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Hypercoiling & Hydrophobically Associating Polymers

Interfacial Synthesis, Surface Properties & Pharmaceutical Applications

STEPHEN RONALD TONGE

Doctor of Philosophy

THE UNIVERSITY OF ASTON IN BIRMINGHAM

September 1994

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The University of Aston in Birmingham

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Summary

The objective of the work described was to identify and synthesize a range of biodegradable hypercoiling or hydrophobically associating polymers to mimic natural apoproteins, such as those found in lung surfactant or plasma apolipoproteins.

Stirred interfacial polymerization was used to synthesize potentially biodegradable aromatic polyamides (M_W of 12,000-26,000) based on L-lysine, L-lysine ethyl ester, L-ornithine and DL-diaminopropionic acid, by reaction with isophthaloyl chloride. A similar technique was used to synthesize aliphatic polyamides based on L-lysine ethyl ester and either adipoyl chloride or glutaryl chloride resulting in the synthesis of poly(lysine ethyl ester adipamide) [PLETESA] or poly(lysine ethyl ester glutaramide) (M_W of 126,000 and 26,000, respectively). PLETESA was found to be soluble in both polar and non-polar solvents and the hydrophobic/hydrophilic balance could be modified by partial saponification (66-75%) of the ethyl ester side chains.

Surface or interfacial tension/pH profiles were used to assess the conformation of both the poly(isophthalamides) and partially saponified PLETESA in aqueous solution. The results demonstrated that a loss of charge from the polymer was accompanied by an initial fall in surface activity, followed by a rise in activity, and ultimately, by polymer precipitation. These observations were explained by a collapse of the polymer chains into non-surface active intramolecular coils, followed by a transition to an amphipathic conformation, and finally to a collapsed hydrophobe. 2-Dimensional NMR analysis of polymer conformation in polar and non-polar solvents revealed intramolecular associations between the hydrophobic groups within partially saponified PLETESA. Unsaponified PLETESA appeared to form a coiled structure in polar solvents where the ethyl ester side chains were contained within the polymer coil.

The implications of the secondary structure of PLETESA and potential biomedical applications are discussed.

Keywords: Amino acids, drug delivery, lung surfactant, surface activity.

Dedication

I dedicate this work to my Mother, Father and brother, Simon, in gratitude for their constant support and encouragement throughout the course of my studies.

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I would like to thank the following people and organizations for their help and support in the furtherance of this work:

Professor Brian Tighe for sharing his thoughts and ideas so readily, and who, in the words of Jorge Luis Borges, "Changed the shapes of my dream",* Dr. Ann Jarvie for advising me so wisely on my journey through the unfamiliar landscape of organic chemistry, Dr. Michael Perry for his tireless enthusiam in identifying the conditions necessary for NMR analysis of the polymers described, Dr. Simon Holland for his help in conducting measurments of interfacial tension and for material support throughout the research, SmithKline Beecham Pharmaceuticals for their financial assistance and finally the Science and Engineering Research Council for their financial support.

* from a poem entitled 'New England 1967'.

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List of Tables.

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List of Abbreviations.

Apo(A,B,C,E)	Apoprotein.
ATR-FTIR	Attenuated total internal reflection - Fourier transform infrared spectroscopy.
^{13}C	Carbon 13 isotope.
CMC	Critical micellar concentration.
COSY	Correlated spectroscopy.
δC	^{13}Carbon-NMR chemical shift.
δH	Proton-NMR chemical shift.
DMF	Dimethylformamide.
DMSO	Dimethyl sulphoxide.
DNA	Deoxyribonucleic acid.
DPPC	Dipalmitoyl phosphatidylcholine.
DSC	Differential scanning calorimetry.
GPC	Gel permeation chromatography.
^{1}H	Proton.
HDL	High density lipoprotein.
LDL	Low density lipoprotein
LETES	Lysine ethyl ester.
NMR	Nuclear magnetic resonance.
NOESY	Nuclear Overhauser enhanced spectroscopy.
PAA	Poly(acrylic acid).
PC	Phosphatidylcholine.
PEAA	Poly(2-ethacrylic acid).
PENDANT	Polarization enhancement nurtured during attached nucleus test.
PG	phosphatidylglycerol.
PLETESA	Poly(lysine ethyl ester adipamide).
PLL	Copolymer of L-lysine and 1,3, benzenedisulphonyl chloride.
POE	Poly(oxyethylene).
POMEG	Poly[(tert-butyloxycarbonylmethyl) glutamates].
PSTY/MA	Styrene/maleic anhydride copolymer.
PVP	Poly(4-vinylpyridine).
RDS	Respiratory distress syndrome.
SEM	Scanning electron microscopy.
SP(A,B,C,D)	Surfactant protein.
STY/MA	Styrene maleic anhydride.

CHAPTER 1

INTRODUCTION

"The study of organic chemistry tends, in its most fruitful and significant latter-day developments, to become less the wide-ranging study of 'the compounds of carbon' and more the study of the 'organic' aspects of carbon compounds, i.e., the study of those aspects bearing on the structure and behaviour of living organisms." *

* Mark, H. & Whitby, G.S. from, Collected Papers of W.H. Carothers on High Polymeric Substances. 1940.[1]

1.1. Degradable Hypercoiling & Hydrophobically Associating Polymers: Analogy to Protein Structure.

Living systems are composed of macromolecules which change their shape and, hence, their function in response to environmental stimuli, for example in the 'unzipping' of the DNA prior to cellular replication, the opening of ion channels in membrane proteins during nerve conduction and the sliding together of actin and myosin during muscle contraction.[2] This dynamic aspect of natural macromolecules is an essential characteristic of all living systems, indeed, the ability to change is the only enduring feature in these systems. To some extent, we may all, at least in part, be considered as the sum of the movements of our macromolecules or when viewed holistically, as a collection of dynamic polymers aware of their own existence.

On a philosophical basis, if biomaterial science is to fully mimic biological systems, in order to replace non-functioning organs, we must employ macromolecules which possess such dynamic behaviour. The ability of certain polymers to hypercoil or hydrophobically associate offers one possible mechanism by which macromolecules could be made to change their conformation, and therefore, their function, in response to local stimuli. Polymers with weakly charged pendant groups, i.e., either weak acids or bases, form an extended chain structure as a result of mutual repulsion between the charged groups. If, in addition, the polymer also bears alkyl pendant groups, then the latter will be subject to hydrophobic interactions which will tend to restrict the alkyl side chains to a minimal volume within an aqueous environment, thereby, allowing maximal hydrogen bonding to occur between the water molecules. This, so called, 'hydrophobic effect', is the principal driving force in the formation of lipid based assemblies such as those found in biological membranes and in defining the conformation of native proteins.

Polymers bearing weakly ionizable pendant groups that form weak bases such as pyridine or imidazole are substantially charged in acid conditions or those below their pK_b value, resulting in a high degree of charge repulsion within the molecule. This ionic repulsion overcomes hydrophobic interactions between alkyl side chains within such polymers and leads to uncoiling of the polymer chain. Conversely, as the pH is raised the proportion of charged pendant groups falls and hydrophobic interactions between the alkyl side chains become the predominant factor, causing the polymer chain to progressively collapse into distinct hydrophobic microdomains. This effect is known as hydrophobic association and is often referred to as hypercoiling, if allowed to proceed this process results in the formation of a compact, insoluble, globular molecule

which precipitates from aqueous solution. A non-uniform loss of charge occurs during the hypercoiling process and leaves some areas of the polymer chain in a substantially charged state, so forming an amphipathic molecule. Amphipathic molecules such as those described are usually surface active. Therefore, functional properties such as surface activity can be 'switched on or off' in response to changes in the pH of the surrounding aqueous solution.

Conversely, polymers possessing pendant alkyl chains and weakly charged, negative pendant groups, e.g. carboxylic acid, exhibit an extended chain conformation at pH values above their pK_a where a substantial proportion of the pendant groups will be charged and intrachain repulsion will ensue. Lowering of the pH to a value below that of the pK_a will result in a net loss of charge and lead to hydrophobic forces becoming predominant, causing the extended polymer chain structure to progressively collapse to form an amphipathic molecule. Hence, by substituting either weakly cationic or anionic pendant groups onto a polymer backbone the polymer can be made to respond to either increases or decreases in pH. This mechanism offers us one method by which polymer molecules can be made to respond to the pH of their environment, and possibly behave in a similar manner to the responsive macromolecules found in nature.

However, it should be noted that protein molecules do not hypercoil, per se, but are subject to hydrophobic associative forces, although, changes in pH may result in the formation of certain secondary structures within proteins, such as the α-helix or β-pleated sheet conformations. Proteins do make use of the hydrophobic effect, not as a result of changes in the degree of ionization of their side chains induced by variation of pH, but in response to a variation of the surrounding hydrophobic environment, e.g. by responding to changes in fluidity of the bilayer lipid annulus surrounding membrane proteins. The latter appears to consist of a tightly bound boundary layer,[3] such as that identified around acetylcholine receptors[4]. Variation of the hydrophobic environment is energetically a far more efficient method of inducing conformational change in macromolecules than that produced by changing the degree of macromolecular ionization and this may offer a teleological explanation for conformation changes observed in nature.

Hypercoiling or hydrophobically associating switchable polymers have two essential features that may be applied to biological systems to confer upon the latter an ability to change their structure, and hence, their function:

1) To act as a simple switch either in an 'on' or 'off' state.
Such molecular switches may be considered analogous to the digital switching mechanism found within integrated circuits and may, when arranged in multiple arrays, enable complex tasks to be performed.

2) To function as the trigger for a cascade mechanism, where uncoiling of the molecule will result in co-operative effects that facilitate further change of molecular shape, and hence, lead to greatly altered function.

Both of the above characteristics are fundamental in determining the dynamic aspects of living systems.

In summary, hypercoiling polymers can be said to mimic the behaviour of some of the functions which account for the essential living processes, and may therefore, be suited for application to living systems and in particular to human medicine. They have considerable potential for use in drug delivery systems and as synthetic apoproteins but have not been applied to living systems, either because they are not biodegradable, or because they are likely to degrade into potentially toxic products. To date, only one hypercoiling polymer which is likely to biodegrade, a polysulphonamide, has been described in the literature[5,6] i.e., the copolymer of L-lysine and 1,3, benzenedisulphonyl chloride (designated PLL) shown in Figure 1.1, but is unlikely to be hydrolysed into physiologically acceptable breakdown products. In general, these molecules may be considered as behaviourally analogous to proteins and other biological macromolecules, in that, they may share the same remarkable ability to change shape and function in response to local stimuli, but achieve this through a series of somewhat different structural modifications.

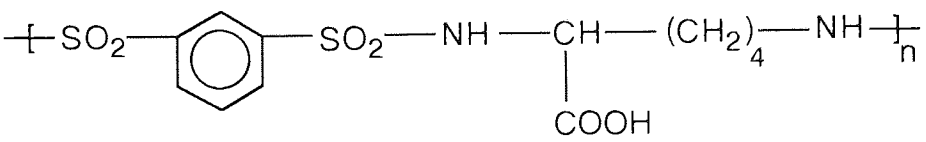


Figure 1.1. Poly(lysine 1,3-benzene disulphonyl chloride) - PLL

1.2. Factors Influencing Polymer Conformation in Solution.

The conformation of a solvated macromolecule can be considered to result from a balance between the following forces acting both within the molecule and between the molecule and surrounding solution:

1) Electrostatic repulsion between charged subgroups.
2) Van der Waals cohesion of uncharged side groups.
3) Hydrogen bonds, e.g. between amide bonds.
4) Interaction with the solvent, e.g. hydrophobic effects.

The solvent-polymer interaction can be regarded as the most important single factor in determining the conformation of solubilized polymers in general, and hypercoiling or hydrophobically associating polymers in particular, since the latter tend to undergo extensive conformational changes in solution.

1.2.1. The Hydrophobic Effect.

The hydrophobic effect can be considered as a restriction of non-aqueous components into the smallest possible area, in order to minimise disorder within the aqueous phase.[7] This property was first recognised by Traube in 1891, who described the ability of water to push out, or to the surface of a solution, components which disrupt bonding between the water molecules. It was soon realised that the attraction of water for itself is energetically the overriding influence in aqueous systems and experimental studies led to the introduction of Traube's Rule, which relates dissolution to molecular size and states that for each additional methyl group in an alkyl chain the surface to bulk ratio rises by a factor of three. Hence, within a body of water, even quite short aliphatic chains will produce a large hydrophobic cavity. Longer aliphatic chains will induce a proportionate increase in the hydrophobic force, thereby limiting, their incursion into the hydrophilic environment. The term 'hydrophobic effect' is somewhat of a misnomer for this phenomenon which in reality is an anti-hydrophilic, rather than a pro-hydrophobic effect.

Application of Traube's Rule to polymers allows us to predict that the presence of sizeable aliphatic components within a polymer will result in exclusion of the polymer from the aqueous environment and cause the polymer to collapse or hypercoil into hydrophobic domains. To observe the magnitude of these changes we need to compare

the free energy of interaction of a hydrophobic group with both water and a hydrophobic solvent. The difference between the free energy of interaction with the two solutions is found to be directly dependant upon the number of carbon atoms in the hydrophobic chain, that is, proportional to the surface area of the hydrophobic cavity.[7] The presence of a double bond will reduce the size of this cavity, as will an aromatic grouping. Therefore, the relative efficacy of pendant groups in forming a hydrophobic centre is as follows: saturated aliphatic chain>unsaturated chain>aromatic group, respectively.

It should be noted, that other non-organic solvents containing polar molecules also have an ability to resist incorporation of non-polar groups. Although, none are as effective as water in excluding hydrophobic components. This property is, in turn, profoundly affected by changes in the nature of the solvent and the presence of additional components. The effect of solvent environment upon polymer conformation will be discussed in detail for each of the hypercoiling polymers reviewed in this thesis. However, some general points are applicable to all hypercoiling polymers and are outlined below by reference to certain specific examples which will be discussed in greater detail in the review section. Particular attention is paid to the polycondensate of L-lysine and 1,3,benzenedisulphonyl chloride,[5,6] which as noted, can be considered to be the only example of a biodegradable hypercoiling polymer.

It should be noted, though, that PLL is not a typical hypercoiling polymer, in that, its compact conformation is only stabilised by the addition of hydrophobic groups such as tetrabutyl ammonium or organic dyes. These agents either bind electrostatically to the pendant carboxyl groups or are entrapped in hydrophobic domains formed by association between the benzene rings within the polymer backbone. Although, a combination of the two mechanisms seems most likely. This is distinct from other hypercoiling polymers where the density of hydrophobic pendant groups is sufficiently high to induce chain collapse directly upon neutralisation of the ionized pendant groups

1.2.2. Effect of pH.

Changes in hydrogen ion equilibria usually induce the most profound conformational transitions within hypercoiling polymers and can easily be monitored by potentiometric titration. Hypercoiling polymers exhibit a deviation from the normal curve of pH change vs. degree of dissociation (α) or ionization. In a simple polyelectrolyte, e.g. poly(acrylic acid) [PAA], the degree of ionization is directly proportional to the pH of the solution. However, in a hypercoiling polymer a

discontinuity in the pK_a is apparent as the polymer changes from a compact to extended chain conformation: as the polymer backbone uncoils ionizable groups become progressively more accessible, and hence, are more readily able to gain or lose protons, resulting in a changed dissociation constant (K), as the polymer effectively becomes a stronger acid.

In the case of anionic hypercoilers, addition of alkali causes the weak acid pendant groups to gradually become ionized and mutually repel one another, leading to an uncoiling of the polymer backbone. This process acts to expose further uncharged pendant groups, which in turn, neutralise more alkali, and as a result additional alkali causes only a small change in pH, leading to the presence of an inflection in the titration curve.[8]

The degree of dissociation (α) can be calculated using the following equation (Equation 1.1):

$$\alpha = \frac{[(NaOH+(H+)-(OH-)]}{Cp} \qquad (1.1)$$

Where Cp = polymer concentration.

When expressed as log pH vs. degree of dissociation (α), the conventional titration curve is linearized, such that the dissociation constant (K) can be deduced directly from the gradient. If data obtained from titrating a hypercoiling polymer is expressed in a this manner a change in slope is observed as the polymer molecules uncoil from a compact conformation to form an extended chain such as that adopted by a conventional polyelectrolyte.[9]

Information regarding the free energy change associated with conformational transitions can also be obtained from titration data by calculating the rate at which the dissociation constant (K) changes with ionization, i.e., the change in slope of pK vs. α. When this technique is applied to poly(ethyl acrylic acid)[10] values of free energy change of around 1500 J/mol have been reported, compared to 500 J/mol for poly(methacrylic acid). Similarly, the ionization value at which conformational transitions occur in alternating copolymers of maleic anhydride and alkyl vinyl ethers is shifted towards higher values with an increase in length of hydrophobic side chains. Using such titration data, Dubin & Strauss[11] compared results from butyl and hexyl vinyl ether copolymers of maleic anhydride and found that a difference in free energy of transition of approximately 1600 J/mol was contributed by each additional methylene

group substituted onto the hydrophobic side chain. Hence, as the side chains become shorter interactions between the chains are less efficient and the corresponding free energy associated with any conformational change is greatly reduced. Ethyl side chains appear to represent the minimum chain length needed to elicit hydrophobic association at least in the case of copolymers of maleic anhydride and alkyl vinyl ethers.[10]

Potentiometric titration also offers a powerful, if indirect method, for deducing the effect of other factors on polymer conformation, e.g. presence of dissolved components within the solvent and the nature of the surrounding media. Such factors will be reviewed below.

1.2.3. Effect of Dissolved Salts.

Changes in the tonicity of solutions containing a hypercoiling polymer, by the addition of salts, leads to a reduction of intrachain electrostatic repulsion within the polymer by a process known as charge shielding. The presence of long chain counter ions capable of binding to ionized pendant groups within such polymers will, themselves, result in hydrophobic association and this will tend to overcome any ionic repulsive effects and so act to stabilise polymers such as PLL[8] in their compact conformation.

1.2.4. Effect of Organic Components.

If water is replaced by an organic solvent or urea then hydrophobic interactions between side chains, e.g. in PLL, will be diminished or destroyed and this results in a reduction or complete disappearance of the compact form of the molecule. In organic media (such as an acetone/water mixture) a monotonic increase in both pH and viscosity is observed upon ionization and this behaviour is indicative of a conventional polyelectrolyte. Similarly, the presence of urea causes a rise in solution viscosity by interfering with water structuring, thereby, minimising the hydrophobic effect which would normally act to collapse the polymer chain. In 8M urea solutions discontinuities in viscosity vs. titration curves completely disappear.[8]

Such conformational effects are also readily studied by observations of optical properties. The optical rotatory power of PLL, for example, is dependant upon the hydrophobic nature of the cation present when measured in a solution of 21% acetone/water. At higher proportions of acetone the hydrophobic effects are overcome

and the optical properties are independent of dissolved cations. In these circumstances conformational changes are directly proportional to the degree of ionization and can be can predicted from the Henderson-Hasselbach equation where K_a is assumed to be a constant (Equation 1.2):

$$pH = pK_a + \log \frac{\alpha}{1-\alpha} \qquad \begin{array}{l} \text{at } 0.5 = \alpha \\ pH = pK_a \end{array} \qquad (1.2)$$

The opposite effect is caused by increasing the number of hydrophobic side chains associated with the polyelectrolyte, e.g. by the addition of counter ions such as tetrabutylammonium or dyes. Lipid soluble dyes such as acridine orange have been shown to reduce the viscosity of PLL over the range of ionization where conformational changes are found to occur. In this case, the conformational changes observed within the polymer occur at higher and higher values of ionization as the concentration of dye is increased and this can be explained by the ability of the dye to stabilise the compact conformation.

1.2.5. Polymer Side Chain Length.

Stabilisation of the compact state of a hypercoiling polymer also becomes more pronounced with the length of the apolar side chains, i.e., hydrophobicity, increases. This is manifest as a shift in the conformational transition zone to higher values of ionization (α), e.g. in PLL, α is normally in the region of 0.15-0.2 but rises to 0.7 upon reaction with butyl ammonium ions.[8] As expected, discontinuous rises in viscosity are also observed with ionization in such polymers as the mean dimensions of the polyion suddenly increase over the range $0.2<\alpha<0.7$, as electrostatic forces overcome cohesive hydrophobic forces which act to stabilise the polymer in its compact state. Similar discontinuities are observed in optical properties.

