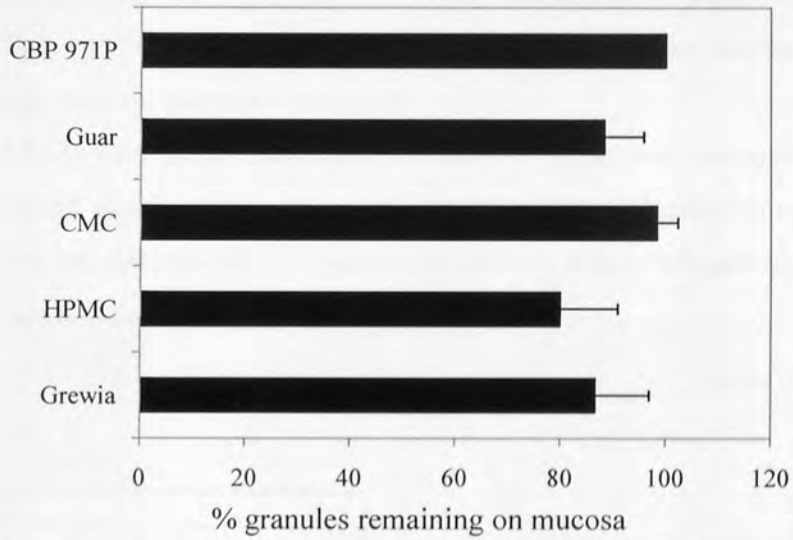


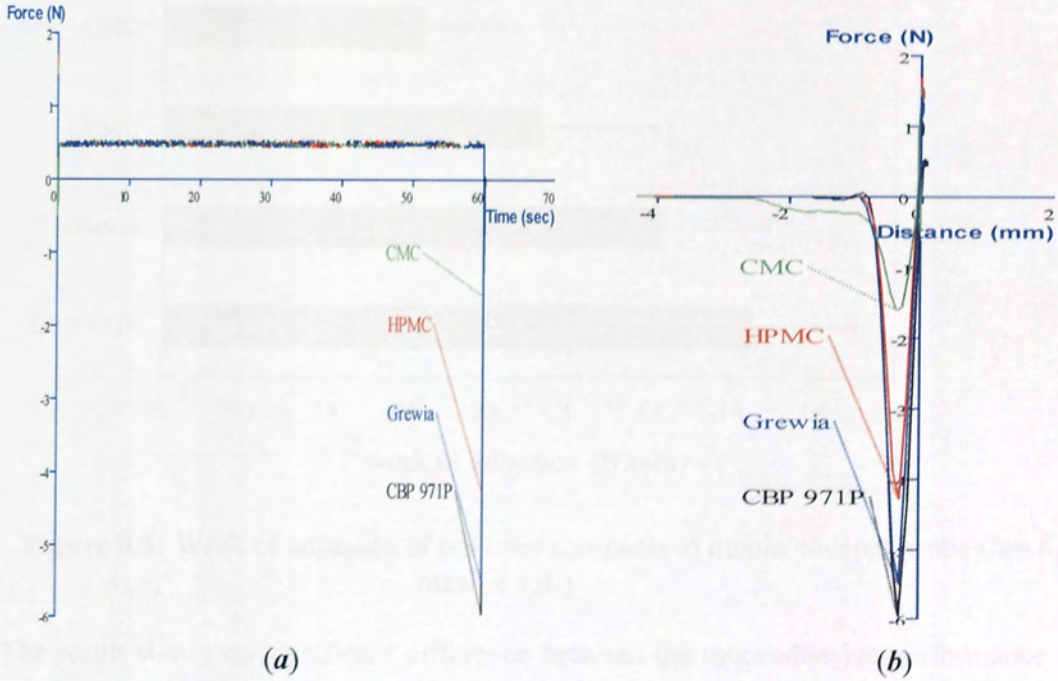
Figure 8.3: The percentage of granules remaining on pig oesophagus (n=6, mean ± s.d.).

The results show that none of the granule made of carbopol 971P (CBP 971P) was flushed from the oesophageal mucosal surface (100% of granules adhered to oesophageal mucosa) at the end of the study. The mean percentages of granules still adhering to the mucosa at the end of the experiment were CMC (98.3%), guar (88.3%), grewia (86.7%), and HPMC (80%). The one way ANOVA of the results however show that there is no significant difference ($P > 0.05$) between the mucoadhesive performance of grewia granules and CMC or guar and HPMC granules, but mucoadhesive performance of CBP 971P granules was significantly greater ($P < 0.05$) than grewia granules.

One factor affecting the observed results is that samples of tissue from the same anatomical region may not necessarily have identical surface morphology, and so identical surface contact between the tissue layers cannot be guaranteed with each determination (Pritchard *et al*, 1996). Furthermore, uniformity in granule size, shape and surface area between granule batches may not be guaranteed given the size range of granules used. This means that intimate contact between granules and tissue may vary widely. Since all other factors which may influence the mucoadhesive property of the materials such as pH of hydration medium and concentration of polymer were kept constant, the differences in the mucoadhesive performance between the polymer compacts will depend on degree of swelling and hydration of the polymer, functional groups making up the polymer, polymer molecular weight, chain length, conformation, and the degree of cross-linking within the polymer (see section 1.17.3).

8.3.2 Tensile tests on polymer compacts

Figure 8.4 (*a,b*) shows the detachment profiles of the polymer compacts. Figure 8.5 shows the work of adhesion to the mucin covered 20 mm aluminium cylinder probe. Results were not obtained for guar gum as the tablets were too fragile to withstand the 0.5 N contact force applied by the texture analyzer.



(*a*) (*b*)

Figure 8.4: Detachment force profile of *a*) force verses time, and *b*) force verses distance for polymer compacts.

In this study the detachment force is represented by the negative force on the force-time or force-distance profile. The positive horizontal line on the force-time profile (figure 8.4*a*) shows the application of contact force of 0.5 N for 60 seconds. Thereafter the probe was withdrawn and the amount of force required to detach the mucin covered probe from the polymer compact is displayed as a profile of force against time or distance.

If cohesive forces within the mucus gel are generally stronger than the adhesive forces between mucoadhesive and mucus, then fracture will tend to occur at the adhesion interface. However, fracture may occur within the mucus gel or within the polymer compact itself, if cohesive forces are weaker than the mucoadhesive forces (Ponchel *et al*, 1987; Helliwell, 1993). This later situation would tend to give rise to artificially low detachment force and work of adhesion and accounts for the work of adhesion seen with CMC compacts. This is shown by the broad detachment profile in figure 8.4 *b*. CMC compacts were not cohesive or hard enough after compressing with the IR press, consequently rupture occurred within the tablet resulting in the low detachment forces observed.

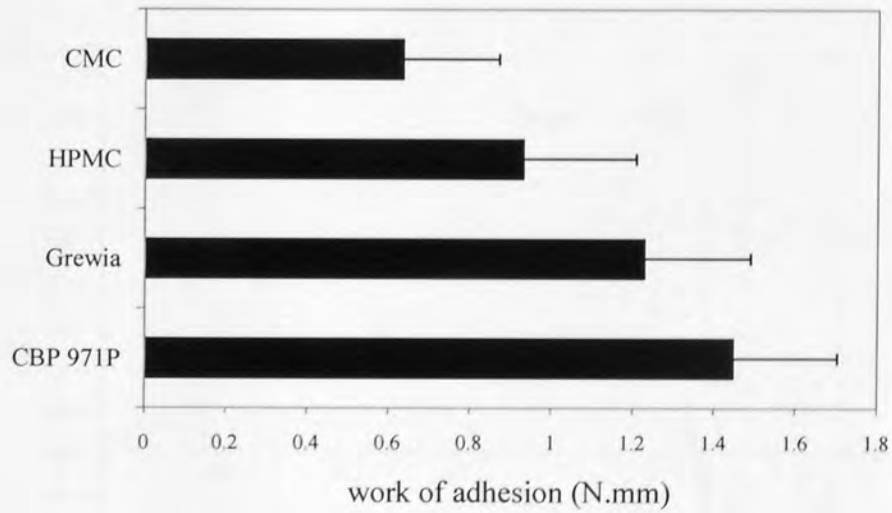


Figure 8.5: Work of adhesion of polymer compacts to mucin covered probe (n = 6, mean ± s.d.)

The result shows no significant difference between the mucoadhesive performance of grewia compacts and HPMC or CBP 971P compacts. Grewia compacts were observed to have significantly greater mucoadhesive performance ($p<0.05$) than CMC. This contrasts with the result obtained for the polymer granules. It was explained in the last

paragraph why CMC compacts gave low work of adhesion; this will be in addition to the effect of compact surface roughness on adhesion to the mucin covered probe. The surfaces of grewia compacts did not only appear rough but were rougher to touch than those of HPMC or CBP 971P.

Detachment force studies have been used as a direct measure of mucoadhesivity or mucoadhesive performance of a material (Smart *et al*, 1984; Pritchard *et al*, 1996; JianHwa, 1994; Cooklock, 1998). The force required to separate surface of the mucoadhesive material from that of the mucoadhesive substrate is measured.

8.3.3 Texture analysis of polymer gels

Texture analyses, a penetrometry technique hitherto used in mechanical characterization of food materials has emerged as a useful technique in the field of pharmaceutical gel characterization (Jones *et al.*, 1996; Tamburic *et al.*, 1996). The technique used by Tobyn *et al.*, (1995) was used to evaluate the mucoadhesive performance of the polymer gels and polymer/mucin mixtures. Figure 8.6 presents the penetration/withdrawal profiles of the polymer gels (3.0% w/w) while the penetration/withdrawal profiles of the polymer/mucin mixtures are shown in figure 8.7.