1.3. Degradable Polymers.

The major disadvantage of using vinyl-based hypercoiling polymers for medical applications is the inability of the body to degrade such molecules. To be, truly, applicable to living systems synthetic hypercoiling polymers must be able to be degraded into harmless non-toxic metabolites. Such an approach would enable these polymers to be directly applied to human subjects without concern over the fate of potentially harmful degradation products. Ideally, such polymers would degraded into components already present in the tissues.

The work described herein attempts to identify potential polymeric structures likely to fulfil the above criteria. To satisfy the first requirement, i.e., to make a polymer with a biodegradable backbone, either ester or amide groups or a combination of the two, could potentially be incorporated into a polymer and so render the polymer susceptible to hydrolytic cleavage by esterases or proteases, respectively. Examples of both enzyme types are widely distributed throughout the body. The existing range of degradable polymers is briefly reviewed below.

1.3.1. Polyesters & Polydepsipeptides.

A wide selection of biodegradable polyesters have been synthesized and include poly α-esters derived from α-hydroxy carboxylic acids, such as poly(glycolic acid) or poly(L-lactic acid) or combinations of the two. The latter materials are principally used as resorbable suture materials (Vicryl^{TM} and Dexon^{TM}). Biodegradable poly α-esters that contain pendant groups may also be suitable for incorporation into hypercoiling polymers, these include carboxylic acid groups, e.g. in poly(tartronic acid),[12] or hydrophobic side chains, e.g. in poly(hydroxybutyrate), poly(hydroxyvalerate), poly(hydroxycaprolate), poly(phenyllactic acid), poly(lactones), poly(valerolactones) and poly(caprolactones).[13] These polymers have primarily been proposed for use as biodegradable implants, e.g. in drug delivery systems.[14]

All of the polymers described above have been synthesized using chemical rather than biochemical techniques, although, a study by Fritzsche[15] has exploited the micro-organism *Pseudomonas oleovorans.* to synthesize polyesters. These organisms produce homopolymers and copolymers with novel pendant groups, including both aliphatic chains and phenyl rings, when grown on a medium rich in either n-alkenes or n-alkanoic acids. This technique, potentially, offers the possibility of synthesizing

polyesters bearing a wide range of pendant groups some of which may possess an ability to hypercoil.

A further subclass of biodegradable polymers which may be of use in the proposed application includes a range of copolymers containing both ester and amides linkages within their backbone, i.e., polyester/polyamides, known as polydepsipeptides. These polymers were initially synthesized by reacting α-amino acid N-carboxy anhydrides with α-hydroxy acid anhydrosulphites.[16] Subsequent work has attempted to define the degradation patterns of various polydepsipeptides,[17,18] in order to fabricate a solid implantable polymer suitable for sustained drug release. These polymers appear to biodegrade in biological environments at three different rates, i.e., parabolic, sigmoidal or linear, depending upon their constituents. Polyamino acid containing polymers exhibit a parabolic degradation pattern, while poly α-esters show a sigmoidal form. It was, therefore, reasoned[17,18] that polydepsipeptides would show a combination of the two, i.e. a linear degradation rate, which would be most advantageous for drug release. In addition, the pattern of degradation in a polymer consisting of alanine, aminoacyl glutamine ethyl ester and hydroxyacyl was found to be retarded by increases in the size of the side chain residues of the aminoacyl and hydroxyacyl units and was more readily degraded by esterases than proteases,[17] and by the incorporation of two alanine residues in place of three.[18]

1.3.2. Polyamides.

Polyamides may, in some, circumstances also be considered as a class of biodegradable polymers, proteins are but one example. Poly(α-amino acids) have been extensively investigated as biodegradable polymers for a wide variety of biomedical applications.[19] Their main advantage is their ability to degrade into physiologically acceptable molecules, a property that is hoped will impart superior biotolerance. Solid inserts prepared from poly(amino acids) are readily degraded from the site of implantation, obviating the need to remove the implant once depleted of its bioactive contents. The polymer backbone used in these materials has generally comprised of repeating polyamide groups arranged in a pattern characteristic of that found in proteins.

Within polyamino acids the pendant side chains are generally modified to give the desired functional or mechanical properties. Examples include random copolymers of γ-benzyl-L-glutamate and L-leucine[19] and modified poly(glutamic acid) derivatives such as poly[(tert-butyloxycarbonylmethyl) glutamates] (POMEG), the hydrophilicity of

which can be adjusted to meet specific *in vivo* release requirements by variation of the ester content.[20]

The presence of a planar amide bond[21] within the backbone of protein molecules and poly(amino acids) constrains free rotation, thereby, limiting segmental rotation of the backbone, and thus, preventing hypercoiling. Consequently, the polyamide backbone within proteins tends to adopt a ribbon-like structure. Conformational changes occur as amino acid side chain residues lose their charge and result in a transition from a random coil to a spiral or helical structure,[22] further coiling is then prevented by the semi-rigid structure of the backbone. Such transitions have been extensively studied in amino acid homopolymers, e.g. poly(L-histidine).[23-25] The latter polymeric agents have been employed as model systems for investigating conformational changes exhibited by proteins. Although, coil-to-helix transitions undergone by both poly(amino acids) and proteins during denaturation may be considered as analogous to the transitions that occur in hypercoiling polymers, they are, in fact, a distinct and far more limited form of the latter.[8]

1.3.2.1. Conformational Changes in Poly(L-histidine).

Poly(L-histidine) undergoes a conformational transition as the side chain residues become deprotonated. Such changes occur as the imidazolinium ion content falls from 60 to 40% and become apparent as a marked discontinuity in various physical parameters, e.g. optical rotation (circular dichroism),[23-26] potentiometric titration,[25] microcalorimetry[25] and solution viscosity.[26] The polymer initially forms an α-helix as the pH is raised between 5 and 6, whether right-handed[24,26] or left-handed[23] remains the subject of some debate, then proceeds to form a β-pleated sheet structure above pH 6,[24,25] and finally precipitates out of solution as the pH is raised still further. This behaviour is in complete contrast to monomeric imidazole which remains in solution above pH 6.

Synthetic hypercoilers and globular proteins both have the ability to form distinct hydrophobic domains within an aqueous environment. The same functional goal is achieved in both cases although the mechanisms employed are quite different. Synthetic hypercoilers can be considered to operate on a molecular scale, to form a locally compact region within the polymer chain. In contrast, globular proteins operate on a macromolecular scale, where large portions of the polymer chain are arranged in fixed conformations in order to orientate hydrophobic residues to a particular zone, as defined by the secondary and tertiary structure.

Cohesive intramolecular forces are recognised as the major factor in determining the compact conformation of globular proteins and, in-part, result from covalent intrachain bonding between cysteine residues as well as from hydrophobic interactions. Protein structures formed in this way have far smaller molecular dimensions than synthetic hypercoiled polymers of similar molecular weight.[27] There is no indication though, that nature makes use of the hypercoiling effect, per se, by using localised changes in pH to collapse protein structure.

1.3.2.2. Polyamino Acids & Polymers Derived from Amino Acids.

Amino acids can be considered as potential monomers for incorporation into synthetic hypercoiling polymers, since in solution they possess a range of both weakly charged and hydrophobic side chain residues. In addition, polymers synthesized from amino acids may release the amino acids monomers upon degradation. To overcome the constraining effect that the amide group has upon rotation of the polymer backbone, and therefore, hypercoiling, amino acids need to be incorporated into the polymer through functional groups located on their side chains and not exclusively via the amine and carboxyl groups substituted at the α-carbon atom.

Recently, Kohn,[28] has reported a series of 'pseudo' polyamino acids in which the conventional repeating peptide bonds of a polypeptide backbone are replaced by a variety of non-amide linkages. Using this technique the author has synthesized a series of polyesters from a hydroxyproline derivative,[29] tyrosine[29] and a serine ester.[30] Poly(iminocarbonates), as shown in Figure 1.2, and poly(carbonates),[29] have also been obtained by using dipeptides as starting materials. In the latter case, the polymerization step involved reacting two phenolic hydroxyl groups located on the side chains of a protected tyrosine dipeptide. The phenolic groups were cyanylated and then reacted with additional diphenol groups to produce a poly(iminocarbonate). The objective of the work of Kohn *et al.* has been primarily to use simple techniques to produce melt processable polymers with good mechanical properties, e.g. by incorporating aromatic groupings into the polymer backbone. All of the 'pseudo' polyamino acids so far reported have been proposed for use as solid, bioerodible implants.

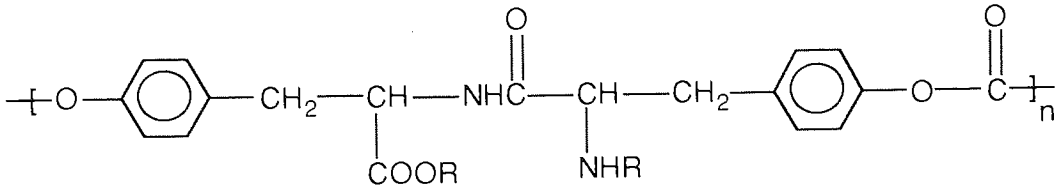


Figure 1.2. Tyrosine-based poly(iminocarbonate).

In addition, other authors[31] have described amine-functionalized aliphatic polyamides synthesized by polycondensation of ornithine and aspartic acid to form water soluble, degradable polymers. These polymers have been proposed as drug carriers but lack hydrophobic centres, and therefore, are unlikely to hypercoil.

Polyanhydrides have also been prepared from amino acids by reactions involving side chain located amine and carboxyl groups.[32] This has been achieved by reacting an amino acid, e.g. β-alanine, with either a cyclic anhydride, to form an amino acid-succinamide, or with a diacid chloride, both by a process of melt condensation. In an alternative method, the pre-polymers were polymerized by direct reaction with diacid chlorides in solution.

Segmental rotation within a polyamide backbone can be increased by partially replacing the repeating amide groups with short interchain aliphatic groups. In this way the rotational constraints imposed by repeating amide groups within the backbone can be negated and the polymer made more susceptible to hypercoiling. Beaumais[5,6] adopted this approach in synthesizing a hypercoiling anionic polymer composed of a polycondensate of L-lysine and 1,3, benzene disulphonylchloride (PLL). This reaction involved an interfacial polymerization and used lysine ethyl ester (LETES) as a precursor[5]. In this case, the polymer produced, incorporated amine groups substituted on both the α-carbon and the ε-carbon atoms of the lysine side chain, into the polymer backbone. The ethyl ester group was partially (66%) removed by saponification in 2M NaOH for 2 hrs. and the resultant polyanion behaved as a diacid, i.e., existing in both a collapsed and extended state. It also formed complexes with copper (II) ions that enabled the microdomains within the polymer to be probed using U.V. spectrophotometry.

As previously described, PLL is likely to be subject to *in vivo* degradation, although its breakdown products may be toxic to biological systems. Other authors have reacted lysine ethyl ester with poly(ethylene glycol) derivatives, again by interfacial polycondensation, to produce a polyurethane copolymer with carboxyl pendant groups suitable for attachment of drug molecules.[33] However, aqueous soluble, synthetic hypercoiling polymers that biodegrade into harmless monomeric components within the body have yet to be reported in the literature.

1.3.2.3. Polyureas and Polyamides Based on Lysine and Lysine Ethyl Ester.

The polyurea shown in Fig 1.3 was described by Pirtskhalava[34] and is based upon L-lysine ethyl ester. Analysis of structural conformation using the methods adopted by Go and Scheraga,[35] for the conformational study of polypeptides, indicated that these polymers were likely to adopt a helical conformation when synthesized at low temperature, where head-tail polymerization of the monomer is favoured. However, the mathematical models applied are related to peptide backbones found in polypeptides, where the dimensions of the helix formed mandate that the side chain groups are exposed to the solvent, whilst the steric arrangement in lysine ethyl ester-based polyureas is quite different. In this case, the extended intra-amide methylene bridges may enable coils to form in which the side chain groups are entirely contained within the core of the helix. The authors do not appear to consider this possibility.

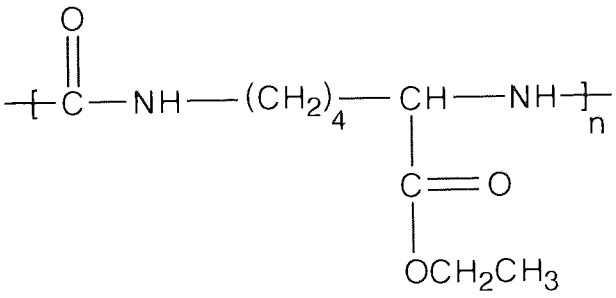


Figure 1.3. Polyurea-based on lysine ethyl ester.

Physicochemical investigations of the polyurea shown in Fig 1.3 reveal that the polymer[36] appears to adopt a random chain or helical conformation depending upon the solvent used for polymerization. The conformation was defined by determining the Mark-Houwink constants and by studies of optical rotation. A gradual transition from random coil to helix was observed as the polar solvent DMSO was diluted with non-

polar benzene, this would suggest that the ethyl ester side chains protrude from the polymer backbone into the non-polar solvent upon helix formation.

A lysine ethyl ester-based poly(urethane) has also been recently reported by Wiggins[37] in the form of poly(ethyl-2,6-diisocyanatohexanoate) and has been proposed for use as a rigid but absorbable tissue scaffold. Poly(lysine ester adipamide) and poly(lysine ester terephthalamide) synthesized by interfacial polycondensation of lysine esters and either adipoyl or terephthaloyl chloride were first reported by Hachihama in 1963[38] with a view to producing solid materials with novel properties.

Katsarava describes a series of polyamides which are particularly relevant to the synthetic work reported in this thesis. The polymers in question were synthesized by solution condensation of bis(trimethylsilyl), methyl and ethyl lysine with either adipic, terephthalic or isophthalic acid chlorides.[39] In an alternative technique, the acid chlorides were replaced with the corresponding diacyl and diaryl esters and a transesterification reaction was conducted. Subsequent saponification of the pendant ester groups of the polyamides yielded polymers with pendant carboxylic acid groups. In no instance, though, does this author suggest partial saponification as a means of producing a surfactant or difunctional molecule with a balance of both hydrophobic and hydrophilic groups. Additionally, saponification of the condensation product of lysine alkyl ester and isophthalic acid is not alluded to. Although, this polymer would, in itself, form a difunctional molecule when fully saponified and is described in this thesis.

Interestingly, the film forming abilities of the polymers described were reported to depend upon the solvent in which they were contained, suggesting that some solvents favour the formation of particular microstructures. Further investigations of the effect of microstructure,[40] based on optical rotatory dispersion studies and Drude co-ordinates, suggested that poly(lysine ester adipamide) adopts a particular microstructure. However, ^{13}C-NMR studies were reported to be non-informative in defining this microstructure. No suggestion is made by these authors that poly(lysine ester adipamide) or related polymers may hydrophobically associate. Coiling is assumed to result solely from intra-amide hydrogen bonding, as a consequence of the regular sequence distribution of amide groups within the polymer backbone. The principal focus of the work by Katsarava *et al.* appears to have been to synthesize biodegradable polymers that release metabolisable breakdown products and are structurally similar to polypeptides.

Other authors, namely, Saotome[41] and Ihara[42,43] have synthesized poly(lysine adipamide) by reacting lysine and adipoyl chloride interfacially. However, the intrinsic viscosity data reported for samples of poly(lysine adipamide)[41] suggest that only low molecular weight material was produced. This work was primarily focused upon studying the optical activity of these polymers, arising from the presence of an asymmetric α-carbon atom within the polymer chain. Nonetheless, these authors observed that in the presence of copper(II) ions an optically active polymer was produced indicating the formation of a regular microstructure. The copper (II) ions appear to co-ordinate the α-carbon amine group and carboxylic acid, and thereby, favour reactions between the amine groups substituted at the ε-carbon atom and so tend to form tail-tail polymer triads. However, the latter are unlikely to form helical sequences. Ihara[42,43] has attempted to use the asymmetric nature of poly(lysine adipamide) for selectively binding amino acids as a method of resolving L isomers. Neither Hachihama, Saotome nor Ihara refer to the likely surfactant properties of the polymers they describe or in the case of the alkyl ester, the possibility of forming a partially saponified derivative.

Copolymers of L-lysine and terephthalic acid have also been reported to form microcapsules by stirred interfacial polymerization of an oil-in-water emulsion of the comonomers.[44] The polyelectrolyte nature of this polymer was expected by the authors to confer pH sensitivity on the permeability of the microcapsules formed and it was hoped that this property could be used to fabricate responsive drug release devices. More recently,[45] Nylon 6 copolymers incorporating L-lysine have been prepared by melt condensation in an attempt produce a biodegradable fibre.

1.4. Functional Properties of Biopolymers.

Natural polymers (biopolymers) are able to achieve a precise control of their molecular architecture partly because they have a precise chain length and monodisperse molecular weight, but also because, they also have a defined sequence distribution and precise stereochemistry.[4] This degree of structural control is obtained by template synthesis, in the form of the genetic code contained in the base pair sequences of DNA. No single chemical technique currently exists to precisely control the parameters of molecular weight, sequence distribution and stereochemistry of synthetic polymers. It is, therefore, unlikely that any polymers synthesized using the techniques currently available in conventional polymer chemistry, will be able to mimic anything but the most basic functions of biopolymers.

The application of recombinant DNA technology to prepare polypeptides with a repetitive sequence distribution has recently been reported by several groups.[46-48] This technique offers the potential of being able to produce synthetic polymer materials which exhibit the same structural regularity and functional control as biopolymers. Control of sequence distribution, molecular weight and stereochemistry should enable the synthesis of polymers which interact with one another to form supramolecular architectures such as those described by Lehn[49] and Percec.[50] Such assemblies are defined by intra and intermolecular non-covalent bonding analogous to the secondary, tertiary and quaternary structures found in biopolymers. We can speculate that groups of hypercoiling synthetic polymers may undergo similar associations to form assembled structures equivalent to those formed by natural polymers and responsible for the essential living processes. Indeed, micellar assemblies between poly(ethacrylic acid) and phospholipid vesicles have been described by Tirrell[51] to form in aqueous solution. The hypercoiling phenomenon exhibited by synthetic polymers may be considered as representative of the secondary and tertiary structures present in proteins, while supramolecular polymer architectures may represent the quaternary structures found naturally.

In order to fully understand the nature of hypercoiling polymers and their relationship to biological surfactants, two literature reviews have been undertaken and are presented in chapters two and three.

CHAPTER 2

HYPERCOILING POLYMERS:

A REVIEW OF CHEMICAL STRUCTURE

& PHYSICAL PROPERTIES

2.1. Cationic Hypercoilers.

The purpose of this review is to demonstrate the hypercoiling phenomenon by illustrating the structure of polymers that exhibit hypercoiling behaviour and, in addition, to describe the techniques used to investigate this effect.

2.1.1. Poly(4-vinylpyridine)

Hypercoiling polymers synthesized by free radical polymerization were first described by Fuoss[52,53] in 1948-49 in a series of studies aimed at elucidating the behaviour of polyelectrolytes in aqueous solution. The term hypercoiling was not, however, adopted at this stage. These authors studied the effects of quaternizing poly(4-vinylpyridine) [PVP] with alkyl halides, e.g. butyl bromide, to form cationic alkylpyridinium pendant groups, see Figure 2.1. The combination of hydrophobic side chains and charged pyridinium groups conferred amphipathic properties upon the polymers, and as a result, these polymers were described as polysoaps.[54,55] By progressively increasing the number of hydrophobic side chains a transition from polyelectrolyte to polysoap was observed and monitored by potentiometric titration.