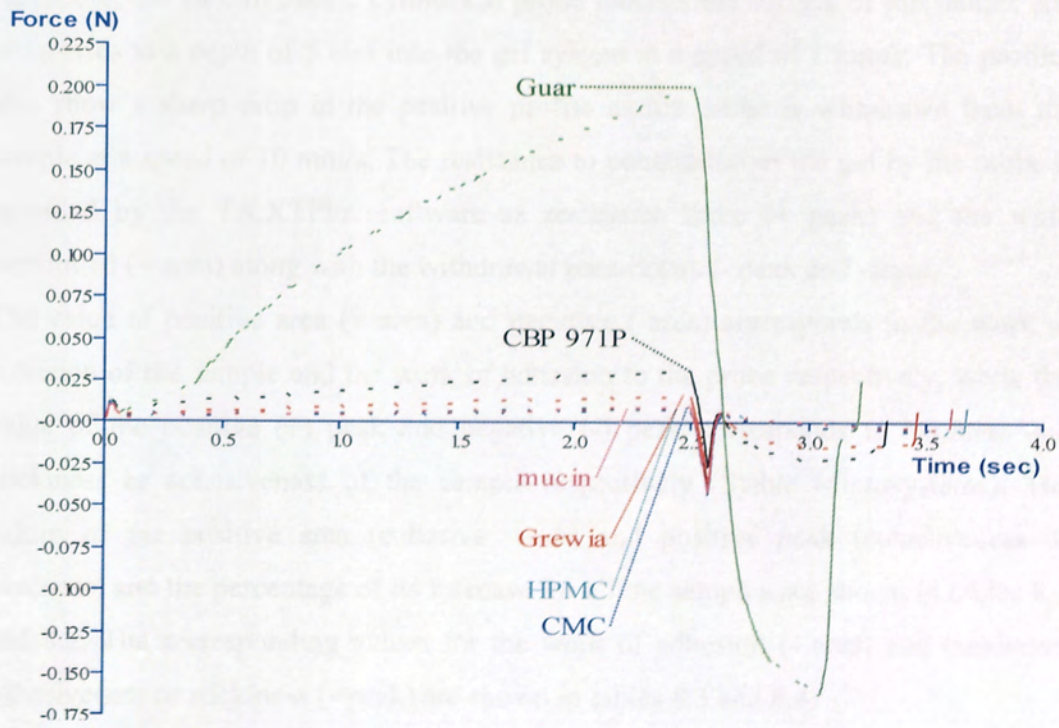


Figure 8.6: Penetration /withdrawal profiles obtained by texture analyzer for the polymer gels and mucin gel

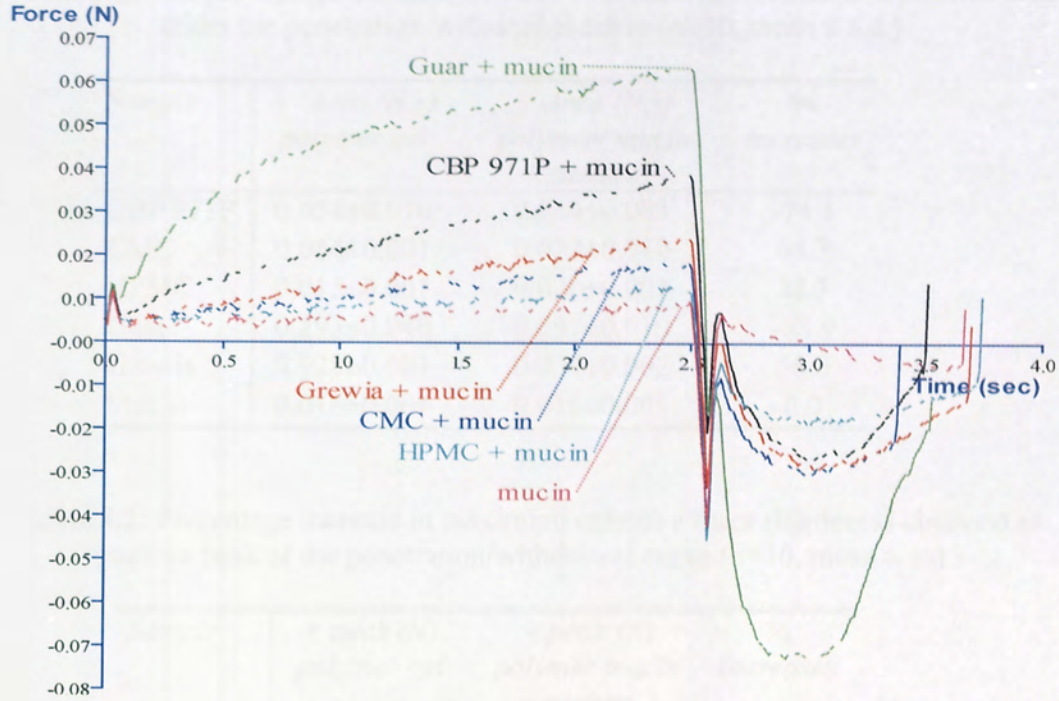


Figure 8.7: Penetration /withdrawal profiles obtained by texture analyzer for the polymer/mucin mixtures and mucin gel

At time 0, the 10 mm plastic cylindrical probe touches the surface of the sample and progresses to a depth of 5 mm into the gel system at a speed of 1 mm/s. The profiles also show a sharp drop in the positive profile as the probe is withdrawn from the sample at a speed of 10 mm/s. The resistance to penetration of the gel by the probe is recorded by the TA.XTPlus software as resistance force (+ peak) and the work performed (+ area) along with the withdrawal parameters (- peak and -area).

The value of positive area (+ area) and negative (-area) corresponds to the work of cohesion of the sample and the work of adhesion to the probe respectively, while the value of the positive (+) peak and negative (-) peak corresponds to hardness and stickiness or adhesiveness of the sample respectively (Stable Microsystems). The values of the positive area (cohesive work) and positive peak (cohesiveness or hardness) and the percentage of its increase for all the samples are shown in tables 8.1 and 8.2. The corresponding values for the work of adhesion (- area) and maximum adhesiveness or stickiness (- peak) are shown in tables 8.3 and 8.4.

Table 8.1: The percentage increase on work of cohesion, obtained as a positive area under the penetration/withdrawal curve (n=10, mean ± s.d.)

Sample	*+ Area (N.s) polymer gel*	*+ Area (N.s) polymer/ mucin mixture*	*% increases*
CBP 971P	0.054±0.010	0.014±0.001	-74.1
CMC	0.014±0.001	0.023±0.010	64.3
HPMC	0.015±0.001	0.020±0.001	33.3
Guar	0.293±0.040	0.091±0.010	-68.9
Grewia	0.025±0.001	0.039±0.002	56.0
Mucin	0.016±0.001	0.016±0.001	0.0

Table 8.2: Percentage increase in maximum cohesive force (hardness) obtained as positive peak of the penetration/withdrawal curve (n=10, mean ± s.d.)

Sample	*+ peak (N) polymer gel*	*+ peak (N) polymer mucin mixture*	*% increases*
CBP 971P	0.034±0.004	0.009±0.0004	-72.1
CMC	0.009±0.001	0.016±0.0030	70.2
HPMC	0.009±0.001	0.013±0.0003	41.3
Guar	0.192±0.020	0.056±0.0040	-70.8
Grewia	0.017±0.001	0.026±0.0010	56.7

It is obvious from the results in table 8.1 and 8.2 that guar gel gave the highest resistance to penetration and indicates that at 3.0 % w/w, dispersion of guar gum gives the most viscous dispersion compared to all the other polymers. The results show that the work of cohesion of guar gels and CBP 971P gels were significantly greater than grewia gels ($P < 0.001$ and 0.01 respectively) while the work of cohesion of grewia gels was not significantly greater than HPMC or CMC gels ($P > 0.05$). However, the interaction between mucin and polymer gels resulted in higher work of cohesion for grewia/ mucin gel mixture which is significantly greater ($P < 0.001$) than the work of cohesion of CMC/mucin, HPMC/mucin and CBP 971P/mucin gel mixtures. Although there was a decrease in the work of cohesion when guar gel was mixed with mucin, the resultant mixture still had a work of cohesion that is significantly greater ($P < 0.001$) than the work of cohesion of all the other polymer/mucin gel mixtures. Similar results were obtained for the maximum cohesive force of the polymer gels and polymer/mucin gel mixtures (table 8.2).

The effect of the interaction with mucin on the viscosity of the polymer gels is shown in figure 8.8. As should be expected there is a relationship between the work of cohesion and the viscosity of polymer gels. It can also be observed that interaction with mucin resulted in a fall in the viscosity of guar and CBP 971P gels while the same interaction resulted in an increase in the viscosity of CMC, HPMC and grewia polysaccharide gels.

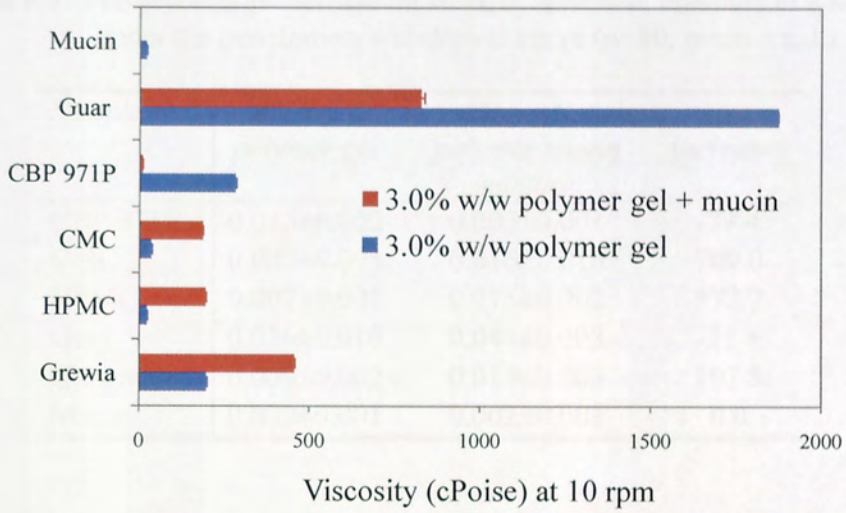


Figure 8.8: The effect of mucin-polymer interaction on the viscosity of the polymer gel + mucin dispersions (n=5, mean ± s.d.).