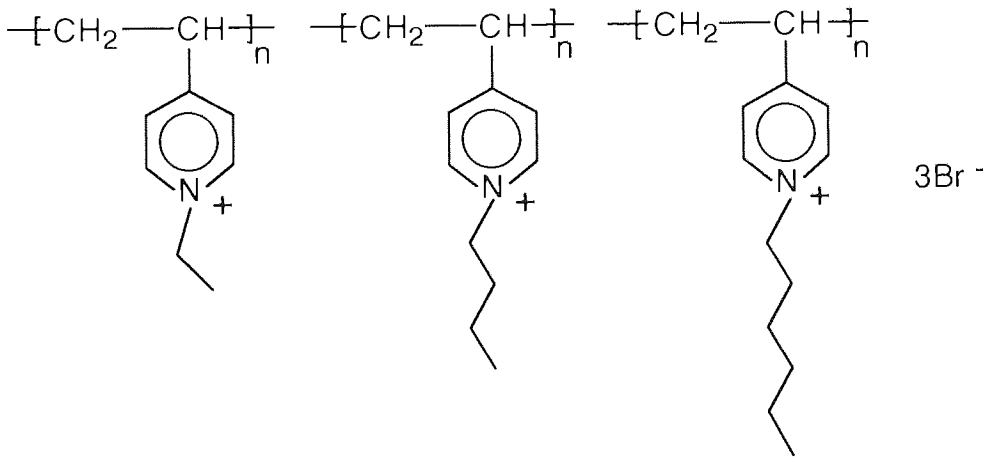


Figure 2.1. Quaternized poly(4-vinylpyridine).

Substituted PVP cannot be considered to be a typical hypercoiling polymer, in that, the molecules do not collapse as a result of deionization arising from variation of solution pH, but from an increase in hydrophobicity that occurs upon side chain substitution. However, many of the techniques used to study PVP have been applied to other hypercoiling molecules and exemplify the methods used to investigate this class of

polymer. Therefore, the role played by each technique in studying conformational changes within PVP molecules will be reviewed in detail.

2.1.1.1. Intrinsic Viscosity Studies of PVP Polyelectrolytes & Polysoaps.

Potentiometric titration, as discussed in the foregoing chapter, is the technique most easily applied to study hypercoiling behaviour. Indeed, this technique was employed by Fuoss[52] in the initial studies of butylated PVP. Measurements of intrinsic viscosity were also conducted to calculate polymer dimensions and this was achieved by applying the Debye Suggestion and dividing the intrinsic viscosity data by the Einstein Coefficient (0.025), to give a calculated specific volume for PVP of 14.4cm^3/g.

Viscosity measurements of butylated PVP in water[52] show that reduced viscosity ($\eta sp/c$) is highest at low polymer concentrations. This is typical behaviour for a polyelectrolyte; upon dilution of a polyelectrolyte below 1% the specific volume defined by a randomly coiled polymer can longer occupy the entire volume of the solution, and hence, the mobile counter ions, e.g. bromide, are free to dissociate into the solution.[56] This process results in the polymer chain developing a net charge and leads to mutual repulsion between charged groups, causing the chain to expand and the specific viscosity to rise, i.e., molecular volume rises upon dilution. Any increase in the concentration of counterions will tend to reverse this effect and cause chain collapse.

In further studies[57] the effect of progressively increasing the content of hydrophobic pendant groups upon a partially quaternized, ethylated PVP, was observed by increasing the proportion of n-dodecyl side chains from 6.7% to 37.9%. This strategy was adopted in order to maintain a constant charge density and observe the effect of hydrophobic substitution rather than electrostatic repulsion. Measurements of reduced viscosity showed that quaternized salts exhibited anomalous behaviour, where an increase in the proportion of dodecyl side chains led to a non-uniform decrease in reduced viscosity, with the greatest decrease occurring between 6.7% and 13.6% dodecyl substitution, indicating a marked fall in molecular volume of the polymer as the hydrophobic chains associate to form a compact structure. This effect may be considered as the intramolecular equivalent of critical micellar concentration.[57] The appearance of this effect at a very low dodecane percentage substitution clearly demonstrates the very strong influence of the hydrophobic effect. The intrinsic viscosities reported by these authors are two orders of magnitude smaller than those estimated for random coils of similar molecular weight. However, with very high

degrees of dodecyl substitution the side chains begin to interact with each other, as the polymer concentration increased, and cause the reduced viscosity to rise.

When salt was added to a solution of PVP, where the PVP had been 6.7% quaternized with dodecyl bromide a linear reduction in viscosity occurred, as expected for a polyelectrolyte, while in PVP quaternized with higher proportions of dodecyl bromide distinct minima became apparent as a result of polymer collapse. Reduced viscosity also fell with the dielectric constant of the solvent, i.e., as the solvent became more hydrophobic the polymer became less viscous. In 91.5% ethanol, butylated PVP appears less viscous than the unquaternized parent polymer. Hence, molecular dimension is essentially a product of counterion, solvent and substitution effects, where the dominant factor must be defined empirically.

It is also possible to monitor the formation of hydrophobic domains in aqueous polymeric solutions by measuring the solubilization of organic solvents. Solubilization of heptanol causes a decrease in reduced viscosity due to a reduction of electrostatic repulsion within the polymer, i.e., the organic effect. In contrast, higher amounts of heptanol encourage the hydrophobic side chains to become exposed, and thereby, interact to increase the reduced viscosity. This intermolecular association can be readily disrupted by a rise in temperature of 20°C and is discussed in greater detail in chapter 6 in relation to physical properties.

2.1.1.2. Light Scattering Studies - The Zimm Plot and Birefringence.

Strauss and Williams[58] investigated the properties of ethylated PVP when partially quaternized with n-dodecyl bromide, by using a Brice-Phoenix light-scattering photometer.* Substitution of more than 8% of the vinyl pyridine monomer units with dodecyl groups resulted in the formation of a compact structure. A Zimm plot of 10.3% dodecyl substituted PVP indicated unusual behaviour, in that the slopes were strongly negative, i.e., showing increased light scattering (reduced intensity) at low polymer concentrations. This behaviour indicated the formation of intermolecular aggregates or micelles as the polymer concentration was increased. More recently, NMR studies have confirmed the tendency for PVP quaternized with alkyl halides to form micelles.[59] In comparison to dodecyl substituted PVP, PVP substituted with 100% ethyl groups, exhibits a normal Zimm plot, where molecular size is seen to increase with dilution, behaviour typical of a polyelectrolyte.

In theta solvent conditions, where molecular dimensions are primarily influenced by short range intramolecular interactions, the dimensions of a 34.1% dodecyl substituted PVP were found to be in close agreement when measured in both isopropyl alcohol (IPA) and a standardized aqueous KBr solvent system. Application of the Flory-Fox equation, with Krigbaum improvements, enabled the molecular dimensions to be calculated. These indicated a sharp reduction in mean end-to-end distance in aqueous KBr solution, as the polymer became progressively substituted. The charge shielding of the polymer backbone induced by KBr enabled hydrophobic side chain interactions to predominate and led to a collapse of the polymer and the formation of micelles. In contrast, IPA presents a favourable environment for the dodecyl side chains, and therefore, in this medium no change in molecular dimensions were observed as the PVP side chains became progressively substituted with dodecyl groups.[58]

* If a molecule is larger than $\lambda/4$ of the incident light, then light rays scattered forward from different parts of the molecule will constructively interfere, while those back scattered will destructively interfere. Therefore, the radiation envelope for such a particle will be unsymmetrical with more light scattered backwards than forwards, such that maxima and minima occur at specific angles dependant upon the wavelength of incident beam. A Zimm plot makes use of this phenomena by comparing the intensity of light scattered at different angles in different concentrations of polymer solution. By extrapolation to a zero scattering angle and zero polymer concentration the molecular dimensions can be calculated, i.e., z-average mean square radius of gyration ($\overline{s^2}$) and the z-average mean square end-to-end distance ($\overline{r^2}_0$), where the dimensions do not reflect the influence of intermolecular effects.[60]

Streaming birefringence studies conducted by Jordan[61] on butylated derivatives of PVP demonstrated that the amount of scattered light (Tan α) fell in proportion to the polymer concentration and with added sodium chloride. Such studies are essentially based upon light scattering and give results analogous to the Zimm plot. Similar changes were also noted in measurements of reduced viscosity. In both cases, the polymer behaved in salt free solution as a simple polyelectrolyte, i.e., it expanded with dilution, but addition of sodium chloride caused charge shielding within the polymer backbone and enabled hydrophobic forces to become predominant, leading to a collapse of the polymer chain. The advantage of birefringence studies over light scattering studies and the use of Zimm plots, or studies of reduced viscosity, is that molecular dimensions can be calculated at high polymer concentrations by application of Peterlin's theory in place of the Flory Equation.

2.1.1.3. Comparison of Viscometric & Photometric Studies

Data obtained from both light scattering and intrinsic viscosity studies have been compared by applying the Flory Equation (Equation 2.2).[58] The results indicate a close agreement between the two methods for both 4.8 and 16.3% dodecyl substituted PVP. However, a difference was noted between the two methods when the 34.1% dodecyl substituted PVP was compared, suggesting formation of a compact conformation that could no longer be represented by the random coil model used for deriving the Flory equation. Interestingly, the 34.1% substituted polymer also failed to exhibit any further reduction in molecular dimensions when the concentration of KBr was increased In this case, charge shielding no longer affected the polymer which was already in a fully collapsed state. The dimensions of the 34.1% substituted polymer can be calculated by applying the Einstein Relation and this gives a value in close agreement to that found using data derived from both intrinsic viscosity and light scattering measurements, indicating that, in this particular instance, the polymer is more likely to exist as a compact spherical structure rather than as a random coil.

2.1.2. Poly(tertiary Amines).

Polymers with tertiary amine pendant groups have also been reported to hypercoil upon either N-protonation or N-alkylation of the amine side chains.[62] One such example is poly[thio-1-(N-N-diethyl)aminoethylethylene], the structure of which is shown in Figure 2.2. This is a polybase containing a thioether backbone and tertiary amine pendant groups, and may be methylated to form quaternary derivatives.

Deprotonation of this polymer results in the formation of distinct hydrophobic microdomains which were found to be readily destabilised or unfolded by adding protonating agents such as a strong acid. Structural changes were observed as discontinuities in potentiometric, viscometric, and photometric, e.g. measurements of laser light scattering, optical rotatory dispersion (ORD) and circular dichroism (CD). These changes were found to be completely reversible, and therefore, this molecule would appear to represent an ideal polymer in which to entrap water insoluble molecules for release at a specified pH.

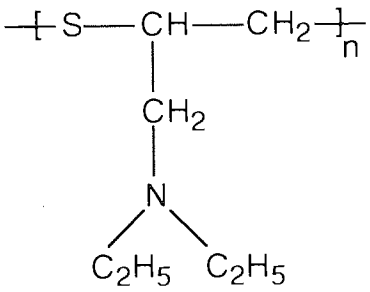


Figure 2.2. Poly[thio-1-(N-N-diethyl)aminoethylethylene]

Unlike the tertiary amine polymers, which become insoluble in aqueous solution upon deprotonation, partially methylated tertiary amines (quaternary amines), remain in solution. The formation of microdomains also occurs as the pH is raised between 6 and 7.5, making these polymers ideal for application to biological systems, e.g. for releasing drugs at a site-specific pH, while remaining in solution in a partially hypercoiled state.

2.1.3. Poly(vinylimidazole) Containing Copolymers.

Poly(vinylimidazole) as shown in Figure 2.3, substituted with alkyl side chains, has been reported to hypercoil,[63] and copolymers of N-vinyl imidazole and styrene have also been found to exhibit an amphipathic nature.[64,65] These effects have been investigated by studying the binding of anionic dyes, e.g. methyl orange.[63] Spectroscopic analysis shows that dye binding is co-operative in nature, whereby, the binding of one hydrophobic dye molecule facilitates the binding of another. Polycations possessing quaternized piperdyl groups in their backbone also behave in a similar manner in the presence of hydrophobic dyes.

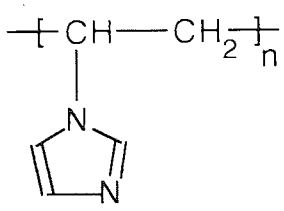


Figure 2.3. Poly(vinylimidazole)

A sharp conformational change is observed in poly(styrene-imidazole) copolymers upon protonation of the imidazole moiety and is manifest as a transition from a 'supercoiled' polymer, existing as an emulsion, to a water soluble extended chain.[65] In non-polar environments the copolymer occupies a smaller hydrodynamic volume due to the aggregation of the styrene side chains. Whereas, in aqueous solution styrene pendant groups form a water insoluble core and water soluble imidazole groups become arranged at the surface. This segmental reorientation is made possible by a high degree of flexibility within the backbone. In acidic media the polymer coil is extended due to electrostatic repulsion between the protonated imidazole groups, although, above pH 4 micelles begin to form and above pH 7 complete deprotonation of the imidazole groups leads to precipitation of the polymer from aqueous media. Further studies have shown that the flexibility of the poly(styrene-imidazole) copolymer chain is reduced when the protonated group is in close proximity to the polymer backbone and increased when the imidazole group is separated from the backbone by a flexible amide linkage.[64]

Fluorescent probes, in which emission is quenched in aqueous environments, e.g. pyrene and N-phenyl-1-naphthylamine, have been used to study conformational changes in these polymers.[65] Fluorescence polarisation offers an alternative technique which can be used to probe conformational change. Using this technique a change of polarisation is observed from low values of 0.05 at low pH, to higher values of 0.35 at high pH. Indeed, the styrene nucleus itself will fluoresce when excited at 260nm and emit as an excimer in a range from 300 to 350nm when 1-pyrenesulphonic acid, complexed at the imidazole site, is used as the acceptor. By applying this technique no energy transfer was found to occur between the two chromophores as the pH was adjusted to 3.5 as a result of chain collapse, which had the effect of separating the two domains.

2.2. Anionic Hypercoiling Polymers.

To date, the only anionic polymers which are known to hypercoil are polyacids containing carboxylic acid pendant groups either in the form of acrylic acid or maleic acid (maleic anhydride). These anionic groupings have been incorporated into alternating copolymers, where the hydrophobic moieties are provided by either methyl or ethyl acrylates, alkyl vinyl ethers, styrene comonomers or alkyl acrylamides. In such copolymers hydrophobicity is controlled by alkyl chain length, and charge density by variation of pH.

2.2.1. Poly(methacrylic) & Poly(ethacrylic) Acids.

Poly(methacrylic acid) solutions have been reported to exhibit a deviation from the behaviour expected of a typical polyelectrolyte upon neutralisation, such as that exhibited by poly(acrylic acid). This behaviour becomes apparent at ionization values of less than 30%.[66] Such deviation has been explained[10,66] in terms of a transition between a compact coil and a more extended conformation, and has been confirmed by NMR spectroscopic analysis.[67] A more pronounced transition is observed in poly(ethylacrylic acid), also known as poly(2-ethacrylic acid), as shown in Figure 2.4, and was first reported by Fichtner and Schonert in 1977[9] and subsequently by Joyce.[10] This polymer has been shown to exhibit a discontinuous change in pH upon neutralisation, characteristic of a hypercoiling molecule, where an apparent shift of the dissociation constant (K) is observed; from a high pK value for the coiled molecule to a lower pK value for the uncoiled molecule, in which state the carboxylic groups are more readily ionized.

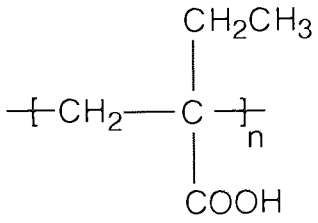


Figure 2.4. Poly (2-ethacrylic acid)

2.2.2. Copolymers of Acrylic Acid & Ethyl Acrylate.

Copolymers containing acrylic acid and ethyl acrylate, as shown in Figure 2.5, offer an alternative means of forming a hypercoiling polymer, where the ethyl ester group provides the hydrophobic nucleus. Dynamic light scattering studies using a 3:1 random copolymer of ethyl acrylate and acrylic acid[68] demonstrate that this copolymer behaves as a simple polyelectrolyte in solutions at low ionic concentration and possesses a high radius of gyration, while at concentrations of sodium chloride higher than 1.2M the polymer coil adopts a compact conformation. The particularly interesting finding of these studies is the sudden break in curves of radius of gyration vs. ionic strength, which clearly indicate a collapse in polymeric dimensions above a specific sodium chloride concentration. The transition observed is less well defined and occurs over a broader range of salt concentrations than similar changes in proteins.[69] Such behaviour is in complete contrast to that observed with poly(acrylic acid), where high salt concentration, i.e., conditions of maximal charge shielding, cause the polymer to adopt dimensions commensurate with that of a random coil conformation and similar to those of the unionized polymer in organic media.

Analogous changes have also been observed in measurements of intrinsic viscosity. In both cases, the effects observed can be explained by charge shielding, where the presence of counter ions in solution act to reduce intrachain repulsion between charged groups within the polymer chain, and thereby, allow hydrophobic interactions between alkyl side chains to predominate.

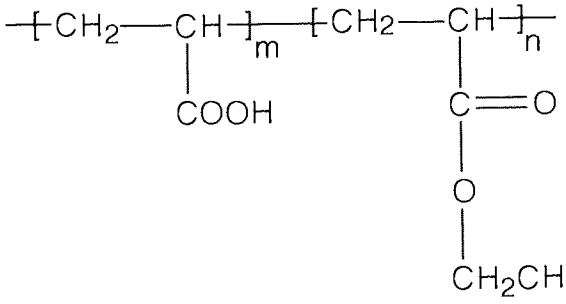


Figure 2.5. Copolymer of acrylic acid and ethyl acrylate

The fluorescent probe toluidinyl naphthalene-6-sulphonate has also been used to investigate the hydrophobic environment formed in the presence of added counterions.[68] These studies show that a change in the relative fluorescence intensity occurs with ionic strength and is most pronounced in the region of 0.3-0.5M sodium chloride, with the coil reaching a minimum size at 0.7M sodium chloride.

Data from both light scattering and intrinsic viscosity studies can be combined and used to calculate molecular dimensions such as the radius of gyration,[69] by applying, both the Flory and Mark-Houwink equations, respectively:

$$[\eta] = KM^{1/2}\alpha^3 \qquad (2.1.)$$

$$[\eta]_\theta = KM^{0.5} \qquad (2.2.)$$

Where values of K can be calculated from double log plots of intrinsic viscosity vs. average molecular weight, and values of average molecular weight calculated from light scattering data.
The Mark-Houwink equation shown is for theta solvent conditions.

By applying this procedure to data obtained in several organic and aqueous media at varying salt concentrations, the effects of both organic and ionic environments have been investigated.[69] Using this technique in theta solvent conditions the average mean square end to end distance ($\bar{r}^2_\theta$) could be calculated and was found to be some 1.3 times greater in organic media than in aqueous media. Light scattering studies confirm these observations and indicate a similar factoral difference (1.4 times greater). Such behaviour is typical of a hypercoiling polymer that only becomes subject to hydrophobic effects when dissolved in aqueous solution.

The free energy change associated with the transition from hypercoil to extended chain can be calculated[68] using equation 2.3:

$$\Delta G = 2.3\ RT \int_0^1 (pH_a - pH_b)d\alpha \qquad (2.3.)$$

Where: pH_a = pH of the A chain in the compact state.
pH_b = pH of the B chain in the extended state.
Both A and B are expressed at the same value of α (degree of ionization).

This technique essentially compares the slopes of the titration curves of the polymer in the compact state (A) with those of the extended chain (B) and from the difference the change in free energy can be calculated. Such calculations indicate that an additional free energy is required to ionize a polyanion that undergoes a conformational transition from a coiled to an uncoiled state. As the acid/ester ratio is decreased in copolymers of acrylic acid and ethyl acrylate, the transition from a compact hypercoil to an extended chain occurs at higher values of ionization, since the charged groups become further

removed from one another, and hence, a higher proportion of the carboxyl groups need to become ionized before the hydrophobic forces can be overcome. Calculations of the fraction of charge per comonomer unit needed to initiate a transition in the polymer, for different copolymer ratios, show similar values and indicate that the same charge per monomer unit is required for the molecule to uncoil irrespective of the type of copolymer used.[68]

2.2.3. Poly(acrylamides).

Extensive studies by McCormick[70] have shown that a series of acrylamide-based polymers substituted with long chain alkyl pendant groups exhibit hydrophobic association. However, the marked increase in solution viscosity caused by these polymers results from interchain association between hydrophobic groups located on separate polymer chains. These materials have been proposed as viscosity modifiers for the drilling fluids used in secondary oil recovery.