Tables 8.3 and 8.4 show the effect of interaction with mucin on the adhesiveness (stickiness) and work of adhesion of the polymer gels. The results show that the work of adhesion of CBP 971P gels was not significantly greater ($P > 0.05$) than grewia gels. Guar gels had work of adhesion which was significantly greater than work of adhesion of grewia gels ($P < 0.001$) but which in turn was significantly greater than the work of adhesion of CMC gels ($P < 0.05$) and HPMC gels ($P < 0.001$). The rank order of work of adhesion of polymer gels is guar > CBP 971P = grewia > HPMC = CMC. Interaction of polymer gels with mucin also resulted in a decrease in the work of adhesion of CBP 971P/mucin gels and guar/ mucin gel mixtures while resulting in an increase of work of adhesion for grewia/mucin, CMC/mucin and HPMC/mucin gel. Consequently, the work of adhesion of grewia/mucin gel mixtures becomes significantly higher ($P < 0.001$) than CBP 971P/mucin gel mixtures. There was no significant difference between the work of adhesion of grewia/mucin gel mixtures and CMC/mucin gel or HPMC/mucin gel mixtures. The rank order of work of adhesion of

polymer/mucin gel mixtures becomes guar > grewia = HPMC = CMC > CBP 971P. As was the case with work of cohesion, guar/mucin gel mixtures still had work of adhesion that is significantly greater than that of grewia/mucin gel mixtures even though interaction of guar with mucin resulted in a decrease of work of adhesion by 21.4% (table 8.3).

Table 8.3: The percentage increase on work of adhesion, obtained as a negative area under the penetration/withdrawal curve (n=10, mean ± s.d.)

Sample	*- Area (N.s) polymer gel*	*- Area (N.s) polymer mucin mixture*	*% increases*
CBP 971P	0.013±0.002	0.003±0.001	-77.4
CMC	0.002±0.001	0.016±0.010	700.0
HPMC	0.002±0.001	0.015±0.002	572.7
Guar	0.056±0.010	0.044±0.003	-21.4
Grewia	0.009±0.002	0.019±0.003	107.8
Mucin	0.002±0.001	0.002±0.001	0.0

Table 8.4: Percentage increase in maximum adhesive force (stickiness) obtained as negative peak of the penetration/withdrawal curve (n=10, mean ± s.d.)

Sample	*- peak (N) polymer gel*	*- peak (N) polymer mucin mixture*	*% increases*
CBP 971P	0.025±0.0020	0.006±0.0004	-74.8
CMC	0.006±0.0010	0.022±0.0070	266.0
HPMC	0.006±0.0010	0.019±0.0010	202.7
Guar	0.155±0.0110	0.070±0.0030	-54.6
Grewia	0.015±0.0004	0.029±0.0010	93.3
Mucin	0.006±0.0004	0.006±0.0004	0.0

The maximum adhesive force (stickiness) of the polymer gels (table 8.4) show the rank order of adhesiveness to be guar > CBP 971P > grewia > HPMC = CMC. The adhesiveness of guar gels or CBP 971P gels were significantly greater ($P < 0.001$) than grewia gels which in turn was significantly greater ($P < 0.01$) than HPMC or CMC. Similarly, the effect of interaction of the polymer gels with mucin given by the % increases in adhesiveness (table 8.4) showed that the adhesiveness (stickiness) of CMC and HPMC were the same ($P > 0.05$) while CBP 971P decreased in

adhesiveness by 74.8%. The rank order of adhesiveness after interaction of the polymer gels with mucin becomes guar > grewia > HPMC = CMC > CBP 971P. The adhesiveness of grewia gel remained significantly greater ($P < 0.001$) than HPMC or CMC.

Leitner *et al.*, (2003) showed from viscosity studies that the viscosity of the polymer/mucin mixtures increases with increasing molecular mass and confirmed an earlier report by Murtazavi and Smart (1994), that shorter chain length polymers with inherent poor gel formimg properties were found to be less effective in promoting gel strengthening. The much higher viscosity of grewia/mucin, CMC/mucin and HPMC/mucin gel mixtures are attributable to the formation of bonds between the polymer and mucin. The type or nature of bond formed may depend on the polymer type or the inherent functional groups present. The formation of disulphide bonds which are covalent in nature have been reported to be accountable for the enhanced mucoadhesive properties of thiomers (Bernkop-Schnurch *et al.*, 1999). Earlier, Murtazavi (1995) had reported the formation of hydrogen bonds between polymer and mucus as essential for the mucoadhesion process.

The decrease in the viscosity of carbopol 971P/mucin mixture or the decrease in adhesiveness and work of adhesion has been confirmed elsewhere (Tamburic and Craig, 1997). According to Bernkop-Schnurch, (2000), the main physical mechanism of mucoadhesion is chain flexibility. Flexible polymer chains favours interpenetration between polymer chains and mucus to a sufficient depth to create a strong adhesive bond. Consequently, he opined that the cross-linking or the covalent attachment of large sized ligands may lead to a reduction in chain flexibility and results in a strong decrease in mucoadhesion. This may explain the decrease in work of adhesion witnessed with carbopol 971P and guar gum.

8.4 CONCLUSION

Several mucoadhesive polymers have been identified and classified as having excellent mucoadhesive performance (Sudhakar *et al.*, 2006) and these include Carbopol 971P, guar, CMC and HPMC. These materials demonstrate excellent mucoadhesive properties both as gels and as compacts. This comparative study indicates that grewia polysaccharide gum may possess excellent mucoadhesive performance comparable to carbopol 971P, CMC, guar and HPMC. Grewia

polysaccharide gum is a high molecular weight polymer that hydrates in water over time to form highly viscous dispersions of the polymer. The result of this study is evidence to suggest that the gum possesses the chain flexibility requisite for interpenetration between polymer and mucus.

CHAPTER NINE

FINAL CONCLUSIONS

9.1 PHYSICOCHEMICAL CHARACTERIZATION

The aim of this research was to evaluate the potential applications of grewia gum as a pharmaceutical excipient and to also characterize the material with particular focus on the effects of drying techniques on its physicochemical properties. The objectives included:

1. Procurement, authentication and processing of the plant
2. Extraction and purification of the gum from the crude pulverized inner stem bark of the plant and drying by three methods- air-drying, freeze-drying and spray-drying.
3. Physicochemical characterization of the processed gum. Properties such as pH, moisture, solubility, ash content, viscosity and flow of dispersions, parameters of powder flow (angle of repose, Carr's compressibility index, Hausner ratio, bulk and tapped densities, true density), elemental composition, intrinsic viscosity and molecular weight, compressibility, and thermal, film and swelling properties.
4. Comparative evaluation of the suspending ability of grewia gum in ibuprofen suspension. The suspending ability of grewia gum will be compared with standard suspending agents such as acacia gum, sodium carboxymethylcellulose and xanthan gum at three levels of concentration (0.5%, 0.75% or 1.0% w/v). The evaluation parameters will include viscosity and rheology, redispersibility, sedimentation volume, degree of flocculation, electrokinetic properties (zeta potential), and microbiological evaluation.
5. Comparative evaluation of the release retardant property of grewia gum in cimetidine and ibuprofen matrix tablets. Standard hydrophilic matrix materials such as guar, acacia, Metolose®, Methocel® and carboxymethylcellulose will be used as reference polymers including ethyl cellulose, a hydrophobic release retardant.
6. Evaluation of mucoadhesive performance of grewia gum. Mucoadhesive systems of grewia gum will be compared with similar systems of hydroxypropyl methylcellulose, carboxymethylcellulose, guar gum and Carbopol 971P®.

Grewia gum from *Grewia mollis* Juss (Fam. Tiliaceae) was obtained from the wild in Mangu local government area of Plateau state in Nigeria and authenticated by the Forestry Reserve Commission. The gum can be extracted and purified according to the method reported (see section 2.3). The mean yield of 32.4% w/w on dry weight basis

by air-drying or freeze drying is good. Okafor *et al.*, (2001) reported a yield of 37% after purification and air-drying. Spray-drying gave a low yield of 3.1% w/w. The resultant product is wheat coloured (freeze dried or air dried) and may require additional processing such as bleaching to give more acceptable colour. The spray dried sample may be preferable in this sense. However, given the ease of processing and the associated cost, air-drying of grewia gum may be more sustainable in the developing world.

The drying technique does not affect the nature of the material but may have some effect on some physicochemical properties of the gum. The present study indicates that grewia gum may be a polysaccharide gum. A combination of techniques such as FT-IR, solid state NMR, ^{1}H and ^{13}C NMR, XPS, SEM indicated it is amorphous in nature and may be a polysaccharide. The confirmation of this may require the use of mass spectroscopic techniques. GC analysis showed the gum is composed of glucose, galactose, rhamnose, arabinose and xylose as neutral sugars. Okafor *et al.*, (2001) reported that the gum contains glucose and rhamnose as neutral sugars with galacturonic acid as the only sugar acid. The gum showed high thermal stability with a thermal oxidation onset temperature >250°C.

The present study also indicated that drying method may have profound effect on the intrinsic viscosity and molecular weight distribution of the gum. The average molecular weight and molecular weight distribution of the gum varied according to the method used to achieve drying. Also the viscosity of the gum dispersions varied with the drying method. Some physicochemical properties of natural gums have been shown to vary with drying methods (York, 1983; Vendruscolo *et al.*, 2009).

Grewia gum powder showed high thermal stability with a thermal oxidation onset temperature >250°C. The gum hydrates slowly and swells gradually to form highly viscous aqueous dispersions with acidic pH of about 5.7. These dispersions exhibited pseudoplasmic (shear-thinning) flow behavior irrespective of drying technique. This is a property desirable for a good suspending agent. Acidic gums contain sugar acids in their structure and carry a negative charge (Izydorczyk, 2005).