2.2.4. Copolymers of Maleic Anhydride & Alkyl Vinyl Ethers.

Alternating copolymers of maleic anhydride and n-alkyl vinyl ethers, as shown in Figure 2.6, when hydrolysed to maleic acid exhibit hypercoiling behaviour in aqueous solution and undergo a transition from a compact polysoap to an expanded polyelectrolyte in response to changes in charge density. This property was first reported by Dubin and Strauss in 1967[71] in copolymers where the alkyl group contained more than twelve carbon atoms. Such polymers form polysoaps and are resistant to pH induced conformational change, such as experienced by conventional polyelectrolytes.[72]

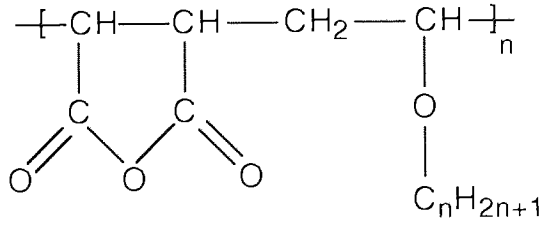


Figure 2.6. Poly(maleic anhydride alkyl vinyl ether)

Intrinsic viscosity measurements of copolymers of maleic anhydride and ethyl vinyl ether indicate that copolymers with short side chains behave as typical, extended chain, polyelectrolytes, i.e., they exhibit a rise of intrinsic viscosity upon dilution.[11,73] Methyl vinyl ether maleic anhydride for example does not show any appreciable conformational change upon dissociation (ionization), presumably because the side chains are too short to be subject to hydrophobic effects. However, measurements of butyl and hexyl copolymers indicate exceptionally low values of $[\eta]$, suggesting an abnormally compact state at low degrees of dissociation. The dimensions increase rapidly between 35% and 65% ionization, indicating destruction of the compact state and formation of an extended chain, while at high charge densities these copolymers exhibit intrinsic viscosities typical of expanded polyelectrolytes. In comparison, octyl and decyl vinyl ether copolymers remain hypercoiled even at high charge density as a result of intense hydrophobic association between the pendant groups.

Potentiometric titration of the ethyl vinyl ether copolymer conducted in sodium chloride solutions at various salt concentrations[11] indicates that conformational change can be largely prevented by the presence of 0.1M sodium chloride. The presence of ions causes charge (Debye-Huckel) shielding which inhibits intrachain repulsion, and consequently, a greater degree of ionization is needed in the presence of high salt concentrations to uncoil the polymer chain. In contrast, the butyl copolymer was found to be far less susceptible to added salt and undergoes a conformational change even at high salt concentrations due to the predominance of hydrophobic effects. Anomalies in both intrinsic viscosity and titration measurements occur over similar values of dissociation and indicate that the conformational changes noted are ionization dependant.

Studies of free energy changes that occur during the formation of the hypercoiled state in these copolymers indicate that the hydrophobic side chains form an environment that is more polar than ethanol and becomes less hydrophilic as the length of the alkyl side chain is increased beyond the hexyl derivative.[11]

Spectrophotometric techniques have also been used to study the nature of the hydrophobic environment within copolymers of maleic acid and alkyl vinyl ethers and confirm the results obtained by potentiometric and viscometric studies.[74,75] In the former studies, absorption conjugates are formed between the test polymer and optical probes that fluoresce in hydrophobic environments, e.g. the dansyl group. These studies demonstrate that the dansyl group is embedded in a non-polar hydrophobic environment at low degrees of polymer ionization and this gradually changes to a polar environment as the degree of ionization of the polymer increases. In the butyl

copolymer, fluorescence was found to fall markedly as the degree of ionization increased and this effect was found to be reduced in the presence of 0.2 and 0.5 M sodium chloride, where the transition occurred only at higher degrees of ionization. Again, illustrating the importance of charge shielding in determining polymer conformation.

Proton-NMR (100Hz.) techniques have similarly been applied to study conformational change in polyelectrolytes, in order, to confirm data obtained from potentiometric and viscometric studies. The line width of proton resonances from the side chains of maleic acid n-butyl vinyl ether were found to exhibit a sharp decrease in the region of conformational transition.[67] This effect was not observed with methyl vinyl ether maleic anhydride, demonstrating the importance of alkyl chain length and showing that the butyl derivative adopts a compact conformation at low ionization which restricts free rotation of its pendant side chains. Using high resolution NMR techniques (400MHz.) shifts in both side chain and backbone protons can be distinguished and the former have been found to be in a more restricted state when the polymer is in the compact conformation.[76]

2.2.5. Copolymers of Maleic Anhydride & Styrene.

Styrene pendant groups also provide a hydrophobic moiety and have been combined with maleic acid to form a hypercoiling copolymer, as shown in Figure 2.7. Indeed, the term hypercoiling, was first used by Dannhauser[77] to describe the behaviour of a styrene/maleic acid copolymer (PSTY/MA). Previous studies by Ferry[78] had observed that PSTY/MA exhibited an intrinsic viscosity some four times less than that predicted by the Flory equation, when the viscosity was assessed in theta solvent conditions containing 0.006N HCl. In a non-ionic theta solvent, viscometric data indicated that the polymer adopted a random coil conformation and under acidic conditions was found to contract by a factor of two.

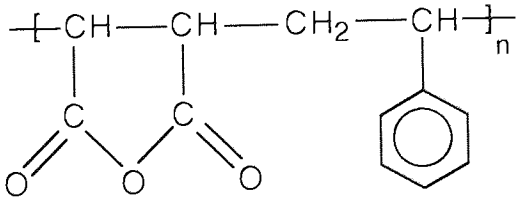


Figure 2.7. Poly(styrene maleic anhydride).

Investigations by potentiometric titration, viscometry and dilatometry indicate that copolymers of maleic anhydride and styrene undergo a pH-induced conformational transition, characteristic of hypercoiling polymers. Potentiometric titration of a STY/MA copolymer shows that anomalous behaviour occurs around 30% ionization, where a change in the pK_a becomes apparent. By conducting titration measurements at various temperatures,[79] the standard enthalpy change of transition could be calculated and was found to be similar in magnitude to the heat of transfer of benzene from a hydrophobic to an aqueous medium, i.e., 1.5 kJ/mole. The energy changes involved in conformational transitions, in the presence of sodium chloride, are of such magnitude that the titration curves observed are not influenced by temperature variations in the range 15-40°C. Hence, gains in free energy favour the formation of a compact structure stabilized by hydrophobic interactions between phenyl pendant groups. In this case, the change in free energy of the system from hypercoil to expanded coil at zero energy is high, in the order of 1.3-4.6 kJ/mol.

Potentiometric titration of copolymers of maleic anhydride and styrene[76] conducted in the presence of increasing concentrations of sodium chloride also provide confirmatory evidence of hypercoiling behaviour. In these studies the curves of pH vs. ionization (α) progressively collapse with increases in sodium chloride concentration as the polymer adopts a more and more compact conformation with increases in the degree of charge shielding. Viscosity studies confirm the observations made, in salt free conditions, and show that a marked increase in intrinsic viscosity occurs with ionization and accompanies the conformational changes, i.e., from a compact coil to a randomly coiled structure.[27] While dilatometry studies[27] indicate a reduction in volume of 0.6 ml/monomol upon collaspe of the polymer conformation. Dilatometry measurements conducted in the presence of sodium chloride solution confirm the potentiometric findings and indicate the formation of a compact structure but, interestingly, one that is not as compact as a globular protein of similar molecular weight.[27]

Spectrophotometric studies also have been conducted by incorporating an optical probe (acridine orange) into a maleic anhydride/styrene copolymer, to enable the presence of particular environments to be identified.[76] Spectra from such studies have been found to exhibit many isoderivative points, indicative of two conformational states, where the compact form demonstrates a hydrophobicity equivalent to that of a 40-50% solution of aqueous ethanol.

2.2.6. Proposed Medical Applications of Anionic Hypercoiling Polymers.

The application of synthetic hypercoiling macromolecules to medicine was first suggested by Seki and Tirrell.[80] This work sprang from a need to synthesize phospholipid vesicles or liposomes which could be made to respond to their environment and used, for example, to selectively release drugs.[81] Such liposomes were complexed with various poly(acrylic acid) derivatives to render the vesicle membrane sensitive to pH, whereby, a change of pH acted as a 'trigger' mechanism to change polymer structure and alter the properties of the liposomal membrane.

Poly(2-ethacrylic acid) [PEAA] (0.1%)[82] was found to adsorb onto the surface of dipalmitoyl phosphatidylcholine (DPPC) liposomes (0.1%) suspended in aqueous solution at pH 7.4, that is, above the pK_a value of the polymer. Such behaviour was explained in terms of hydrogen bonding between the charged carboxylic acid pendant groups within the polymer and the phosphodiester head groups of the phospholipid. Lowering the pH to 6.5, i.e., towards the pK_a of the polymer, caused a loss of charge, at which point hydrophobic interactions between ethyl pendant groups then predominated and led to a collapse of the polymer chain. The resultant hydrophobic domains acted to disrupt the liposomal membrane and caused a release of its contents, e.g. carboxyfluorescein.[82] Concomitant differential scanning calorimetry studies showed a sudden broadening of the melting endotherms as the pH of the system was lowered, indicating a reorganization of vesicle structure.[82,83]

The presence of hydrophobic domains within PEAA molecules also results in an association of the polymer with the aliphatic side chains of the phospholipid, causing the lipid and polymer components to form discoidal polymer-lipid assemblies, analogous to those found in lipoproteins.[51] Electron microscopy provided a further insight into these pH induced structural changes, and showed the presence of small (125-400 Å) diameter micellar particles, similar to those observed in high density lipoproteins.[51] Such pH induced macromolecular reorganization resulted in the formation of optically clear, aqueous suspensions.[82] The pH at which conformational transition in polymer/liposome structures takes place was found to depend, not only upon the chemical structure of the polymer, but also upon its tacticity.[83] Incorporation of the photophysical probe pyrene, into PEAA, enabled the presence of hydrophobic environments to be identified. Studies using this technique[51,84] revealed that a large increment in fluorescence occurred as the pH was lowered below 6.5. This was concomitant with the conformational transition of the polymer. Binding of DPPC to PEAA was also found to result in a shift in the pK_a value of the polymer causing a decrease in its apparent acidity,[84] i.e., it behaved as a weaker polyacid.

The need for a biodegradable hypercoiling polymer and its potential application to medicine has been noted by Seki,[85] who describes the use of poly(β-malic acid) as a polymeric trigger capable of lysing DPPC vesicles. However, poly(β-malic acid) cannot, strictly, be considered as an example of a hypercoiling polymer, because it does not contain pendant hydrophobic side chains. It is most likely to undergo a similar pH induced conformational change to that experienced by poly(methacrylic acid), which cannot be classified as a hypercoiling polymer.

2.3. Surface Activity of Synthetic Hypercoiling Polymers in Solution.

2.3.1. Background Considerations Relating to Polymeric Surface Activity

Monomeric surfactants form micelles above their critical micellar concentration (CMC) and this phenomenon is characterised by a fall in surface tension, and hence, a rise in surface activity. However, the micelles themselves are not expected to be surface active, since they expose only their hydrophilic segments to the aqueous phase. This contention cannot be tested using conventional surfactants, because the micelles formed under these conditions are in a dynamic equilibrium with highly surface active unassociated surfactant molecules which readily associate with the surface and lower the surface tension, so masking any changes. Whereas, in polysoaps such as those described by Jorgensen,[86] where the soap molecules are attached to a polymer backbone, no 'free' surfactant molecules are available and so the surface activity of micelles can be measured directly. Indeed, these polymers exhibit no wetting, foaming or detergent properties and it is probably correct to assume that they do not orientate themselves at the aqueous\air interface.[56] They do, unusually, possess an ability to solubilize non-polar materials and adsorb onto surfaces from aqueous solution, which is somewhat paradoxical for non-surface active molecules.

2.3.1.1. Surface Activity of Polyelectrolyte Solutions.

The influence of polymer structure on surface activity and use of surface activity as a means of monitoring polymeric conformation in solution has received scant attention in the published literature. Early studies on the conformation of polysoaps, by Jorgensen[86] in 1961 observed that 0.0025-0.75% poly(4-vinylpyridine) partially quaternized with n-dodecyl and ethyl bromide had very little effect on the surface

tension of water, except in the presence of KBr, where charge shielding and chain collapse become important. Even in this instance, the surface tension did not fall below 62 dynes/cm. A slightly greater percentage (as a proportion of the starting value) reduction was noted in interfacial tension, although it was suggested that this difference could be accounted for by the presence of solubilized hydrocarbon within the aqueous phase. However, the addition product of poly(2-vinylpyridine) and bromopropylbenzene depressed surface tension to approximately 45 dynes/cm. It should be emphasised, though, that in neither case were surface or interfacial tensions monitored while changing pH, which may have a limited effect on polysoap conformation and a much greater effect on non-polysoap/hypercoiling polymers.

In the studies of Pop[87] and Boiko[88] the effect of percentage ionization (pH) and polymer concentration upon the surface tension of polyelectrolytes in solution was, however, investigated. These authors focused principally on the ammonium and potassium salts of a styrene maleic acid copolymer. Both authors reported that a reduction in ionization led to a sharp fall in surface tension. The studies of Pop reported a fall from 71 to 52 dynes/cm, which was also accompanied by a fall in reduced viscosity and indicated the formation of more compact molecules.

Increasing the concentration of the predominantly charged polymer (>50% ionized) was found to cause a progressive fall in surface tension in a concentration dependant manner.[87] At this level of ionization the molecules were assumed to be in their fully extended state exposing their hydrophobic groups and could, therefore, pack at the air/liquid interface to reduce the surface tension in direct proportion to the concentration of polymer present, until a critical concentration was reached at very high polymer concentrations. At this point, conventional intermolecular micelles were formed and a further reduction in surface tension occurred. Closer inspection of this data reveals a reversal of this trend as the charge is reduced, i.e., at 50% ionization, the values of surface tension were generally higher than those observed with the 100% ionized polymer.[87] The surface tension vs. neutralisation curves reported by Boiko[88] also reveal that at high polymer concentrations the fully charged polymer exhibited a lower surface tension than the values observed at 75% ionization. Boiko accounts for this change by assuming that high degrees of charge shielding occur upon neutralisation and that this leads to a collapse in the polymer chain. An alternative explanation of the rise in surface tension noted upon acidification may be provided thus; as the molecule transforms from an extended chain to a dumb-bell shape it adopts an intramolecular micellar structure, which is not, itself, surface active. Both sets of data would tend to support this theory.

In contrast, in the predominantly uncharged polymer (<25% ionized), a sharper reduction in surface tension occurred in response to increases in polymer concentration and this occurred at lower concentrations than was observed in the fully charged polymer. In this case, there is insufficient charge on the molecule to extend the chain, resulting in partial collapse and formation of distinct hydrophobic domains, possibly forming dumb-bell shaped amphipathic molecules. The latter, readily associate to form polymolecular micelles and lower the surface tension to a much greater extent than that caused by fully charged molecules. Boiko ascribes the overall fall in surface tension on raising the polymer concentration to polyelectrolyte unfolding and does not propose the formation of intramolecular micelles, although these are most likely to account for his findings. Both authors (Pop & Boiko)[87,88] also identified a CMC, with typical CMC values of 0.07-0.16%, [88] and found this effect to be concomitant with a fall in reduced viscosity. Pop[87] claims that monomolecular (an equivalent term to intramolecular) micelles only form at <50% ionization and in polymer solutions of high concentration.

Okubo[89] accounts for the low surface activity of polyvinyl electrolytes by concluding that their structures are unable to sterically separate their hydrophobic and hydrophilic groups into defined regions, except when above their critical micellar concentration, where intermolecular associations achieve this effect. Observations by Aidarova[90] are in agreement with these findings and show that the surface activity of methacrylic acid/methacrylate copolymers is largely dependant on the hydrophobic/hydrophilic balance resulting from the level of ester incorporation. Surface tension values in the 40-46 dyne/cm range are reported by this author.

2.3.1.2. Solubilizing Ability of Polyelectrolytes.

The early studies by Strauss[72] showed that the polysoap poly(2-vinylpyridine) partially quaternized with n-dodedyl bromide was able to solubilize normally water insoluble hydrocarbons and it was not found to be necessary to achieve a critical micelle concentration before solubilization occurred. The degree of solubilization was found to be proportional to the polysoap concentration, suggesting that the micelles were intramolecular in nature.[91] The concept of intramolecular micelles as proposed by Strauss, in which the micelles are not surface active but can solubilize hydrophilic agents within their hydrophobic domains, is consistent with the surface tension findings of Pop[87] and Boiko.[88]

Solubilization of isooctane and n-octane[92] was observed to result in a fall in reduced viscosity, supporting the concept of polymer collapse around a hydrophobic core. In contrast, solubilization of benzene in an aqueous polysoap solution,[93] resulted in a reduction of reduced viscosity at low polymer concentrations and a raised viscosity as the concentration of polysoap was increased. This difference is said to arise from the ability of aliphatic groups to become solubilized within a micellar core and so reduce the molecular dimensions, compared to benzene, which was considered to span hydrophobic regions of neighbouring micelles and lead to aggregation, resulting in an apparent rise of molecular volume.

Intriguingly, Richlin[94] reports that such polysoaps form metastable fibre-like precipitates at the air/liquid interface upon compression of the interface and concludes that this results from polymer folding. This behaviour may be analogous to the lipoidal phase change observed by Bangham[95] upon interfacial compression of DPPC and is almost certain to result from an association of hydrophobic groups.

It is now considered that intramolecular micelles do not exist as independent units, unlike micelles within conventional monomeric surfactant systems, and as a result, their molecular dimensions cannot be determined by conventional light scattering or hydrodynamic methods.[96] Consequently, luminescence quenching has been employed as an alternative method of assessing micellar size, by incorporating both luminescent probe and quencher molecules into the polymer chain.[96] In the presence of excess micelles the chance of both molecules being present in any one micelle is inversely proportional to the number of micelles and the degree of quenching observed reflects this relationship. Such studies demonstrate that micellar size is independent of polymer concentration and this, once again, suggests intramolecular micellation.

The studies of surface tension and solubilization, when viewed together, demonstrate that surface and interfacial tension measurements offer a simple method of monitoring conformational change within macromolecules. As a result, the poly(isophthalamides) and poly(adipamides) described in the later chapters of this thesis have been investigated using these techniques.

CHAPTER 3

BIOLOGICAL SURFACTANT SYSTEMS:

PROPOSED APPLICATIONS OF DEGRADABLE HYPERCOILING & HYDROPHOBICALLY ASSOCIATING POLYMERS

3.1. General Characteristics of Surfactant Systems.

It is important when considering biosurfactants to compare their surface activity to that of synthetic surfactants. The lowest equilibrium surface tensions recorded for the latter are around 20 dynes/cm for water soluble hydrocarbon surfactants. The surface tension of these water soluble surfactants shows no difference upon surface compression[97] because, the interfacial surfactant molecules can rapidly equilibrate with those molecules in the bulk phase. Hence, the superlow surface tensions reported in certain biological systems, e.g. in the mammalian lung, must result from a distinct mechanism which is outside the behaviour diplayed by conventional synthetic surfactants.

3.2. Survey of Natural Surfactants.

3.2.1. Bacterial Lipopeptides.

In comparison to synthetic systems the surface tension of globular proteins in aqueous solution seldom falls below 50 dynes/cm[98] and under equilibrium conditions the most surface active natural material is a lipopeptide known as surfactin[99] and a group of related compounds known as the Iturins.[100] Both materials possess antifungal properties and are produced by *Bacillus subtilis*. These compounds consist of a cyclic heptapeptide, containing anionic amino acids linked to a C_{10}-C_{12} alkyl chain and exhibit an equilibrium spreading pressure (π) ranging from 45 dynes/cm, for surfactin, to 9 dynes/cm for Iturin A. Both materials can essentially be considered as monomeric.

3.2.2. Lung Surfactant.

3.2.2.1. Physiology of Lung Surfactant

The lungs essentially consist of a series of minute interconnecting fluid-lined sacks known as the alveoli. This arrangement maximises the surface area for gaseous exchange across a fluid/air interface, while at the same time, minimising the space occupied by the lungs within the body. However, such an arrangement presents a

physico-chemical problem for the body; from Laplace's law, see equation 3.1, we can see that as the diameter of a bubble (of which the alveolar sack approximates) decreases, the pressure increases. Pressure disparities between the alveoli would, therefore, tend to force air from the smaller alveolar sacks into the larger ones, resulting in a collapse of the former. If this situation occurred *in vivo* then subsequent expansion of the lungs would be more difficult as their compliance would have decreased.[101]

$$P = \frac{2\gamma}{r} \qquad (3.1)$$

Where: P = pressure, γ = surface tension, r = radius of bubble.