The gum is only dispersible in water with extremely low solubility. It may be composed of large proportion of structural material from the plant. This in conjunction with high moisture content increases the risks of microbial growth. Low solubility may limit the application of the gum as excipient where solubility is a requirement.

Slow hydration and swelling of the gum may be of significance in controlled release delivery of medicaments. The gum contain metals such as Ca, Mg, P, and N. These have been confirmed elsewhere (Okafor *et al.*, 2001).

9.2 SUITABILITY AS PHARMACEUTICAL EXCIPIENT

Grewia gum has good suspending ability and can stabilize pharmaceutical suspensions when used in concentrations between 0.5% - 1.0% w/v. It was superior to acacia gum and sodium carboxymethylcellulose at the ~~level of~~ concentrations evaluated. The viscosity imparting property of grewia gum was second only to xanthan gum in the ibuprofen suspension formulations. Stable suspensions have been reported to have zeta potential that is more negative than -30 mV or more positive than +30 mV. The mean zeta potential of suspension formulations containing 0.5% - 1.0% w/v of grewia gum were more negative than -30mV which was comparable to xanthan at the same concentrations. These were far more stable than suspension formulations containing sodium carboxymethylcellulose or acacia (less than -20 mV) at the same concentrations. Therefore, grewia gum may be preferable to acacia gum or sodium carboxymethylcellulose when suspending ability is indicated, thus meeting the fourth objective of this study (see section 1.20). In Nigeria where grewia gum is in relatively abundant supply, grewia gum can be an alternative to xanthan gum in suspension formulations. Although natural gums share a common disadvantage of microbial growth, this can be overcome by the use of preservatives such as benzoic acid.

Another objective of this study was to evaluate the release retardant property of grewia gum and to compare this with standard hydrophilic or hydrophobic polymers. The present study showed that grewia gum can provide sustain release of ibuprofen from monolithic matrix tablets for up to 24 hours. The gum produces tablets with good physical properties. The ability of grewia gum to sustain the release of ibuprofen from monolithic matrices was superior to Metolose® and guar gum. The gum can also provide sustained release of cimetidine from the monolithic matrices for 12 hours. Results from this study showed that the gum has greater release retardant power when compared to Methocel®, CMC, Metolose®, or acacia gum in cimetidine matrices.

Grewia gum does not interact with ibuprofen or cimetidine. The present study indicated no evidence of interaction or incompatibility between grewia gum and ibuprofen or cimetidine. There was also no evidence to suggest any incompatibility between grewia gum and the other excipients in the formulation such as lactose

monohydrate, microcrystalline cellulose or magnesium stearate. Grewia gum can be used in tablet formulations containing any of these ingredients without risk of incompatibilities.

Comparative studies of the mucoadhesive and tensile properties of granules, compacts and gels of grewia polysaccharide gum showed that grewia polysaccharide gum has some advantages compared with HPMC or CMC or CBP 971P when mucoadhesive performance is an indication. Grewia polysaccharide gum formed more cohesive gels on interaction with mucin, suggesting that the gum possesses adequate chain flexibility for interpenetration between polymer and mucus.

Grewia polysaccharide gum provides a novel but viable alternative to its natural counterparts like guar gum, acacia gum, tragacanth or xanthan gum. The gum reveals a reservoir of potential that can be exploited in both food and the pharmaceutical industry. Its relatively abundant availability, low cost and non-toxicity compared with the semi-synthetic and synthetic counterparts, means that the development and use of the gum as excipient will apart from providing an alternative source, save foreign exchange and provide a means of livelihood to the local people in those places where the plant is abundant and can both be cultivated or found growing wild.

9.3 RECOMMENDATIONS

The purification and drying of grewia polysaccharide gum results in a product that is coloured. This may reduce the appeal of the material. It may be desirable to exploit techniques that will bleach the colour or achieve a product with less intense coloration.

Grewia polysaccharide gum hydrates and swells in aqueous media. It does not however dissolve completely in water (low solubility) but disperses slowly upon hydration and swelling to give a highly viscous dispersion. It is insoluble in ethanol and most organic solvents. The material contains a high proportion of insoluble matter, this may place a limitation on its useand increases the risk of microbial contamination. It will be desirable to have a product that is more soluble in aqueous media and that is readily dispersible for some applications.

The high moisture uptake capacity of grewia polysaccharide gum may increase susceptibility of the gum to microbial growth and contamination. A combination of these two factors may threaten product stability and decrease product shelf-life.

This work has only taken a step in what remains a long journey towards exploitation of grewia polysaccharide gum. It has provided some answers, left some questions unanswered and probably created new ones. Further work on linkage patterns and uronic acid composition are necessary to elucidate the structure of this polymer. It may be necessary in the end to exploit the possibility of developing a better product by chemical modification of the parent material.