To avoid these problems mammals produce a natural surfactant which lowers the surface tension of the alveolar surfaces, as the alveolar surface area is constricted during exhalation, thereby, preventing a collapse of the lungs, which would otherwise occur.[102] In converse, the force needed to inflate the lungs is also equalised. In both cases the lungs are able to deflate and inflate uniformly with a variation in terminal size of different alveoli, since the compliance of the lungs remains equivalent during both events. Such a degree of functional control is achieved by reducing interfacial or surface tension in direct proportion to the reduction in surface area and this, in turn, is achieved by an increase in the concentration of surfactant per unit area of the surface.

The surfactant nature of lung fluids was first recognised by Pattle[103] in 1958 who noted the stability of bubbles formed in the foam expressed from the lungs of mature animals. Clements[104] suggested that this property resulted from the presence of a surfactant, while Avery[105] observed that lungs from foetuses of less than 1000g usually lacked surfactant. Pattle[106] went on to identify a lecithin containing lipoprotein as the major component of lung surfactant and Buckingham[107] demonstrated that the appearance of osmophilic secretory bodies within cells lining the alveoli of mouse foetuses were associated with the fall in surface tension of lung fluids, noted during later periods of gestation.

In human neonates, lung surfactant is synthesized around two months prior to term, enabling the lungs to inflate and normal breathing to commence at birth. In infants born more than two months premature the quantities of lung surfactant may be greatly reduced or completely absent. This situation prevents the lungs from inflating and results in the development of a condition known as neonatal respiratory distress syndrome (RDS) or hyaline membrane disease. Prematurity remains the most common

cause of neonatal mortality, with RDS resulting in some five thousand deaths per annum in the U.S.A. alone. Until quite recently treatment for RDS involved the use of mechanical ventilators, in addition, to placing such infants under conditions of hyperbaric oxygen, in an attempt to forcibly inflate their lungs and so prevent complete exhalation. The latter procedure stops the lungs from collapsing, although it invariably damages their delicate structure and should, ideally, be avoided.

3.2.2.2. Development of Artificial Lung Surfactants.

Over the past fifteen or so years researchers and clinicians have attempted to define the precise structure of lung surfactant in order to synthesize an artificial agent capable of replacing native surfactant or supplementing deficiencies in premature neonates. These investigations have shown that lung surfactant is largely composed of a mixture of phospholipids which become orientated at the air/fluid interface. Endogenous surfactant consists of 90% lipid in combination with 10% protein. The lipoidal fraction is 90% phospholipid of which 80% is phosphatidylcholine (PC), some 40-45% in the form of the dipalmitoyl ester (DPPC) and the remainder as monenoic PC. The lipid also contains 10-15% phosphatidylglycerol (PG) and 7-8% cholesterol.[108] Films composed of surfactant exhibit an equilibrium spreading pressure approaching 70 dynes/cm and at such elevated pressures Bangham[95] has suggested that a phase change may be induced, forming a solid lipid film, by effectively lowering the phospholipid transition temperature (T_C). As a consequence of these investigations, mixtures of synthetic phospholipids analogous to those found in native surfactant have been widely tested as potential artificial surfactants in premature rabbits[109,110] and lambs.[111]

Although these attempts have resulted in considerable success, synthetic phospholipid-based surfactants have been found to be less effective than the natural product. It appears that the added phospholipids fail to completely adsorb and spread at the alveolar air/fluid interface without the presence of a series of apoproteins, termed surfactant proteins (SP). The latter, modify the assembly of phospholipids such that a lipid monolayer is formed at the interface. However, it is not advisable to add animal derived apoproteins to artificial surfactant mixtures because of their potential to elicit undesirable allergic responses. Notwithstanding, such potentially adverse side effects, clinical workers in Japan[112] have found semi-synthetic lipoprotein mixtures to be highly effective in treating clinical RDS. Two commercial products containing bovine and porcine derived apoproteins in combination with DPPC are now commercially available in the U.K. under the trade names Survanta and Curosurf, respectively. Alternative solubilizing agents, such as tyloxapol and hexadecanol, have also been used

to replace the apoproteins and facilitate lipid monolayer formation.[111] An artificial surfactant containing these agents in combination with DPPC is also available for clinical use in the U.K. under the tradename Exosurf. In addition, a product containing DPPC in combination with PG and without any additional components is sold under the trade name of ALEC.

Clinical studies[113] have shown that the artificial surfactant ALEC is particularly effective in treating neonates born after 25-30 weeks gestation, where the effect of therapy is to increase the survival rate to a level equivalent to that of one extra week of gestation, reducing overall mortality from 27% to 14%. Neonates born before 25 weeks suffer from additional complications which increase their mortality rate to approximately 40%, such neonates do not benefit from therapy to the same extent as those born after 25 weeks, where RDS represents the principal complication. Post 30 week gestation neonates produce endogenous surfactant and so do not normally require exogenous material. Hence, there appears to be a 'window of therapeutic opportunity' when artificial surfactants can be used to greatest clinical benefit.

3.2.2.3. Characterization & *In Vitro* Behaviour of Apoproteins

The high price of the current range of animal derived, apoprotein-containing, surfactant products, coupled to their lack of availability to a large portion of the world's population, creates an overriding need for a cheap synthetic alternative. One group of investigators[114] have recently suggested using polyamino acid homopolymers with hydrophobic residues as synthetic analogues or model apoproteins, to study the interactions between apoprotein and lipid. Their results show that both poly(leucine) and poly(phenylalanine) greatly enhance the interfacial adsorption of DPPC, and therefore, suggest that the requirement for a hydrophobic grouping is rather non-specific.

Synthetic peptides consisting of alternating residues of leucine and lysine are the simplest structures capable of adopting protein-like conformations with a hydrophobic interior and hydrophilic exterior. Unlike native proteins though, this is not a fixed arrangement[115] and the orientation can change in response to the polarity of the surrounding environment. A related 21 residue polypeptide consisting of LeuLeuLeuLeuArg repeat sequences was found to reduce surface tension both *in vitro* and *in vivo* when combined with DPPC.[116] Computer modelling of this sequence, carried out as part of the work reported in this thesis, has shown that this polypeptide forms an amphipathic helix.

The importance of apoproteins in facilitating the action of lung surfactant has led to an intensive effort to extract, characterize and sequence these proteins. Recent studies[117,118] have succeeded in identifying the active segments of some of the apoproteins involved. A portion of one of the apoproteins has now been synthesized by solid-phase synthetic techniques (Merrifield) and has been shown to facilitate the adsorption of phospholipid at the air/fluid interface.[118]

The primary, and to some extent, secondary and tertiary structures of lung surfactant apoproteins have now been largely defined,[119] primarily by complementary DNA sequencing techniques.[120-122] These studies indicate the presence of three separate proteins, nominated: SP-A, SP-B and SP-C. More recently, a fourth apoprotein designated SP-D, has been identified and appears to be functionally similar to SP-A.[123] SP-B and SP-C[120] are principally involved in spreading lipid at the air/fluid interface, and therefore, are of prime interest to the work described herein. SP-B and SP-C[124] are much smaller hydrophobic proteolipids than SP-A; SP-B comprises 381 amino acid residues, the fragment from amino acid 210 to 289 represents the active site of the molecule in relation to surface active properties. This region, in turn, consists of two separate regions of 23 amino acids and 24 amino acids, respectively, both of which are predominantly composed of hydrophobic and cationic residues.

The periodicity of charged and hydrophobic residues throughout the active site suggests that this region of the molecule may be amphipathic in nature. Molecular modelling studies by Takahashi,[125] Keough and Perez-Gil[108] and Whitsett[124] suggest that SP-B forms an amphipathic helical structure where the hydrophobic residues are arranged on one facet, perpendicular to the axis of the coil, and the hydrophilic residues are arranged on the opposite facet. The molecule may be inserted into the bilayer membrane so that the hydrophobic faces adjoin the acyl chains of the lipoidal groups leaving the hydrophilic groups to interact with one another to form disc-like micelles, a similar arrangement to that found in plasma apoproteins (see section 3.3). These proposals are supported by the appearance of micellar structures revealed by electron microscopy of lung fluids[126] and in SP-B or SP-C containing DPPC recombinants.[127]

Addition of SP-B to aqueous suspensions of phospholipid *in vitro* cause the latter to spread spontaneously at an air/fluid interface, and thus, to be adsorbed.[117,118] The SP-B apoprotein can be considered as a polymer possessing defined cationic regions and hydrophobic domains, and we may reason, that a synthetic hypercoiling polymer may act in a similar manner. Indeed, micellar structures resembling those found in lung fluids have been reported to occur between PEAA and DPPC.[51]

In contrast to SP-B, SP-C appears to be a membrane bound protein, because of the presence of a central hydrophobic region of 34 amino acid residues that possess an ability to span a lipid bilayer.[128] FT-IR deconvolution studies indicate that 60% of SP-C is in the form of an α-helical coil, when associated with lipid bilayers[129] and appears to possess a perpendicular orientation to the bilayer. SP-C increases the elasticity of the bilayer and may possibly play a role in surface spreading of phospholipids.

Hence, both SP-B and SP-C can be considered small, amphipathic, cationic proteins and both facilitate adsorption of phospholipids at the air/fluid interface, destabilise lipid bilayers and modify the lipoidal environment,[130] presumably by making use of the hydrophobic effect. SP-A and SP-D appear to be principally involved in transporting surfactant from the Type II cells found lining the alveolar space to the outer surface of the overlying liquid.[108]

The central contention of this thesis is that biodegradable hypercoiling or hydrophobically associating polymers may behave in a similar manner to SP-B and in combination with DPPC may also form micellar structures with a high surface activity. The application of such polymers to the development of an affordable artificial lung surfactant will be explored as the principal objective of the work described herein.

3.3. **Plasma Lipoproteins.**

3.3.1. Macromolecular Structure & Function.

Serum lipoproteins are somewhat analogous to the apoprotein/lipid recombinants found in lung surfactant. Their apoproteins are structurally similar to those of surfactant protein, but are distinct from SP-C, in that, they do not contain regions of an exclusively hydrophobic nature, but are more like the proposed structure for SP-B, possessing hydrophilic and hydrophobic facets within the same area of the polypeptide chain.[131] The serum apoproteins can be divided into four categories, apoprotein A, B, C and E. The basic function of these molecules is to transport lipids within the bloodstream and interstitial spaces. The apoprotein/lipid complexes that form are organised into four distinct structures, namely; chylomicrons, low density lipoprotein (LDL) - mainly apo-B, very low density lipoprotein (VLDL) or high density lipoprotein (HDL) - mainly apo-A.[132]

Complementary base pair DNA sequencing of lipoprotein encoding genes across a series of animal species indicates a remarkable similarity of repeated segments, more so than in any other protein group so far investigated. A dimer of 22 amino acid residues, consisting of two 11 amino acid sequences is regularly repeated. This sequence is conserved across species for the same apoprotein subtype, indicating a common primary heritage for the apoprotein genes.[133,134]

Segrest[135] attempted to explain the mechanism by which these macromolecular lipoprotein assemblies carry lipid components in aqueous media. He observed a change in measurements of circular dichroism as apoproteins bound phospholipids, indicating an increase in the α-helical content of the protein. Specific amino acid sequences were identified which formed helical structures and exhibited an amphipathic character. This led to the suggestion that apoproteins form a series of α-helices, each arranging their amino acid side chains such that hydrophobic groups are exposed on one facet, perpendicular to the axis of the helix, while hydrophilic groups are arranged at the opposite facet, so forming an amphipathic helix. A number of such helices then surround a bilayer of lipid, in a 'doughnut' arrangement, which prevents contact between the hydrophobic portions of the lipids and surrounding water molecules. The term amphiphilic helix is sometimes used, although, amphipathic is a more appropriate term, since it correctly implies that the force responsible for defining this structural motif is the exclusion of hydrophobic segments from the aqueous environment rather than an affinity between non-polar segments.[131]

Removal of lipid from an aqueous environment gives a far greater gain in free energy to the overall system than that lost by the removal of the hydrophilic head groups. Such changes minimise the free energy of the system, by maximising hydrogen bonding between water molecules within the surrounding solution, and are biological examples of the hydrophobic effect.[132] Consequently, the binding of lipoproteins to lipids is primarily dependant upon hydrophobic interactions, hydrophilic head-group specificity does not appear to be important, although the presence of charged groups in the amino acid side chains of lipoproteins does make their conformation sensitive to changes in pH.

The co-operative behaviour of apoproteins on binding lipid suggests that they are quite dynamic structures and that lipid binding induces a conformational change within apoproteins which facilitates further lipid binding and results in an increase in the overall size of the lipoprotein complex. As a result of the reversible conformational changes undergone by some apoproteins (Apo-A) these proteins are often referred to as exchangeable apoproteins.[136] Their conformation changes in response to both

association with lipid[137] and in response to the solvent environment, e.g. Apo-E unfolds as the pH is changed from 7.4 to 4.8.

One method adopted to graphically depict polypeptide sequences that enables likely putative amphipathic structures to be identified is known as an 'Edmundson Plot',[138] where a helical wheel is used to project side chain residues onto a plane perpendicular to that of the long axis of the helix. Application of this technique has led to the identification of such helical forms in many protein and polypeptide sequences and suggests that amphipathic coils represent a common structural motif in nature. An exhaustive computerised search[139] of all known protein sequences, using the helical hydrophobic moment technique to compare the mean hydrophobicity of adjoining sequences,[140] showed that the most hydrophobic proteins were those associated with lipid, e.g. in the C-terminal portion of human immunodeficiency virus glycoprotein, in membrane spanning ion channels or in lytic peptides. Unusually, such peptides have been reported to increase surface tension at the air/water interface.[131] Hence, these agents may not be viewed as surface active in the conventional sense except when associated with lipid.

The importance of macromolecular assemblies is also emphasised by the observation that the hydrophobic portions of both cholesteryl esters and triglycerides are too large and to be solubilized by single chain amphipathic proteins, and hence, solubilization of mixed lipids can only be achieved by macromolecular assemblies of proteins, in order to create hydrophobic domains of sufficient size to accommodate the lipid molecules.

3.3.2. *In Vitro* Studies of Plasma Lipoproteins.

To assess the structure of such assemblies, artificially produced recombinants of DPPC and partially purified apoprotein A-1 have been produced.[141] These appear as optically clear solutions and when examined by SEM and can be seen to be made up of discoidal phospholipid bilayers. Differential scanning calorimetry studies indicate a decrease in enthalpy of the phospholipid gel-liquid crystalline transition which is a feature associated with the presence of co-operative lipoprotein complexes.[137] A linear decrease in diameter of the discoidal complexes is also observed as the lipid/protein ratio is reduced and is consistent with the localisation of apoprotein in an annular arrangement around the edge of the lipoidal disc. Studies by Brasseur[142] using ATR-FTIR to determine the orientation of apo-AI confirmed the discoidal mode of assembly of lipoprotein particles.

Recombinant DNA technology has recently been used by Logan *et al.*[143] to synthesize amphipathic α-helical peptides analogous to those found in apolipoproteins and proposed to undergo reversible hydrophobic interactions. Peptides containing 11 amino acid tandem repeats have been formed, in which leucine residues constitute a hydrophobic face along the longitudinal axis of the helix.

3.4. Other Naturally Occurring Lipid-Associating Polypeptides.

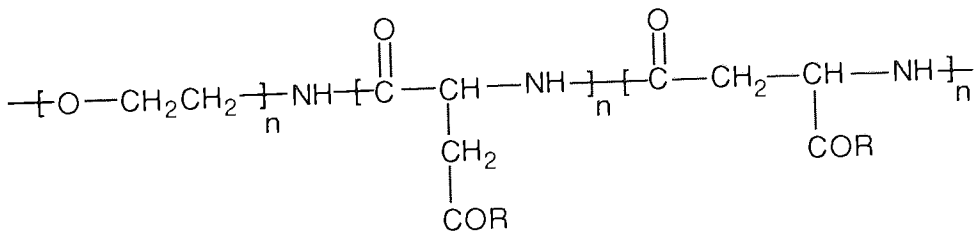
A number of naturally occurring polypeptides possess amphipathic α-helical structures, these include defensive venoms and toxins, e.g. the bee venom, melittin,[144] antimicrobial lytic peptides such as the cecropins[145] and toxins secreted by the skin of the African Clawed Toad, *Xenopus laevis*.[146] The amphipathic structure shared by these peptides enables them to span cellular bilayer membranes and associate to form pores, thereby, negating the semi-permeable nature of these membranes and causing cell lysis.

Amphipathic segments of protein molecules also associate to form transmembrane pores, e.g. the five transmembrane segments of the nicotinic acetylcholine receptor operate holistically as a gate mechanism and upon interaction with acetylcholine or nicotine activate the nerve impulse.[147] In contrast, certain natural polyesters such as poly(β-hydroxybutyrate) can also form helices. However, the latter exhibit a greater internal diameter because backbone rotation is not constrained to the same extent as is the case in, amide-based, polypeptides. Such helices have sufficient space within their core to contain their hydrophilic side chains, and therefore, form an exolipophilic-endopolarophilic helix around an inner framework helix of polyphosphate, the two structures being linked by calcium ions.[148] As in the receptor proteins such channels may play a role in ion transport and in bacterial cell membranes have been implicated in the transport of calcium, phosphate and DNA.

3.5. Hypercoiling Polymers: Lipid-Related Applications.

Biodegradable hypercoiling polymers, possessing hydrophobic regions may be able to mimic the functions of endogenous apoproteins by acting as biological surfactants in solubilizing lipids and lipophilic compounds. This property has two potential applications in the field of drug delivery:

1) As a simple drug carrying polymer for exogenous lipophilic agents, acting in an analogous way to endogenous LDL, which has itself, been proposed as a vehicle for carrying purine and pyrimidine antineoplastic drugs.[149] A synthetic hydrophobically associating, degradable polypeptide has recently been developed to deliver the anthracyclene, anticancer agent, adriamycin.[150] In the latter case, the polymer consists of two blocks, a hydrophilic block composed of amino poly(ethylene glycol) covalently bound to a block of drug bound ω and α bonded poly(aspartic acid). The drug molecule is covalently bound to pendant carboxylic acid groups within the poly(aspartic acid) segment and forms a hydrophobic block, as shown in Figure 3.1. The resultant amphipathic molecule forms micelles in which the reactive drug is buried within a hydrophobic core. The small size of these micelles (20-100nm) renders them resistant to clearance from the blood compartment by the reticuloendothelial system and this may result in sustained drug release. Such conjugates may offer a unique benefit in the administration of drugs that would normally cause extravasation at the site of injection. Attachment of cell-specific antibodies to such conjugates has been proposed as a method of achieving site-specific drug delivery.[151,152]



where R = OH or drug molecule

Figure 3.1. PEG/Poly(aspartic acid)/drug conjugate.

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2) Targeted drug delivery at a site-specific pH. A more sophisticated application of the biodegradable hypercoilers would exploit their ability to uncoil at specific pH values to target drugs to those regions of the body where a localised pH anomaly is found. In this respect, their application to neoplastic disease is particularly favoured, since diseased tissue is often characterised by a poor or inadequate blood supply, and therefore, has a tendency to become dependant upon anaerobic respiration,[153] leading to a localised reduction in pH. Poly(ethacrylic acid), a non-degradable negatively charged hypercoiling polymer, in combination with drug loaded liposomes, has been suggested as a method of targeting such areas with antineoplastic agents.[81] However, on theoretical grounds, biodegradable hypercoiling polymers offer a better prospect for targeted delivery of antineoplastic agents, since the latter can be directly incorporated into the hydrophobic portion of the polymer without the use of liposomes.

Hence, the polymers proposed in this thesis have potential applications as a replacement pulmonary surfactant for treating RDS and for targeted drug delivery in cancer chemotherapy.