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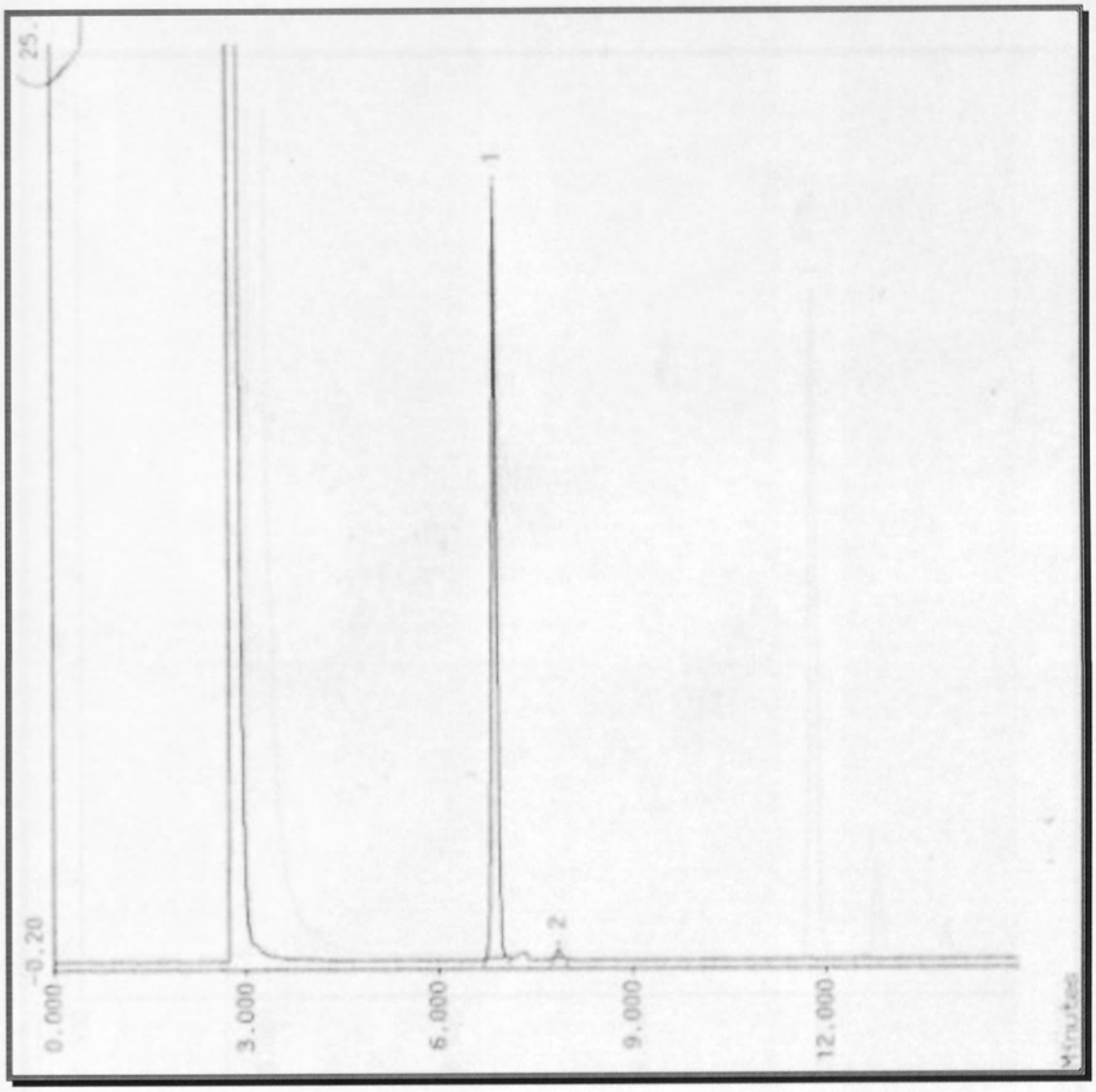
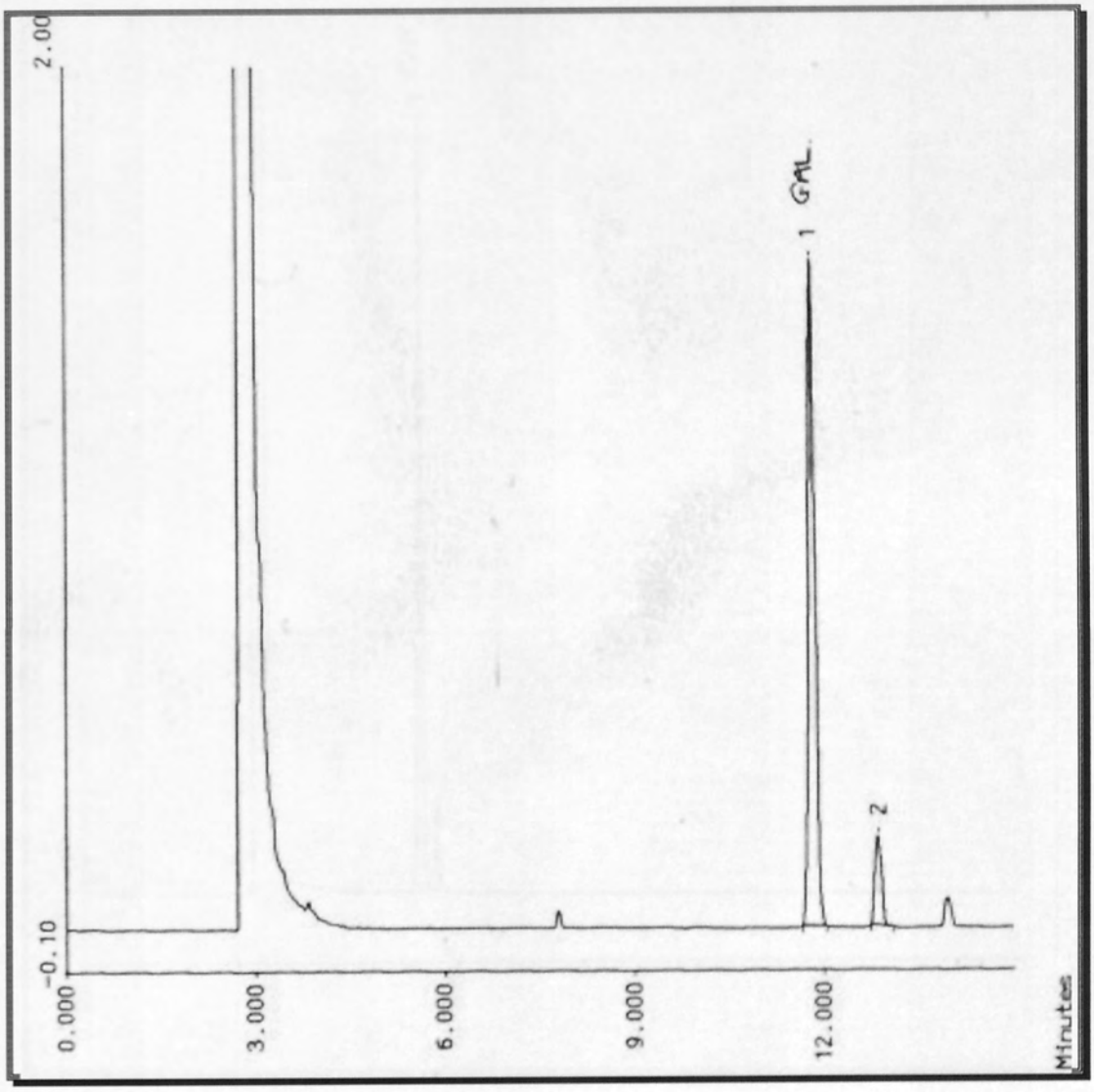
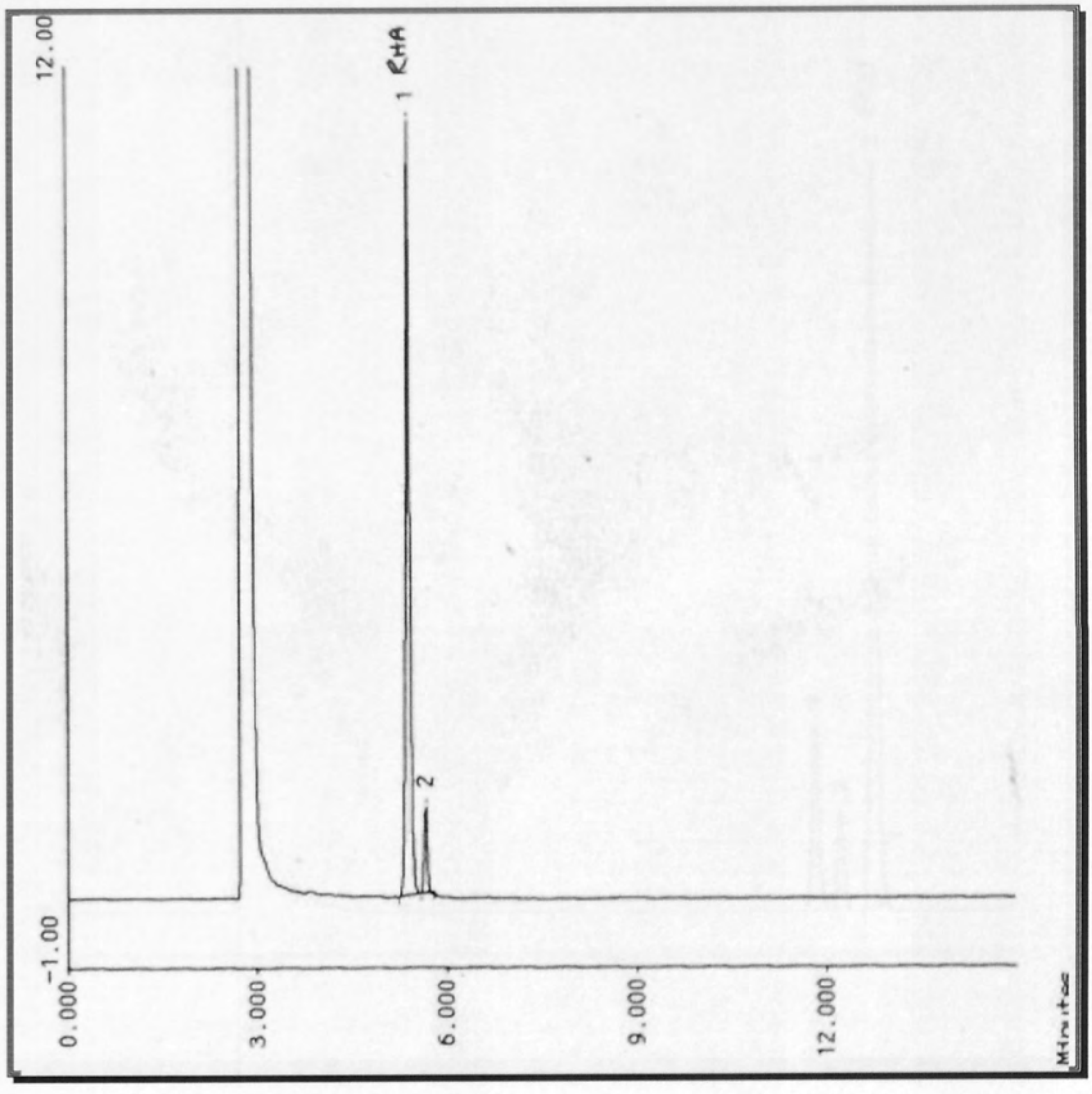
APPENDIX



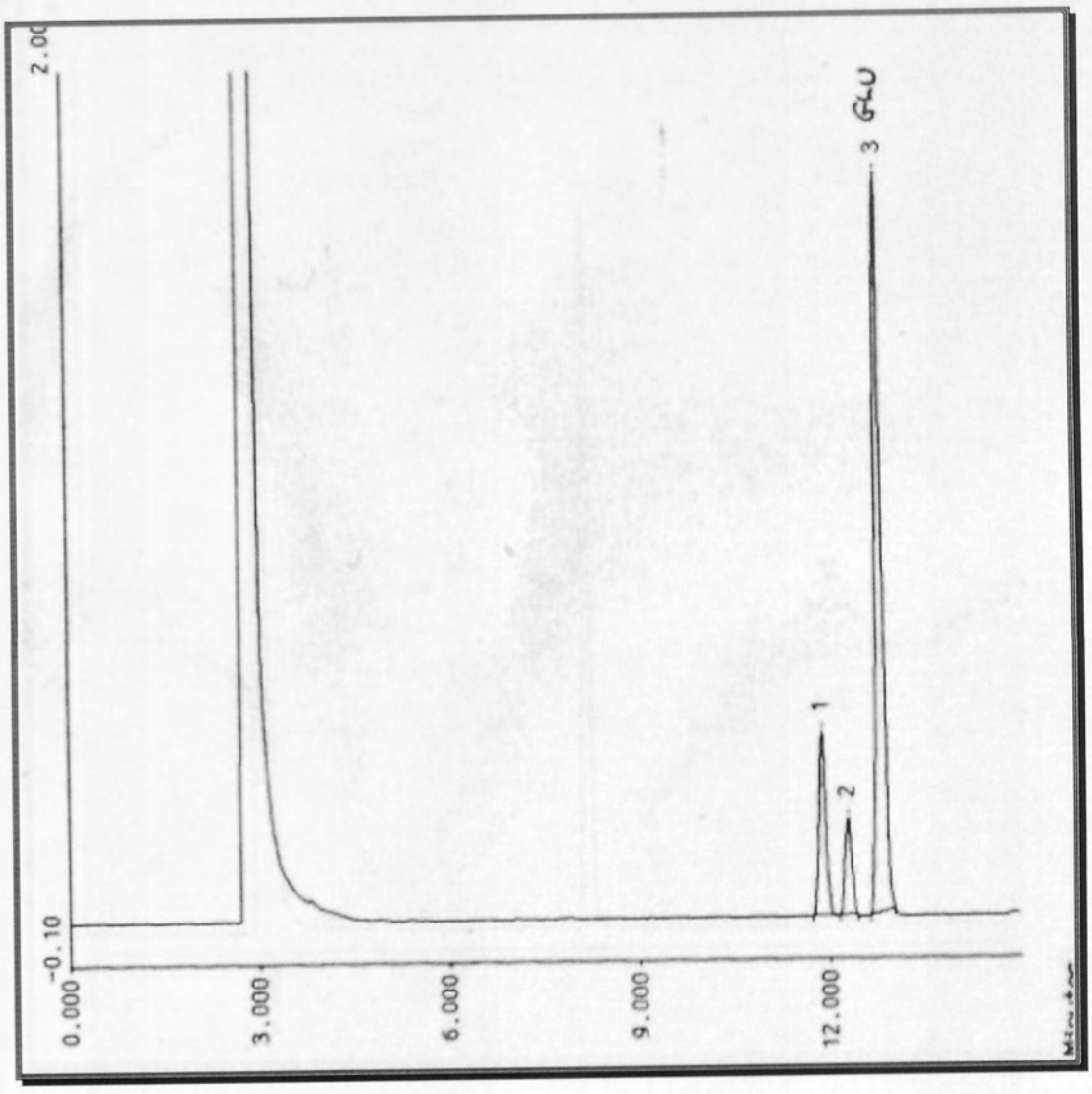
APPENDIX A1.1: GC PEAK OF ARABINOSE



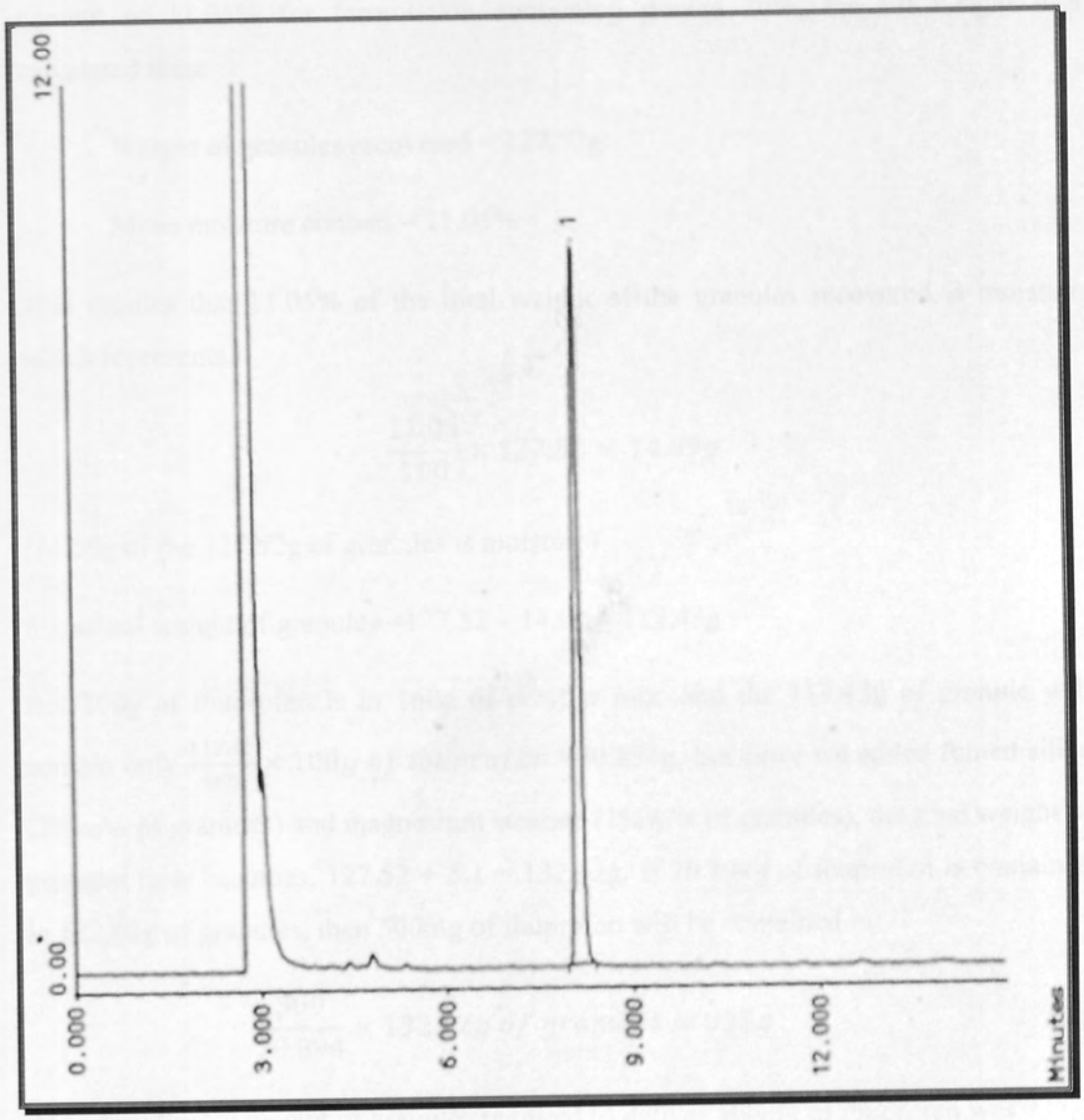
APPENDIX A1.2: GC PEAK OF GALACTOSE



APPENDIX A1.3: GC PEAK OF RHAMNOSE



APPENDIX A1.4: GC PEAK OF GLUCOSE



APPENDIX A1.5: GC PEAK OF XYLOSE

APPENDIX A1.6: DETERMINATION OF TABLET FILL WEIGHT

The amount of granules fed into the die was determined taking into consideration the moisture content of the powders or granules. As an example, given a mean moisture content of 11.05% for formulation containing grewia 20%, the fill weight was calculated thus:

Weight of granules recovered = 127.52g

Mean moisture content = 11.05%

This implies that 11.05% of the total weight of the granules recovered is moisture which represents,

$$\frac{11.05}{100} \times 127.52 = 14.09g$$

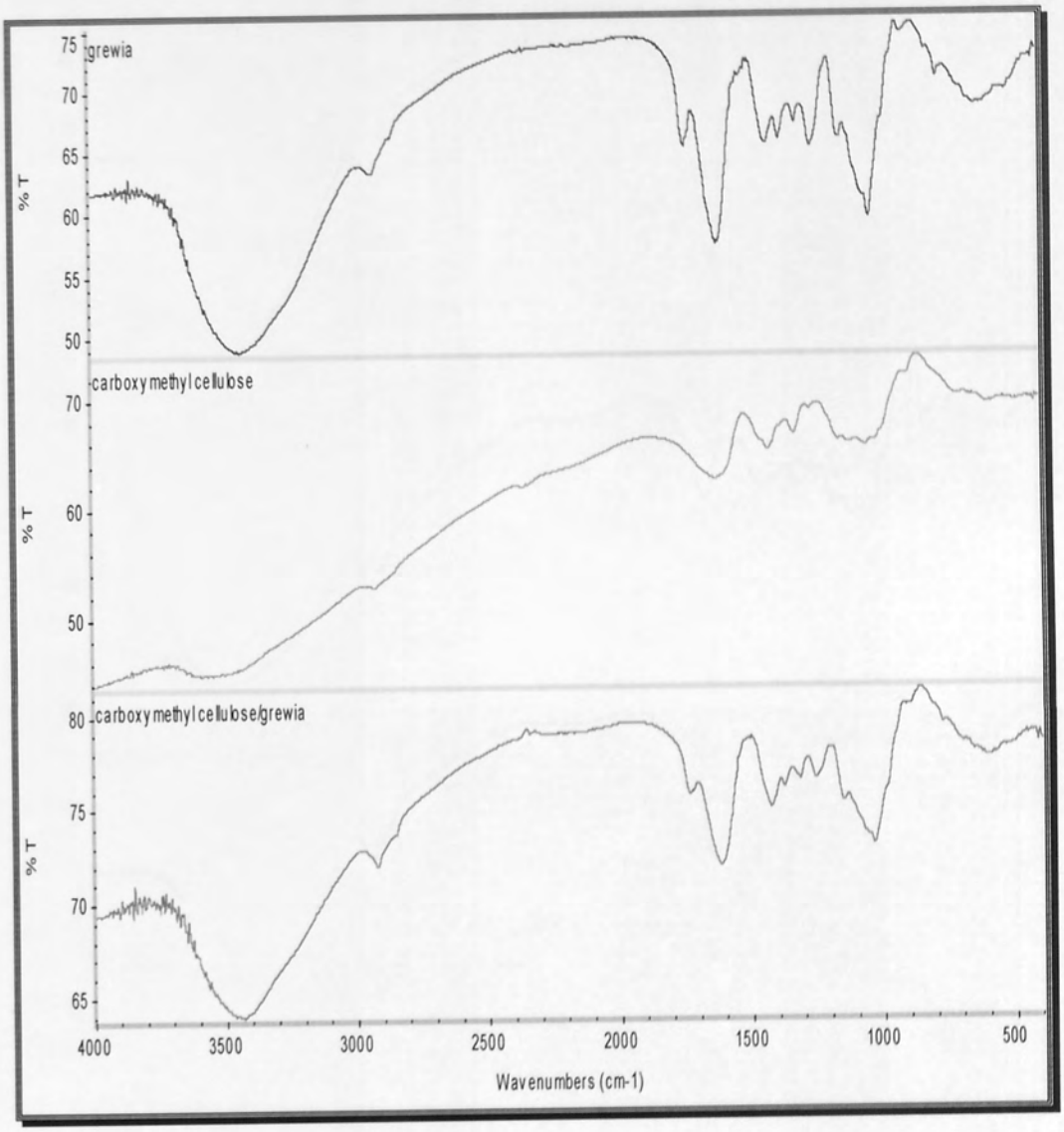
(14.09g of the 127.52g of granules is moisture)