3.6. Types of Surface/Interfacial Activity Found in Nature: An Overview Comparison with Synthetic Surfactant Systems.

In summary, there appear to be essentially two types of solubilizing molecule and representatives of both are found in nature and in synthetic systems. The first type is the conventional monomeric amphipathic substance which associates at interfaces, is surface active, and forms micelles above a specific critical micellar concentration. Examples found in nature include the bile acids and low molecular weight cyclic peptide systems substituted with long chain fatty acids, exemplified by surfactin. The conventional synthetic surfactants, e.g. soaps, fall into this category. The polymeric surfactants such as the block copolymers of ethylene oxide and propylene oxide (Pluronics™) are also probably best considered in this category by virtue of their amphipathic nature.

The second category of surfactants are somewhat more obscure and less readily comprehended, these are the polymeric hydrophobically associating polymers which can assemble into a macromolecular structure where a distinct hydrophobic facet or domain is juxtaposed to a charged or hydrophilic domain. The former associate with hydrophobic materials so as to envelop them in hydrophilic shell, and thereby, render them water soluble. We can refer to these as the 'associating surfactants', in that, they

only behave as surfactants when in contact with hydrophobic materials and are not intrinsically surface active. In nature the associating surfactants are exemplified by amphipathic peptide helices contained in either polypeptides or proteins. The bee venom polypeptide, melittin, is an example of the former, while apolipoprotein-A is an example of the latter.

The apoproteins that form a constituent of lung surfactant are one particular example of an associating surfactant, in that the lipid being solubilized is itself amphipathic and assembles into lamella sheets in water. The latter, will slowly associate at air/water interfaces to lower the surface tension, but when associated with apoproteins do this rapidly and appear to be highly surface active. It is intriguing to speculate as to the possibility of forming synthetic correlates of these materials; synthetic hypercoiling surfactants may function as apoproteins and associate with amphipathic surfactants to form macromolecular assemblies which, like lung surfactant, may be highly surface active and display superlow surface tensions especially at interfaces under compression. It is interesting to note that although Tirrell *et al.*[51] described an association complex between poly(ethacrylic acid) and DPPC, and with great foresight, likened this to the micellar structures observed in plasma apolipoproteins, particularly HDL, they did not report the surface activity of these complexes.

CHAPTER 4

INTERFACIAL POLYMERIZATION I

THEORETICAL CONSIDERATIONS AND SYNTHESIS OF AROMATIC POLYAMIDES

4.1. Interfacial Polycondensation: Background and Theoretical Considerations.

Interfacial polycondensation is a potentially valuable technique for synthesizing high molecular weight polyamides and polyesters.[154-156] This technique was first developed by the E.I. du Pont de Nemours Company in the early 1950's (to Kirby)[157,158] as a small scale, laboratory alternative to the melt polycondensation methods developed by Carothers in 1929.[154] Low temperature interfacial polycondensation requires both an acid chloride and active hydrogen containing compound, e.g. amine, alcohol, phenol or thiol, which react together by elimination (the Schotten-Baumann reaction). If the two components are bifunctional then a polymer is formed.

The usual synthetic procedure adopted consists of interfacially reacting a diacid halide, dissolved in a water immiscible phase, with a diamine or diol in an alkaline, aqueous phase. The presence of an alkali such as sodium hydroxide, in the aqueous phase, acts as an acceptor and shifts the equilibrium of the reaction towards polymer formation. Detergents may also be added to increase polymer yield by enhancing interpenetration of the two phases, and in this sense, the formation of a surface active hypercoiling polymer may enhance the rate of its own production. Polymerization is said to occur exclusively in the organic phase,[159] but in the case of polyester formation a high partition coefficient exists for the diol between organic and aqueous phases and so favours polymer formation in the aqueous phase.

Interfacial polymerization offers an attractive technique for synthesizing degradable hypercoiling polymers, since monomeric components such as modified amino acids are unlikely to be stable at high temperatures, and therefore, low temperature synthesis must be considered. A further advantage of the interfacial technique is the high reaction rate, usually proceeding to completion within minutes and resulting in formation of a high yield of high molecular weight polymer, combined with less stringent requirements for monomer purity than those demanded for solution polymerization. In addition, there is no necessity for molecular equivalence of the monomers, since the stoichiometry of the reaction is optimised about the interface where the reaction occurs.

4.2. Experimental Technique Used.

The stirred interfacial technique offers an improvement over unstirred interfacial synthesis, which is most often used to demonstrate this methodology. In the stirred technique the surface area between the two reactant phases is markedly increased. It was, therefore, decided to use this method in order to obtain maximal polymer yields.

The procedure most often used in the polymerizations described in the following two chapters, based upon amino acids or their derivatives, involved interfacially reacting a 0.1M solution of acid chloride dissolved in either 50 mls of a chlorinated solvent or hydrocarbon, with a 0.1M solution of diamine dissolved in 50 mls of alkaline water at pH 10-11.

The procedure used to remove any polymeric material consisted of terminating the reaction by combining the reaction mixture with approximately 400 mls of single distilled water. The material that remained within the reaction vessel was rinsed with additional water and the washings combined with the reaction material already in aqueous solution or suspension. The latter solution was then stirred and the pH measured. Sufficient amounts of concentrated hydrochloric acid (38%) were then added to acidify the aqueous solution to a pH of 1.5-2.5, and more particularly a pH 1.75. Anionic material was then precipitated out of solution, i.e., in conditions substantially below the pK_a of weak acids (the pK_a of the carboxylic acid groups is approximately pH 4-6). The precipitate was recovered by Buchner filtration, rinsed in distilled water and dried *in vacuo* at 30°C. The dried product was weighed and dissolved on excess methanol (approximately 200 mls), in order to remove hydrolysed acid chloride, starting amine and any low molecular weight oligomers. After selective dissolution of the low molecular weight components into excess methanol, any remaining polymeric material was recovered from the methanol, usually in the form of a yellow or pale brown coloured, resinous material.

The latter material was either removed directly or separated by filtration, rinsed with excess fresh methanol and dried *in vacuo* at 30°C. The resulting, potentially, polymeric material was then subjected to the analytical procedures described in section 4.3.

4.3. The Analytical 'Focusing' Process.

A sequence of analysis was identified to focus efforts on 'the candidates most likely to succeed', i.e., those substances produced by the interfacial synthetic technique which were most likely to be high molecular weight polyamides with difunctionality, and therefore, most likely to behave as biodegradable hypercoiling polymers. This sequence was necessary to avoid an avalanche of analytical work that would otherwise have threatened to curtail or hinder progress of the synthetic work, which was considered as the core activity. In order to avoid the latter, a targeted screening approach was adopted.

The sequence of analytical techniques used depended upon the physical nature of the material produced in any particular synthesis. Virtually all the materials produced were initially subject to ATR-FTIR spectroscopic analysis.* If the infra-red spectrum indicated the presence of amide I and II bands, and in addition, the extracted material was resinous in nature and soluble in both methanol and DMF, then this was taken as an indication, albeit a rather approximate one, of the polymeric nature of the material. Such materials were then subjected to gel permeation chromatographic (GPC) analysis. If the latter revealed a high molecular weight then a further sample of the material was, in some instances, investigated by proton and ^{13}carbon-NMR spectroscopy in order to define the precise primary, and in some cases, secondary structure of the material. This 'functionally driven' analytical pathway focused attention on those materials most likely to meet the original specification.

Use of the analytical screening sequence described enabled those reactions that succeeded in producing potentially useful polymeric materials to be fully investigated, whilst those that failed received only cursory investigation. Thus, in most cases it is known why a reaction succeeded but not always why it failed. This approach mimics the synthetic impetus found in nature where only modifications that offer some degree of functional advantage evolve, while unsuccessful modifications literally wither away. This has been the philosophical basis for both the synthetic and analytical programme adopted.

* Characteristic infra-red resonant frequencies used for spectral analysis were those reported by Socrates,[160] Williams[161] and Nakanishi.[162] All spectra were collected using the ATR-FTIR technique.

4.4. Aims and Objectives of Synthetic Work:

The practical objective of the work described in this thesis has been to synthesize potentially biodegradable hypercoiling or hydrophobically associating polymers which may function as protein correlates. To achieve this a pseudo-biological approach has been adopted, in that a spatially defined process, i.e., interfacial polymerization, was used to produce amphipathic polymers that may themselves associate at interfaces. By adopting this strategy a large number of different molecules were synthesized.

The initial focus of the work was to produce amide derivatives of the degradable sulphonamide hypercoilers, described by Beaumais *et al.*,[5,6] using interfacial polycondensation as the synthetic technique and employing amino acids and their derivatives as the monomeric starting materials. The goal of this work was to synthesize a series of polyamides with the capability of degrading by hydrolysis into biologically acceptable breakdown products.

There are no literature references to lysine-based poly(isophthalamides) produced by interfacial polycondensation. The only related work refers to the synthesis of poly(isophthalamides) based on either lysine or lysine ethyl ester[39] produced by solution polymerization of a silyl activated lysine ethyl ester.

4.5. Aromatic Polyamides: Introduction and Rationale.

The highly reactive aliphatic acid chlorides were not initially considered suitable for use in stirred interfacial reactions due to their high rates of hydrolysis on contact with the aqueous phase. It was, therefore, decided to use the less reactive and more hydrolytically stable aromatic acid chlorides, namely, phthaloyl and isophthaloyl chloride. These offer the additional advantage that the organic acid hydrolytic breakdown products which may be released on contact with biological fluids, e.g. phthalic and isophthalic acid are expected to be non-toxic The phthalamide esters have been extensively used as plasticisers in biomedical polymeric devices and have not been reported to cause adverse tissue reactions.

4.5.1. Synthesis Based on Phthaloyl Chloride.

4.5.1.1 Development of Synthetic Strategy.

The initial experiments in this series involved reacting diamino acids; lysine, diaminopropionic, diaminopimelic, ornithine and the indole-containing amino acid, tryptophan, with phthaloyl chloride. In view of the lower reactivity of phthaloyl chloride, and consequently, lower rate of hydrolysis, it was expected that more acid chloride would be available to react with the diamine.

4.5.1.2. Synthetic Procedure.

The acid chlorides used in these studies were purchased from the Aldrich Chemical Company and the amino acids, diamines and carboxylic acids were purchased from the Sigma Chemical Company. The solvents used were of analytical grade, unless otherwise stated. All materials were used as supplied and without further purification.

The general synthetic procedure involved reacting 50mls of a 0.1-0.2M aqueous solution of the test diamine, containing 0.3-0.82M sodium hydroxide, with a, approximately, 10% molar excess of acid chloride, dissolved in 75mls of carbon tetrachloride. The reaction was conducted in a 1.2L Waring commercial blender (Model No. 7011G) operated at the maximum rotating speed of 2000 rpm. Blending was usually continued for 5-10 minutes, although in the initial series of ranging experiments, blending times of up to 30 minutes were employed.

4.5.1.3. Extraction Procedure.

It was generally anticipated in these reactions that any polymeric material formed should contain pendant carboxylic acid groups, and would therefore, behave as weak polyanion. As a result, the polymeric products were extracted by precipitation in acid conditions. On completion of the blending, the blended mixture was quenched in approximately 500mls of water at neutral pH. The pH of the quenched mixture was then adjusted to approximately pH 2. All acid insoluble precipitated material was collected by filtration, dried *in vacuo* and subjected to ATR FT-IR spectrophotometric analysis using a Nicolet 510 FT-Spectrometer.

4.5.2. Experimental Procedures and Results: Phthalamides.

4.5.2.1. L-Lysine.

The initial experiments were conducted using L-lysine and carried out on a fairly large scale, by interfacially reacting 100mls of aqueous lysine solution (0.1M), containing sodium hydroxide (0.3M) as the acid acceptor, with 150mls of carbon tetrachloride, containing phthaloyl chloride (0.11M). A white crystalline material was deposited on the walls of the blender during the interfacial synthesis. The results of FT-IR analysis of this deposit are shown in tabulated form in the Appendix, Table A1 (spectrum 30). Out-of-plane bending frequencies of ortho substituted aromatic groups were identified along with a five membered imide ring and a C-O stretching frequency, characteristic of the amino acid/carboxylic acid grouping. A weak signal for the secondary amide I and II bands was also apparent.

Acidification of the aqueous phase failed to precipitate any material. However, dilution with excess acetone caused formation of a white precipitate, which formed as a resinous material on evaporation of the acetone. FT-IR analysis, reported in the Appendix, Table A1 (spectrum 44), indicated that this resinous precipitate was composed of carboxylic acid salts, showing both asymmetric and symmetrical stretches, in addition to hydrogen bonded dimers. These findings suggest the presence of phthalic acid, combined with aliphatic groups, that are probably present as methylene groupings within unreacted lysine. The imide identified was assumed to be produced by reaction of both acid chloride groups from a single molecule of phthaloyl chloride with a single amine group. The reaction is depicted in Figure 4.1.

Further experiments used the same amounts of reactant in half the volume of solvent, i.e., 50mls of aqueous sodium hydroxide solution containing lysine (0.2M) with some two and a half times the molarity of alkali (0.82M). This was interfacially reacted with phthaloyl chloride (0.23M) in 75mls of carbon tetrachloride, but failed to yield any substantial quantities of material. The FT-IR spectrum, shown in the Appendix, Table A1 (spectrum 155), of the material deposited on the wall of the blender during this reaction demonstrated the presence of secondary amide I and II bands, carboxylic acid and carboxylate groups, possibly present as amino acids, in addition to methylene groups. These data suggests that low molecular weight phthalamides, phthalic acid and unreacted lysine were the principal components of the reaction mixture.

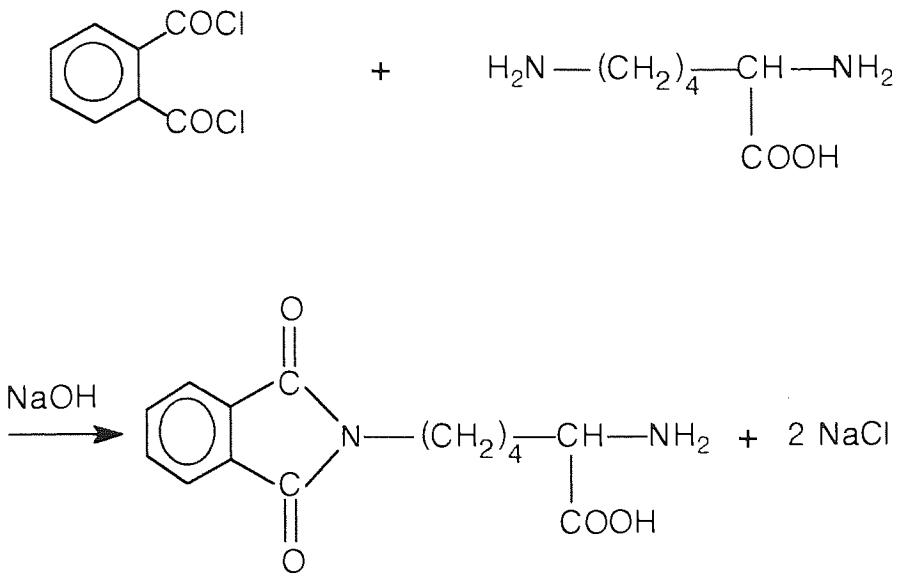


Figure 4.1. Reaction of phthaloyl chloride with lysine: showing imide formation and reaction 'end-capping'.

4.5.2.2. DL-Diaminopropionic Acid.

Replacement lysine with DL-diaminopropionic acid (0.2M) and use of a reduced quantity of phthaloyl chloride (0.14M) also led to the formation of an acetone insoluble, white, resinous material upon reaction, which was soluble in both methanol and acidified water. FT-IR analysis, as reported in the Appendix, Table A1, of both the resinous aqueous extract and the organic phase (spectra 43 and 34, respectively) showed similar spectra to those obtained with the resinous material extracted when lysine was used as the starting material, suggesting that phthalic acid and unreacted diaminopropionic acid were the principal reaction products.

The mole ratio of diamine to diacid chloride was then changed to 1:1.75, in an attempt to minimise the effect of acid chloride loss by hydrolysis. Under these conditions a small amount of 'rope-like' fibrous material was removed from the interface between the two phases at the end of the experiment. This material was pale brown in colour, soluble in methanol and formed sheet-like films on drying. FT-IR analysis of this material, as shown in the Appendix, Table A1 (spectrum 59), confirmed the presence of amide I and II bands and a mixture of carboxylic acid and carboxylate groups. These observations tend to suggest that a poly(phthalamide) had been produced, albeit, in very low yields.

In the case of diaminopropionic acid, the short side chain and close proximity of the amine group substituted at the β-carbon atom to the carboxylic acid groups substituted at the α-carbon atom may provide a degree of steric hindrance, and so prevent reaction end-capping by imide formation. This may account for the small amount of 'polymeric material' that appeared to have been produced.

4.5.2.3. DL-Diaminopimelic acid.

The effect of increasing side chain length was investigated by observing the reaction of the diamine derivative of heptanedioic acid, i.e., DL-α,ε-diaminopimelic acid, using similar reaction conditions to those initially employed with lysine, but doubling the concentration of the reactants used to 0.2M. No material was precipitated during this reaction, but a precipitate formed upon dilution of the aqueous phase with acetone. The organic phase was only precipitated by acetone in the presence of methanol. FT-IR analysis, see Appendix, Table A1 (spectra 56 and 48), suggested that the precipitate from both phases was largely composed of carboxylic acid, probably aryl in nature, in addition to amine groups. Raising the diamine to diacid chloride mole ratio to 1:1.75, in order to minimise the loss of acid chloride by hydrolysis, failed to produce any polymer-like material, unlike the situation noted with diaminopropionic acid. Although a small quantity of fibrous material was formed when this experiment was repeated using half the volume of reactants. FT-IR analysis of the organic phase, see Appendix, Table A1 (spectrum 48), showed the presence of amide I and II bands and suggested that a very low yield of, possibly oligomeric, phthalamide was formed.

4.5.2.4. L-Ornithine.

L-Ornithine was chosen as a model compound to fully investigate the reaction between diamino acids and phthaloyl chloride because, it possessed a side chain with an intermediate length between that of lysine and diaminopropionic acid. Ornithine (0.18M) dissolved in 56g of aqueous sodium hydroxide (0.71M) solution, and was interfacially reacted with phthaloyl chloride (0.23M), dissolved in 75mls of carbon tetrachloride. A yellow coloured material was deposited on the walls of the blender, as observed in reactions involving other diamino acids. Both the aqueous and organic phases were precipitated upon dilution with acetone and formed a methanol soluble product.

FT-IR analysis revealed that acetone precipitation removed amide material from the aqueous phase, as can be observed from the spectral data collected before and after precipitation, as shown in the Appendix, Table A1 (spectra 33 and 35). While analysis of the organic phase (spectra 54 and 36) showed that it contained a five-membered imide ring, combined with aliphatic groups. When this organic phase was partitioned against water the aqueous extract was found to contain mainly carboxylic acid salts, as shown in spectrum 54. From the spectrophotometric analysis it was concluded that phthalamide and phthalic acid were the principal reaction products.

This experiment was repeated using fresh acid chloride, to negate any effect caused by degradation of the staring materials. Once again, ornithine (0.2M) dissolved in 50mls of water, containing sodium hydroxide (0.8M), was interfacially reacted with phthaloyl chloride (0.22M) dissolved in 75mls carbon tetrachloride. A small amount of insoluble resinous material precipitated onto the walls of the blender during the reaction and also collected at the interface between the two phases upon termination of the reaction. FT-IR analysis of these two samples, after washing with carbon tetrachloride and extracting into methanol, see Appendix, Table A2 (spectra 65 and 66), demonstrated the presence in both samples of the amide I and II bands, combined with carboxylic acid and carboxylate salts, with a stronger carboxylate-carbonyl signal present in the wall deposit. This may indicate that a small amount of ornithine-based phthalamide was formed under these experimental conditions. The dominance of carboxylic acid groups in the wall precipitate suggests that this material is mainly phthalic acid.

In an attempt to reduce the rate of acid hydrolysis, compared to the rate of amidation, sodium hydroxide (0.67-0.8M) was replaced as the acid acceptor with the weaker base sodium carbonate (0.76M) and the reaction between ornithine (0.2M) and phthaloyl chloride (0.25M) retested, using two separate experimental conditions. One reaction used 50mls of water and 50mls of carbon tetrachloride, respectively. The second reaction replaced the carbon tetrachloride organic phase with chloroform, so as to limit the number of toxic materials used in the synthesis. In both cases a white precipitated material collected in the lower organic phase. Acidification of the upper aqueous phase also resulted in the formation of a large volume of white precipitate.