So, actual weight of granules =127.52 – 14.09 = 113.43g

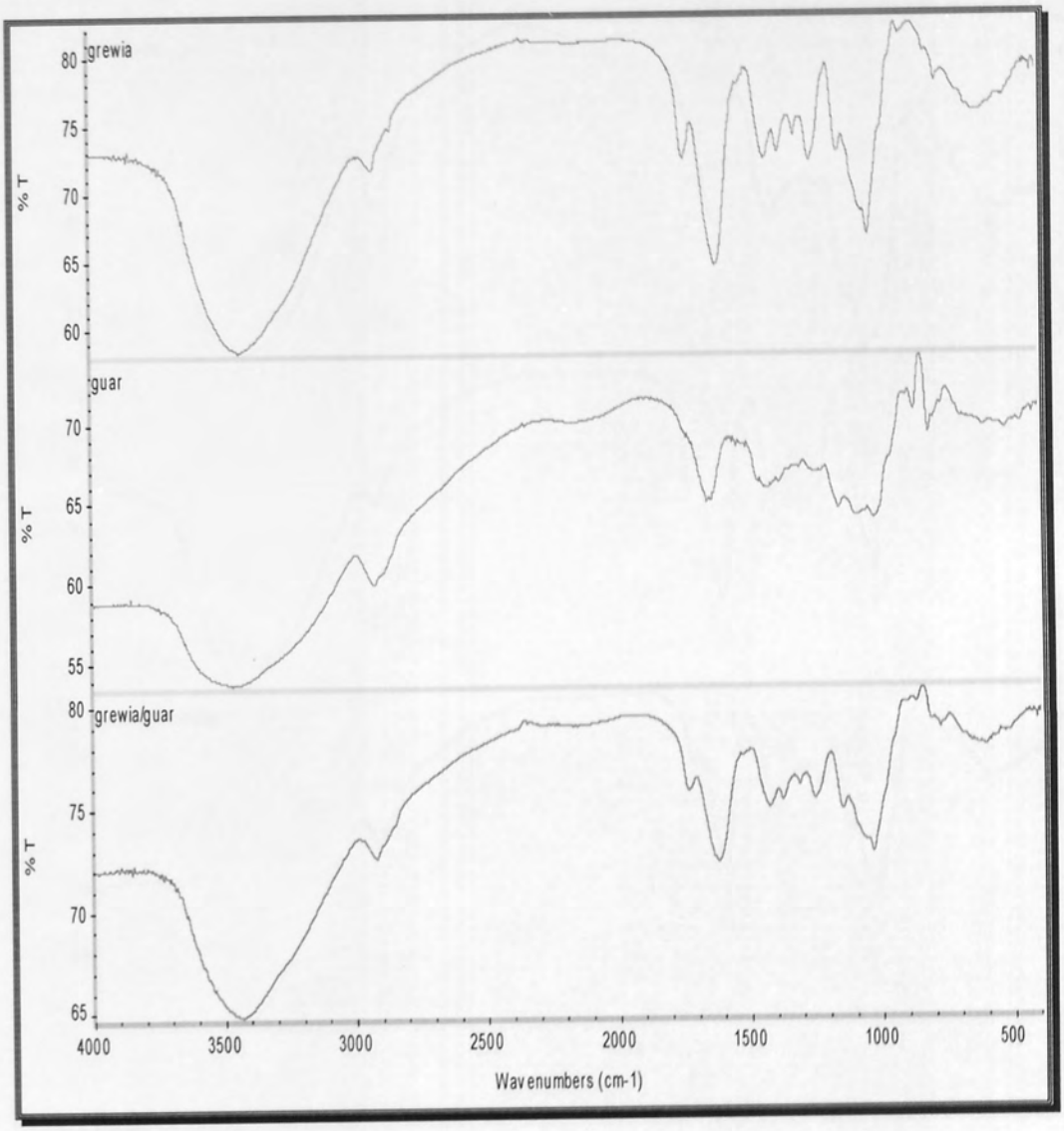
But 100g of ibuprofen is in 160g of powder mix, and the 113.43g of granule will contain only $\frac{113.43}{160} \times 100g\ of\ ibuprofen$ =70.894g, but since we added fumed silica (3%w/w of granules) and magnesium stearate (1%w/w of granules), the total weight of granules now becomes, 127.52 + 5.1 = 132.62g. If 70.894g of ibuprofen is contained in 132.62g of granules, then 500mg of ibuprofen will be contained in:

$$\frac{500}{70.894} \times 132.62g\ of\ granules = 935g$$

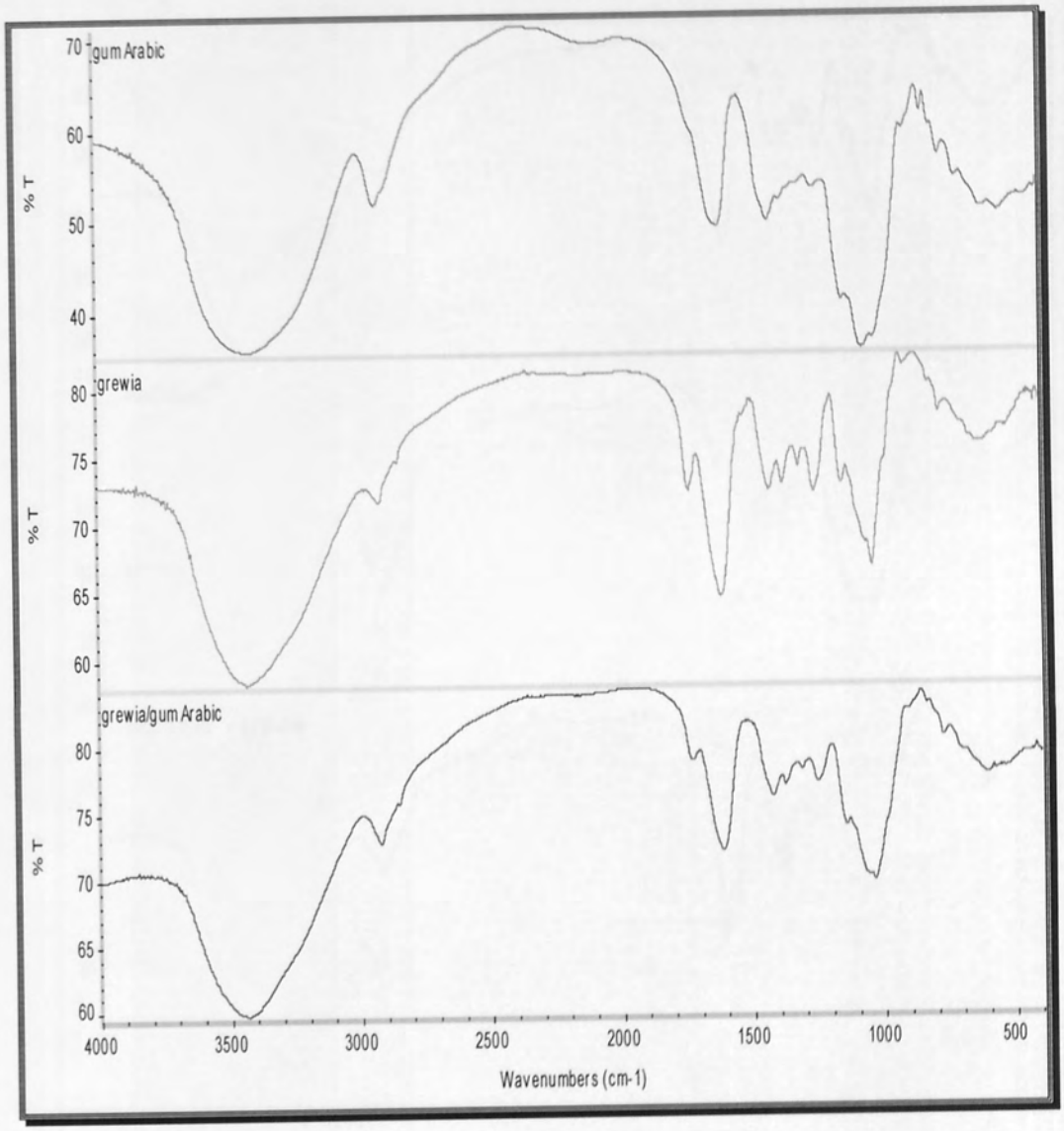
Therefore the fill weight of granules required to deliver 500mg of ibuprofen was 935 g.



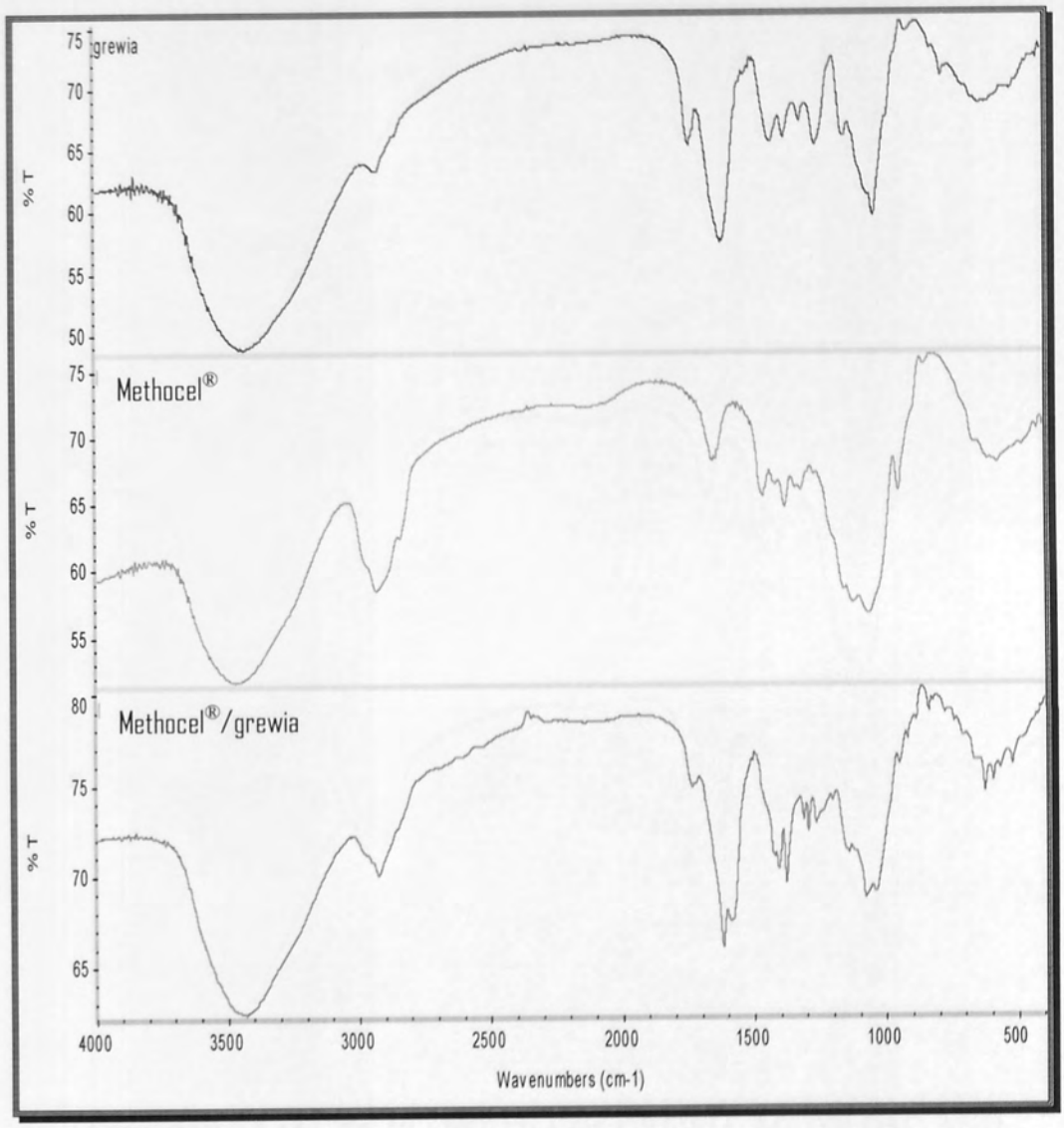
APPENDIX A1.7: FT-IR SPECTRA OF GREWIA, CMC, AND CMC/GREWIA (1:1) PHYSICAL MIXTURE



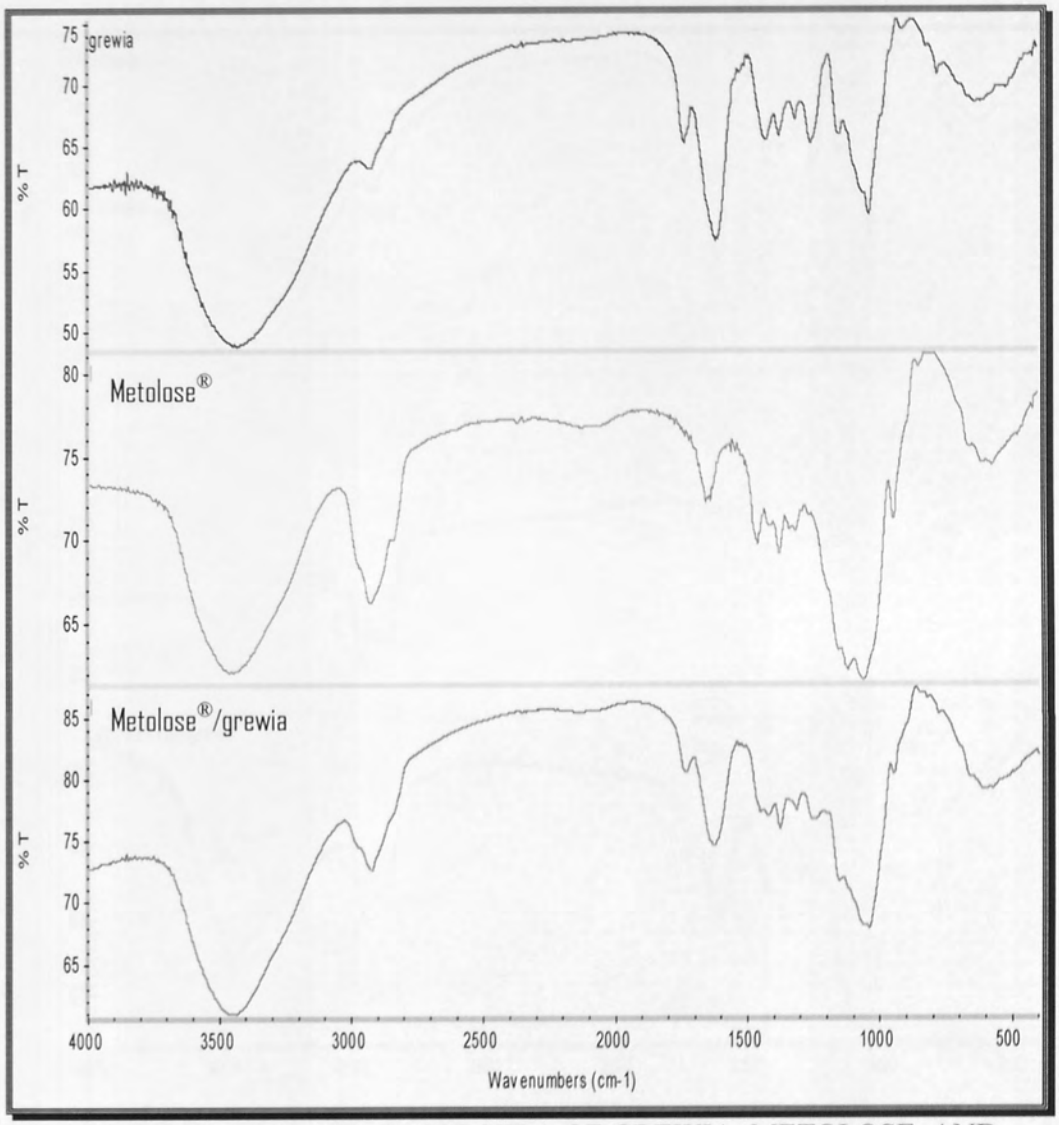
APPENDIX A1.8: FT-IR SPECTRA OF GREWIA, GUAR, AND GREWIA/GUAR (1:1) PHYSICAL MIXTURE



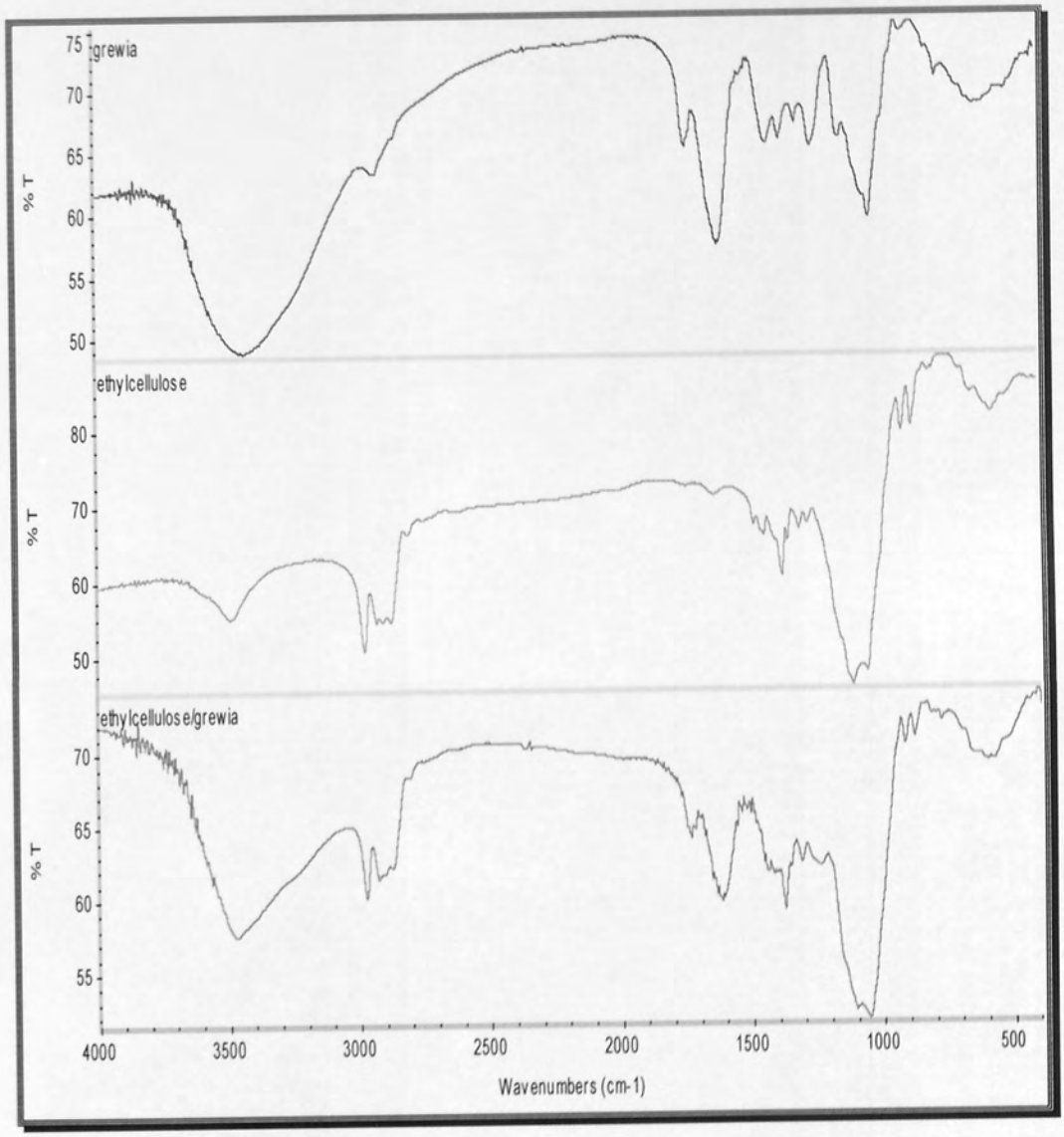
APPENDIX A1.9: FT-IR SPECTRA OF GREWIA, GUM ARABIC, AND GREWIA/GUM ARABIC (1:1) PHYSICAL MIXTURE



APPENDIX A1.10: FT-IR SPECTRA OF GREWIA, METHOCEL, AND METHOCEL/GREWIA (1:1) PHYSICAL MIXTURE



APPENDIX A1.11: FT-IR SPECTRA OF GREWIA, METOLOSE, AND METOLOSE/GREWIA (1:1) PHYSICAL MIXTURE



APPENDIX A1.12: FT-IR SPECTRA OF GREWIA, ETHYL CELLULOSE, AND ETHYL CELLULOSE/GREWIA (1:1) PHYSICAL MIXTURE