The results of FT-IR analysis, shown in the Appendix, Table A2 (spectra 97 and 98), indicated a similar pattern of spectral resonances as those obtained when sodium hydroxide had been used as the acid acceptor. Where the chloroform phase exhibited a stronger signal at 1393cm^{-1} and a weaker signal at 1589cm^{-1}, than was observed in the aqueous phase, indicating the preponderance of carboxylic acid dimers in the organic

phase and carboxylate salts in the aqueous phase. This was combined with a reduction in hydrogen bonding. There is also some spectral evidence to suggest the presence of a 5-membered imide ring. These results suggested that both phases contained phthalamides with carboxylic acid groupings, possibly in the form of those shown for the lysine-based phthalamide in Figure 4.1.

Hence, similar reactions occurred between phthaloyl chloride and ornithine as between phthaloyl chloride and both diaminopropionic and diaminopimelic acids. In all cases, where the pH of the aqueous phase was measured, it was found to be acidic on termination of the reaction. This was intended to result in precipitation of the polymer from the interface, and thereby, promote further polymer formation at the locus of polymerization. However, these conditions may have had the opposite effect by limiting the availability of acid acceptor and so preventing the reaction from proceeding to completion.

4.5.2.5. L-Lysine Ethyl Ester.

Studies conducted by Beaumais[5,6] made use of L-lysine ethyl ester to form poly(sulphonamides) by an interfacial reaction with 1,3, benzene disulphonylchloride. The experimental procedure adopted by these authors was repeated using phthaloyl chloride in place of 1,3, benzene disulphonylchloride, in an attempt to produce a poly(phthalamide). However, no material appeared to be formed under these reaction conditions, either as a result of the extreme dilution of the reactants used (0.02M) or due to the insufficient quantities of acid acceptor present.

4.5.2.6. L-Tryptophan.

L-Tryptophan was used in place of a diamino acid to incorporate an indole ring structure into the polymer and, potentially, to produce a polymer with hydrophobic indole groups as an integral part of the polymer backbone, combined with pendant carboxylic acid groups. The amine group substituted at the α-carbon atom and the NH grouping within the indole ring could be considered to function as a diamine for the purposes of the reaction with phthaloyl chloride.

Tryptophan (0.2M) was dissolved in 50mls of aqueous solution, containing sodium hydroxide (0.6M), and interfacially reacted with phthaloyl chloride (0.22M) dissolved in 75.3mls of carbon tetrachloride. A yellow-orange coloured precipitate was formed

during the reaction and adhered to the walls of the blender. In addition, a small amount of fibrous 'rope-like' material collected at the interface between the two phases upon termination of blending. This material was soluble in both methanol and strongly alkaline water. Curiously, solvation of this product in water was accompanied by colour changes and the solution showed an ability to form a foam.

FT-IR analysis, as shown in the Appendix, Table A2 (spectra 69 and 70), indicated that both the precipitate and rope-like material contained amide I and II bands at 1646 and 1526cm^{-1}, respectively, with a carbonyl stretching frequency at 1709cm^{-1}. Although the wall deposit exhibited a stronger amide signal, it is also lacked a carboxylate signal at 1596cm^{-1}, suggesting that it consisted of mainly carboxylic acid. Both materials exhibited resonant frequencies around 1450cm^{-1} and 2950cm^{-1}, indicative of aliphatic groups. The spectral data would tend to suggest that the principal differences between the two samples resulted from the presence of uncharged carboxylic acid groups in the interfacial sample, probably as a result of the acidic extraction procedure.

The possibility that the material produced by the interfacial reaction described was, in fact, a triad of one phthaloyl chloride grouping bonded to two tryptophan residues, via amine groups substituted at the α-carbon atom, was investigated by repeating the interfacial reaction with a sample of the precipitated material produced in the reaction described above. A sample (0.54g) of this material was reacted with a further phthaloyl chloride (0.05M), dissolved in 40mls of carbon tetrachloride. Fibrous stranded material was, once again, produced and appeared to be identical to that observed in the initial reaction but of somewhat darker colouration. FT-IR analysis (spectrum 71) also showed that this fibrous material was chemically identical to that recovered in the first experiment. Although, higher molecular weight material may well have been formed. No further studies were conducted.

4.5.2.7. L-Tyrosine.

The diamine used in pervious experiments, was replaced with tyrosine in an attempt to synthesize a poly(ester/amide) on interfacial reaction with phthaloyl chloride. The target structure is shown in Figure 4.2. This reaction produced a foam-forming, and possibly, surface active product which was soluble in methanol and formed yellow crystals on drying. However, the FT-IR spectrum of this material failed to indicate the presence of either ester or amide groups.

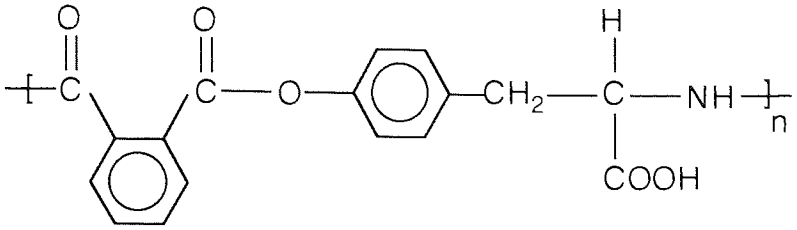


Figure 4.2. Proposed structure of poly(tyrosine phthalamide).

4.5.2.8. Summary of Experimental Work with Phthaloyl Chloride.

These experiments suggested that it may be possible to form phthalamide-based polymers using the interfacial technique, but only from short chain amino acids, i.e., diaminopropionic acid, in the presence of excess acid acceptor (0.8M NaOH). Although, only small amounts of material appear to have been formed. The low yields observed are probably due to the predominance of side reactions, leading to the formation of low molecular weight phthalamide-based triads and by the formation of imides (as shown in Figure 4.1) which would act to end-cap any developing polymer chain and prevent further chain extension. The formation of imides has also been proposed by Katz[163] as a possible explanation for the failure to obtain high molecular weigh poly(phthalamides) an interfacial reaction with aliphatic diamines. Excessive loss of acid chloride by hydrolysis in the strongly basic conditions used may also have contributed to the low yields of material produced.

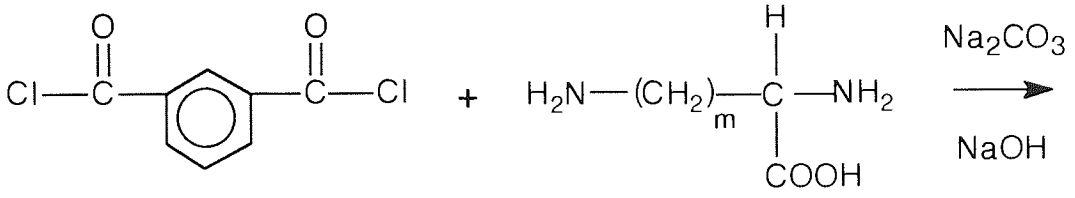
In addition, to the polymerization, which appeared to occur between diaminopropionic acid and phthaloyl chloride, polymer-like material was also collected from the interface when ornithine and tryptophan were used, although lesser amounts of material were formed. In contrast, no such 'polymeric' material was formed when diaminopimelic acid was used. One possible explanation for these results may be that the short side chain length in these amino acids results in a high degree of steric hindrance about the amine group substituted at the ε-carbon atom and this prevents phthalamide (imide) formation and favours polymerization. No such constraint is imposed by the longer side chain present in diaminopimelic acid. A similar argument can be applied in the case of tryptophan, where the bulky indole ring may also exert steric effect. However, the use of insufficient quantities of acid acceptor may, in part, have contributed to the failure of the diaminopimelic acid to react with phthaloyl chloride.

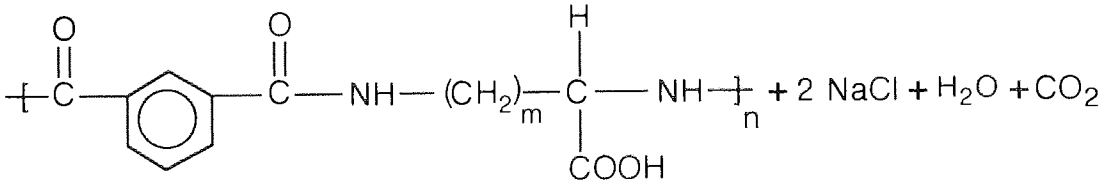
4.5.3. Synthesis Based on Isophthaloyl Chloride.

4.5.3.1. Development of Synthetic Strategy.

The propensity for phthaloyl chloride to form short chain phthalamides or imides led to the use of isophthaloyl chloride, i.e., the meta, rather than the ortho substituted isomer. Where the reactive acid chloride groupings are separated by an additional aromatic carbon atom. Molecular modelling studies suggested that this spatial separation would prevent the two acid chloride groups from within the same molecule reacting with a single amine group to form an imide. A typical reaction scheme illustrating the synthetic work undertaken is shown in Figure 4.3.

Figure 4.3. Interfacial reaction of isophthaloyl chloride with amino acids.



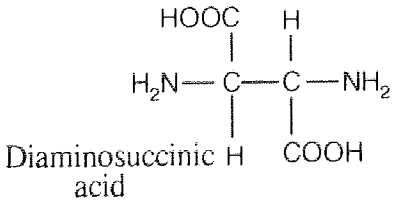


Where : m = 1- Diaminopropionic

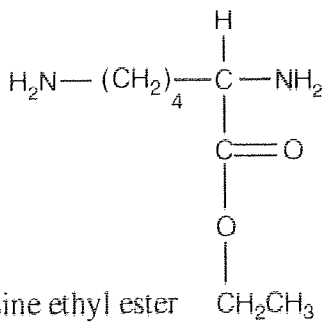
= 1 - Diaminosuccinic

= 3 - Ornithine

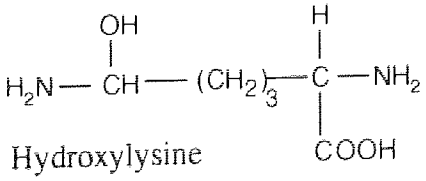
= 4 - Lysine/Hydroxylysine/
Lysine ethyl ester



Diaminosuccinic acid



Lysine ethyl ester



Hydroxylysine

4.5.3.2. Synthetic Procedure.

The basic reaction procedure was similar to that outlined in section 4.5.1.2. with the exception that 50mls of a 0.2M aqueous solution of diamine was usually employed, although in a few cases 0.76M and 1.0M concentrations were used. With the exception of the first experiment in this series, sodium hydroxide was not used as the acid acceptor but was replaced with high concentrations of sodium carbonate, i.e., 0.76M. Sodium carbonate was chosen because it is a weaker base and was less likely to hydrolyse the acid chloride, before the latter could react with the diamine. Isophthaloyl chloride was always used at 10% or 25% molar excess when compared to the diamine, i.e., by use of 50mls of either a 0.22M or 0.25M solution in carbon tetrachloride. Blending times of 3-30 minutes were used, although 10 minutes was the period most frequently employed.

4.5.3.3. Extraction Procedure.

In those reactions where it was anticipated that a polymer with pendant ester groups would be produced, then any precipitated material was filtered, washed with excess carbon tetrachloride, water and methanol. The methanol was expected to extract lower molecular weight oligomeric material, because of the greater preponderance of polar end groups in such oligomers. Methanol extraction also removed any isophthalic acid formed by hydrolysis of the acid chloride during the reaction. In reactions where pendant carboxylic acid groups were anticipated, the aqueous phase formed after quenching the reaction mixture with excess water, was precipitated by lowering the pH to approximately pH 2 (by the addition of concentrated hydrochloric acid), followed by Buchner filtration to remove any precipitate. The aqueous phase of the reaction medium often contained insoluble flocculated material, prior to acidification. This often occurred with polymers that were subsequently found to contain charged carboxylate pendant groups, and presumably, resulted from a saturation of the aqueous solution with product, causing excess material to be precipitated out of solution. In these instances, the flocculated insoluble material was combined with the acid precipitated material prior to extraction into excess methanol. Any insoluble material collected by filtration was dried *in vacuo* and subjected to FT-IR spectrophotometric analysis.

4.5.4. Experimental Procedures and Results: Isophthalamides.

4.5.4.1. L-Lysine Ethyl Ester (LETES).

An aqueous solution of LETES (0.2M), containing sodium hydroxide (0.4M) was interfacially reacted with isophthaloyl chloride (0.22M) dissolved in carbon tetrachloride and formed an insoluble, 'cotton wool-like' precipitate. The dihydrochloride salt of LETES was used in this and all subsequent experiments. After washing and drying *in vacuo* the extracted material was found to be insoluble in alkaline water, some 20% of the material was also found to be insoluble when extracted in excess methanol, but was soluble in DMF. These findings suggested that an amide with ester side chains may have been formed and this was confirmed by FT-IR spectroscopy, see Appendix, Table A3 (spectra 94), where amide I and II bands, at 1640 and 1532cm^{-1}, were identified along with strong signals characteristic of the ester carbonyl and C-O stretching resonances at 1722cm^{-1} and 1217cm^{-1}, respectively. The signal from the ester bond appeared somewhat shifted downfield by an overlying carbonyl-carboxylic acid group. The presence of a carboxylic acid grouping was further supported by a C-O stretching frequency at 1299cm^{-1}. Aliphatic stretching and asymmetric deformation resonances were also apparent at 2928/2865cm^{-1} and 1438cm^{-1}. In addition, there is evidence of hydrogen bonding between amide groups with characteristic resonances at 3332cm^{-1} and 3061cm^{-1}.

Gel permeation chromatography (GPC) was conducted on the resinous, methanol insoluble material, and on a dried sample of the methanol used in the extraction procedure. The results of these investigations are shown in Table 4.1, and referred to under the sample codes LYI-2 (5382) and LYI-3 (5383), respectively. The GPC results showed a M_W and M_n of 25,900 and 13,650, respectively, for the resinous material, and 7,300 and 1,500, respectively, for the methanol soluble material. Confirming the differential solubility of the lower molecular weight material in methanol.

4.5.4.2. L-Lysine.

The presence of carboxylic acid groups in the resinous polymeric material produced using lysine ethyl ester suggested that lysine itself may be a possible diamine candidate for reaction with isophthaloyl chloride. L-Lysine (0.76M), was therefore, reacted with isophthaloyl chloride in an unstirred interfacial experiment according to the procedure

outlined in 4.5.3.2, with the exception that no acid acceptor was present, other than excess lysine. FT-IR analysis of the aqueous phase, shown in the Appendix, Table A3 (spectra 74 and 75), demonstrated the presence of both amide I and II bands and also a carbonyl stretch at 1722cm^{-1}, indicating the formation of amide bonds and carboxylic acid groups. This finding was confirmed when the solution was made alkaline by the appearance of carboxylate resonances at 1589cm^{-1} and 1400cm^{-1} (spectrum 75).

Table 4.1. GPC Molecular size analysis: Aromatic polyamides.*

Sample Code	Sample No.	M_W	M_n	Polydispersity
LYI-2	5382	25,900	13,650	3.08
LYI-3	5383	7,300	1,500	4.80
LYS/ISO	4100	14,900	3,600	3.12
HYDROXY	4276	4,130	1,300	3.15
12N/ISO	3956	13,050	3,500	2.14
ST/ORNA	4277	12,050	3,650	3.35
DIA/ISO	3955	12,950	3,600	2.02
TYRO/ISOPH	3573	250	200	1.15
TYRO/ISO	4099	150	150	1.05

* All samples were analysed using DMF as the solvent medium. Each value shown is the mean of two determinations. Values of M_W and M_n are shown rounded to the nearest 50. Poly(ethylene oxide) and poly(ethylene glycol) were used to calibrate the GPC system and all results are expressed as the PEO/PEG equivalent molecular masses. All GPC molecular size determinations were conducted by RAPRA Technology Ltd.

This reaction was repeated in the presence of sodium carbonate. L-lysine (0.2M) dissolved in 50mls aqueous solution, containing sodium carbonate (0.6M), was reacted by the stirred interfacial technique with isophthaloyl chloride (0.22M), in 50mls of carbon tetrachloride. A precipitate was rapidly formed. On termination of the reaction further material was readily precipitated from the aqueous phase upon adjusting the pH to 4.8. Methanol soluble, and insoluble fractions were formed. FT-IR analysis, shown in the Appendix, Table A3 (spectrum 77), indicated strong amide I and II bands, a carbonyl stretch characteristic of a carboxylic acid, along with C-H stretching and deformation frequencies indicative of aliphatic chains. The methanol insoluble material was readily soluble in DMF but insoluble in acetone and THF, again suggesting the presence of an amide. The aqueous supernatant remaining after acid precipitation, was dried and subjected to FT-IR analysis, the results (Appendix, Table A3, spectrum 76)

indicated the presence of the amide I and II bands with a weak carbonyl carboxylate resonance at 1589 cm^{-1} and an absence of any ester resonances. These results suggest that an amide containing material possessing both carboxylic acid and aliphatic groups was formed in the reaction.

GPC analysis of the resinous material conducted in DMF, and shown in Table 4.1, sample code: LYS/ISO-(4100), indicated M_W of 14,900 with corresponding M_n of 3,600, confirming the formation of high molecular weight polymeric material.

In contrast, when 0.6M sodium hydroxide was used as the acid acceptor, the reaction did not appear to proceed to completion and a white crystalline material was formed. The latter was almost certainly isophthalic acid formed by hydrolysis of the acid chloride in the strongly basic conditions.

4.5.4.3. δ-Hydroxylysine.

δ-Hydroxylysine was used in place of lysine in order to assess the effect that an additional hydrophilic grouping would have upon the interfacial reactivity of lysine and also on the solubility characteristics of any resulting polymer.

δ-Hydroxylysine (0.1M), dissolved in 50mls aqueous sodium carbonate (0.38M) solution, was reacted interfacially with isophthaloyl chloride (0.125M), dissolved in 50mls of carbon tetrachloride. The reaction led to the formation of a white precipitate. Quenching the reaction with excess water and subsequent acidification of the mixed aqueous/organic phase led to further precipitation of material and a phase separation. The separated phase accounted for 50.3% of the total carbon tetrachloride added, while the remaining 49.7% appeared to be solubilized in the aqueous phase. The filtered material was extracted in excess methanol and the methanol insoluble product constituted a 69.1% product yield.

FT-IR analysis of the methanol insoluble and soluble material, as shown in the Appendix, Table A3 (spectra 151 and 119, respectively) indicated the presence in both samples of amide I and II bands, with a carbonyl stretch at 1716cm^{-1} and C-O stretching resonance at 1090cm^{-1}, indicative, of a carboxylic acid. Aliphatic stretching and deformation resonances were also apparent. The spectrum from the material extracted into the methanol phase during the separation process appeared to be similar to that of the methanol insoluble material, but exhibited a much stronger carboxylate resonance at 1539cm^{-1}, along with stronger carboxylic acid signals at 1722cm^{-1}

(carbonyl), 1292cm^{-1} (O-H def) and 1090cm^{-1} (C-O Str). This is probably as a result of the presence of lower molecular weight material and a greater proportion of carboxylic acid end groups, or possibly due to the presence of dissolved isophthalic acid.

GPC analysis of the methanol insoluble resin, shown in Table 4.1, sample code HYDROXY-(4276), indicated a M_W of 4,130 with corresponding M_n of 1,300. These results indicated that largely oligomeric material had been formed, perhaps as a result of the increased solubility of hydroxylysine in the aqueous phase as compared to the solubility of lysine, leading to a reduced rate if ingress into the organic interface.

4.5.4.4. L-Ornithine.

L-Ornithine was, again, chosen as the model compound to fully investigate the reaction between diamino acids and the acid chloride under investigation (isophthaloyl chloride). 50mls of aqueous ornithine (0.2M) solution containing sodium carbonate (0.6M) was interfacially reacted with isophthaloyl chloride (0.22M), dissolved in carbon tetrachloride. A white precipitate was formed during the interfacial reaction and further material precipitated upon acidification (<pH4) of the quenched reaction mixture. The precipitate was extracted into excess methanol and formed, both a methanol soluble, and insoluble fraction. The latter material was found to be resinous in nature, soluble in DMF and formed films on drying.

FT-IR analysis of both phases, shown in the Appendix, Table A3 (spectra 154 and 80), demonstrated similar spectra to those observed with lysine and hydroxylysine-based isophthalamides. Both aqueous and organic phases exhibited amide I and II bands, a carboxylic acid/carbonyl resonance, in addition to C-H stretching and deformation frequencies, indicative of aliphatic groupings. Once again, the methanol soluble phase lacked the symmetrical stretch at 1387cm^{-1} indicative of a carboxylate grouping, but exhibited a stronger carboxylic acid C-OH resonance at 1299cm^{-1}, possibly as a result of the lower molecular weight and a greater proportion of carboxylic acid end groups or perhaps due to the presence of dissolved isophthalic acid.

The differential methanol solubility of the products formed during the reaction is likely to result from differences in the molecular weight range of the two materials. Further experiments to investigate the effects of varying the experimental conditions included; raising the concentration of sodium carbonate to 0.76M, increasing the blending time from 10 to 30 minutes, increasing the molar quantities of the reactants by

a factor of five and using hexane in place of carbon tetrachloride as the organic phase. In all instances, the reaction proceeded as described above and the products were extracted with methanol the to remove lower molecular weight material.

FT-IR spectroscopy was conducted on all samples and the results are shown in the Appendix, Table A3 (spectra 107, 112, 99, 101 and 102). The results reveal spectra typical of a poly(isophthalamide) such as that shown in Figure 4.4. (spectrum 154). In the case of the reaction employing hexane, IR spectroscopy (spectra 107 and 112), indicated a similar spectral profile to that found when using carbon tetrachloride as the organic phase. The material remaining in the aqueous phase after acidification and removal of the precipitate appeared to be mainly composed of a mixture of carboxylic acid, carboxylate groups and amides. Probably representing isophthalic acid and low molecular weight isophthalamides that remained dissolved in aqueous solution.

The precipitated material formed by the reaction between ornithine (0.2M) and isophthaloyl chloride (0.25M), in the presence of high concentrations of sodium carbonate (0.76M) was extracted into excess methanol and the molecular weight of the methanol insoluble phase analysed by GPC. The results, shown in Table 4.1, sample code 12N/ISO-(3956), indicated a M_W of 13,050 with corresponding M_n of 3,500. Confirming the formation of high molecular weight polymeric material.

A sample of the methanol insoluble material made when using the 0.76M sodium carbonate technique was further purified by Soxhlet extraction in methanol. FT-IR analysis of the resin before Soxhlet extraction, and the methanol insoluble and soluble extract after Soxhlet extraction, are shown in the Appendix, Table A3, spectra 99, 101 and 102, respectively. These spectra demonstrated the presence of an aliphatic ester carbonyl stretch at 1734cm^{-1}, combined with an ester C-O stretching frequency at 1299cm^{-1} in the Soxhleted material, these resonances were particularly prominent in the resinous material. In addition to the characteristic amide I and II bands, both C-H stretching and deformation frequencies, indicative of aliphatic groupings, were also present. These results indicate that refluxing in hot methanol, possibly in the presence of residual acid, causes methylation of the pendant carboxylic acid groups to form the corresponding methyl ester. The strong signal observed at 1172cm^{-1} in the IR spectrum is characteristic of this ester. It is interesting to note that methylation occurred predominantly in the resinous material which was in contact with distilled methanol and not to the same extent in the methanol soluble material which was in contact with small amounts of residual water in the Soxhlet flask. The methanol soluble material, was therefore, less likely to be fully esterified.

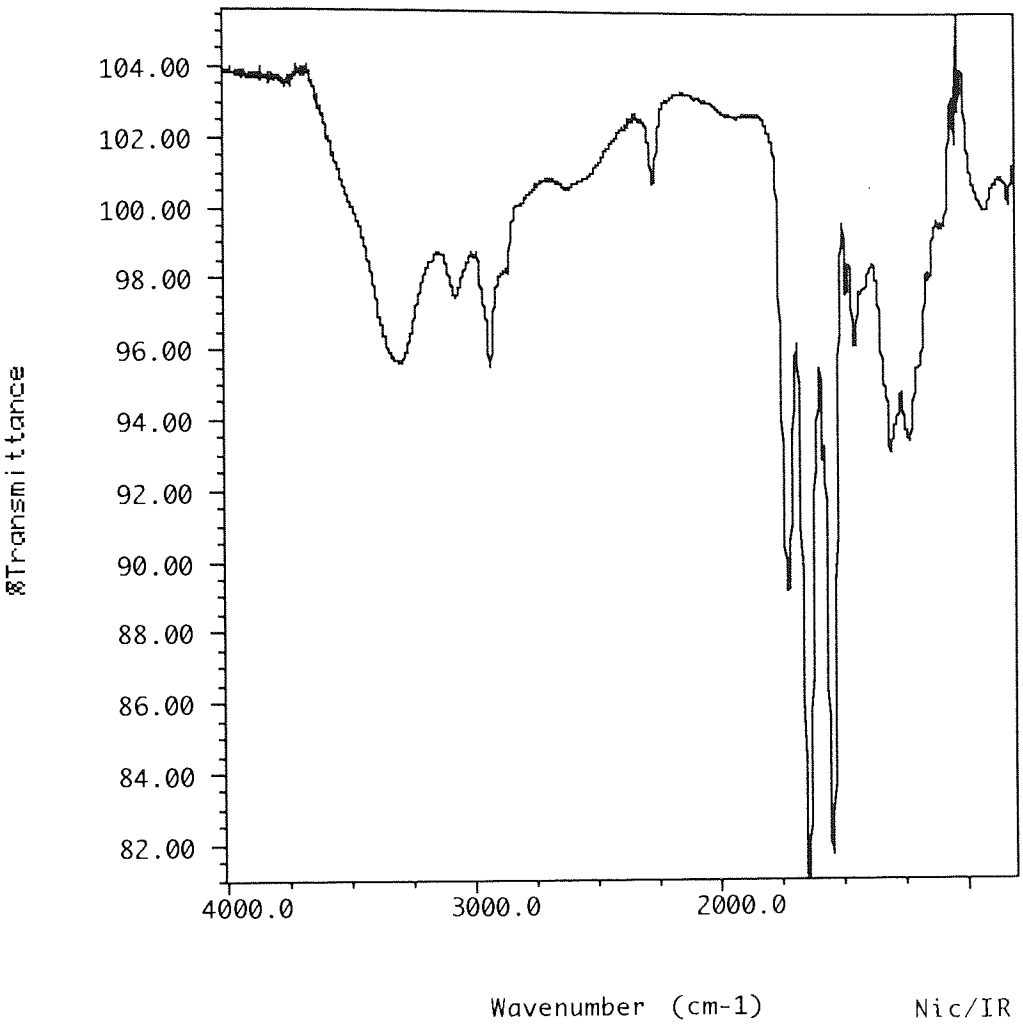


Figure 4.4. FT-IR spectrum of poly(ornithine isophthalamide) - resinous precipitate (spectrum 154).

GPC analysis of the base polymer, prior to Soxhlet extraction, was conducted in DMF, and the results are shown in Table 4.1, sample code ST/ORNA-(4277). These results indicated a M_W of 12,050 and the corresponding M_n 3,640. Confirming that polymeric material had been formed, but with lower molecular weight than that observed in the pre-Soxhleted polymer. It can be assumed that Soxhlet extraction resulted in partial hydrolysis of the polymer backbone in addition to side chain esterification.

A sample of the Soxhlet extracted resin was subjected to analysis by proton-NMR. which showed a resonance at 3.316 ppm. characteristic of the δH of a methyl ester.

4.5.4.5. DL-Diaminopropionic acid.

DL-Diaminopropionic acid was used as a model compound to observe the effect of a minimal intrachain distance on diamine reactivity. An aqueous solution of diaminopropionic acid (0.2M), containing sodium carbonate (0.76M), was interfacially reacted with isophthaloyl chloride (0.25M), dissolved in 50mls of carbon tetrachloride. A flocculated yellow-coloured mass was formed during the reaction and completely dissolved on quenching the reaction mixture with excess water, presumably because the increased charge density within the polymer led to a high aqueous solubility. The product was then recovered from the aqueous mixture by acidification to pH 1.7. The resultant precipitate was extracted into excess methanol and formed both a methanol soluble and insoluble fraction. The latter, appeared as a yellow resinous material which was soluble in DMF.

FT-IR analysis of the resinous phase, as reported in the Appendix, Table A4, spectrum 95, demonstrated the presence of amide I, II and III bands, in addition to a carboxylic acid carbonyl resonance and C-O-H deformation resonances, all characteristic of carboxylic acid groupings. Aliphatic groupings were also present in the form of C-H stretching and deformation resonances. The results of GPC analysis of the resinous, methanol insoluble material, are shown in Table 4.1, sample code DIA/ISO-(3955), and indicated a M_W of 12,950 with corresponding M_n of 3,600. These data confirm the formation of a high molecular weight polyamide, bearing carboxylic acid groupings, probably present in pendant form.

4.5.4.6. L-Tryptophan.

L-Tryptophan was used in an attempt to incorporate an indole ring structure into a polymer, by interfacial reaction with isophthaloyl chloride, and thereby, produce a polymer with two hydrophobic groups as an integral part of each repeat unit of the polymer backbone combined with pendant carboxylic acid groups.

50mls of aqueous L-tryptophan (0.2M) solution, containing sodium carbonate (0.76M), was interfacially reacted with isophthaloyl chloride (0.25M), dissolved in 50mls of carbon tetrachloride. A bright yellow precipitate formed immediately upon blending and further precipitated material was deposited upon acidification of the quenched reaction mixture. The precipitate appeared to be unstable and its surface layers slowly became discoloured, turning pink, possibly due to an oxidative reaction with air or perhaps as a result of a photochromic reaction, or both. The coloured material was soluble in ethanol and resulted in the formation of a deep purple colouration after several days in ethanolic solution.

The results of FT-IR analysis, as shown in the Appendix, Table A4, spectrum 110, indicated the presence of both amide I and II bands at 1646 and 1526cm^{-1}, respectively, and a carbonyl stretching frequency at 1716cm^{-1}, combined with a C-O stretching frequency at 1097cm^{-1}, indicating the presence of a carboxylic acid dimer. C-H stretching and scissoring deformation frequencies were also present at 2928cm^{-1} and 1438cm^{-1}, respectively, indicative of aliphatic groupings. However, the C-O stretching frequency at 1223cm^{-1} would tend to suggest the presence of carboxylic acid groups present within an amino acid.

In conclusion, it would appear that the recovered product was a mixture of monomeric isophthalamides, possibly with pendant carboxylic acid groups, combined with unreacted amino acid.

4.6. Isophthaloyl Chloride-Based Ester-Amides, Esters and Anhydrides.

Amino acids containing side chain hydroxyl groups, e.g. tyrosine and serine were used to investigate the possibility of synthesizing ester/amides or depsipeptides by stirred interfacial polymerization with isophthaloyl chloride. The experimental conditions used were identical to those described for the isophthalamides in sections 4.5.3.2. and 4.5.3.3. The synthetic strategy adopted was designed to investigate the reactivity of phenoxide and hydroxyl groups towards acid chlorides, in order to assess the ability of these groups to act as hydrogen donors in comparison to the amine group. These studies also give some indication as to the likelihood of side reactions. In addition, the reactivity of aliphatic alcohols towards isophthaloyl chloride was further investigated by using the diol, butane-1,4-diol, in place of serine. The ability of aliphatic diacids to react interfacially with isophthaloyl chloride and the potential to form anhydrides or poly(anhydrides) was also investigated by using malonic acid in place of a diamine.

4.6.1 Ester/Amides.

4.6.1.1. L-Tyrosine.

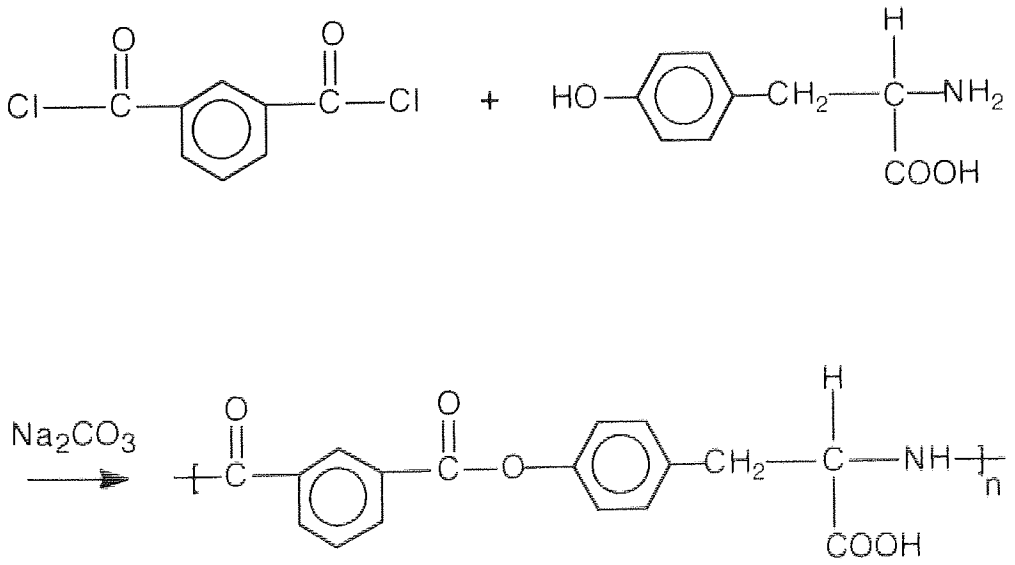
L-Tyrosine (0.2M) dissolved in 50mls aqueous solution, containing sodium carbonate (0.6M), was interfacially reacted with isophthaloyl chloride (0.22M), dissolved in 50mls of carbon tetrachloride. A white precipitate was formed during the reaction which dissolved in the aqueous phase (pH 10-11) on standing for twelve hours. The material was subsequently reprecipitated on acidification to pH 4. These observations may be explained by the formation of an anionic poly(ester/amide). Alternatively, the ester bonds may have hydrolysed in such highly alkaline conditions to form a low molecular weight amide. However, clear interpretation is complicated by the poor solubility of the tyrosine starting material in aqueous solution.

GPC analysis was conducted on a sample of the extracted material and the results, shown in Table 4.1, sample code TYRO/ISOPH-(3573), indicated a M_W of 250 and a corresponding M_n of 200. These results confirm that no polymeric material had been formed.

Further experiments investigated the effect of changing the pH of the reaction mixture. A high post-reaction pH, i.e., >pH12, was maintained by using a higher concentration of sodium carbonate (0.76M), thereby, maintaining any unreacted tyrosine in solution. This approach was not entirely successful, because the post-reaction pH was only, in fact, maintained at pH 10 and this was insufficient to retain the tyrosine in aqueous solution. The precipitated white material collected from the aqueous phase of the reaction mixture was extracted into excess methanol and formed a methanol insoluble material that was resinous in nature and soluble in DMF.

FT-IR analysis, of this resinous material, as shown in the Appendix, Table A4 (spectrum 90), indicated a resonance at 1728cm^{-1}, which suggests a mixture of carbonyl bonds from both carboxylic acid and ester groups. The presence of an ester grouping is also suggested by ester C-O symmetric and asymmetric stretching frequencies at 1216cm^{-1} and 1059cm^{-1}, respectively. Amide I and II bands are also apparent and there is a strong signal in the 1540 to 1500cm^{-1} region that represents an asymmetric deformation within an amine grouping, probably present as residual unreacted amino acid. Aliphatic stretching and deformation frequencies were also identified. This spectral data points towards the formation of a poly(ester/amide), such as that shown in the reaction scheme illustrated in Figure 4.5.

Figure 4.5. Proposed reaction scheme for synthesis of poly(tyrosine isophthalamide).



GPC analysis of the resinous material synthesized when using high concentrations of acid acceptor is shown in Table 4.1, sample code TYR/ISO-(4099), where a M_W of 150 and corresponding M_n of 150 are reported. These results suggest that any ester or amide that is present is likely to be low molecular weight material, possibly present as a dihydroxy trimer.

The highly basic environment used in the second synthetic process appeared to result in the formation lower molecular weight material than that produced by the initial synthetic technique using 0.6M sodium carbonate, possibly as a result of hydrolysis of amide/ester based oligomer/polymer under these conditions. In a subsequent experiment, no precipitated material formed in the quenched aqueous phase until the pH dropped below 6, which would indicate an absence of any unreacted tyrosine. Although, FT-IR analysis (spectrum 88) failed to confirm the presence of any ester bonds and found that the material principally contained amino acid groups.

If tyrosine is to react rapidly with isophthaloyl chloride then it must be retained in solution and this can only be achieved by keeping the aqueous phase at a high pH throughout the reaction period, to maintain the phenoxide ion in its charged state. In order to achieve this, the reaction between an aqueous solution (100mls) of L-tyrosine (0.2M) and isophthaloyl chloride (0.22M), dissolved in 100mls of carbon tetrachloride, was repeated in the presence of elevated concentrations of firstly, sodium hydroxide (2.0M) and then, in a separate experiment, sodium carbonate (6.25M).

In the reaction conducted in the presence high levels of sodium hydroxide the post-reaction pH was 12.52, and no white precipitate was formed in the upper aqueous phase during the experiment (unlike in previous experiments). In this instance, an aqueous emulsion of creamy-white colouration formed and a distinct lower organic phase was also apparent. On termination of the reaction, acidification of the aqueous phase resulted in formation of a precipitate which was recovered as a methanol insoluble phase after extraction into excess methanol.

FT-IR analysis conducted on a dried sample of the aqueous phase showed a strong resonance at 1598cm^{-1} indicative of a carbonyl asymmetric stretching frequency within a carboxylate group. Acidifying this material caused a shift in the resonance peak from 1589cm^{-1} to 1684cm^{-1}, see Appendix, Table A4 (spectrum 178). An accompanying carboxylate resonance was also present at 1305cm^{-1}, suggesting the formation of a carboxylic acid. There was no evidence of amide I or II bands. This data would seem to indicate that the reaction produced predominantly isophthalic acid as a result of the rapid hydrolysis of acid chloride in the strongly basic conditions. Replacing the high concentrations of sodium hydroxide with sodium carbonate (6.25M) failed to maintain adequate basicity throughout the reaction, i.e., >pH12, and this led to precipitation of tyrosine.

In conclusion, the interfacial technique does not appear to be suitable for the synthesis of poly(ester/amides) based upon tyrosine, because of the poor aqueous solubility of this amino acid. In order to overcome this limitation, the pH must be adjusted to strongly alkaline conditions, which in turn, cause a rapid hydrolysis of isophthaloyl chloride, and thus, prevents polymerization from occurring.

4.6.1.2. L-Serine.

Serine was used in place of a diamino acid to assess the interfacial reactivity of aliphatic hydroxyl groups towards acid chlorides. L-serine (0.2M) dissolved in 50mls of aqueous solution, containing sodium carbonate (0.76M), was interfacially reacted with isophthaloyl chloride (0.25M), dissolved in 50mls of carbon tetrachloride. A pale yellow fibrous precipitate was collected upon termination of the reaction which dissolved in excess water and could not be reprecipitated upon acidification, suggesting an absence of any carboxylic acid groups. The pale yellow precipitated material was largely soluble in methanol, although a small fraction of the material remained insoluble.

It is most likely that the principal products formed in this reaction are monomeric compounds, namely, serine isophthalamide or diserine isophthalamide and isophthalic acid. All three contain carboxylic acid groups and could be expected to be readily precipitated from aqueous solution upon acidification. In addition, these products are relatively polar in nature and would be expected to dissolve in the polar solvent methanol. The failure of any material to reprecipitate from aqueous solution may be the result of a dilution effect.

4.6.2. Esters.

4.6.2.1. Butane-1,4-Diol.

Butane 1,4 diol was used in place of a diamine or aminol to assess the interfacial reactivity of aliphatic hydroxyl groups towards acid chlorides in the absence of a competing amine group. This gave some indication of the suitability of the interfacial route for the synthesis of aliphatic polyesters and the potential for side reactions. The reaction conditions were identical to those noted above, for the reaction between serine and isophthaloyl chloride, with the exception that butane-1,4-diol (0.2M), dissolved in 50mls aqueous solution, was used in place of serine. A precipitate was formed during the blending process and further material was precipitated upon acidification of the reaction mixture after termination of the reaction. The precipitate was found to be soluble in methanol.

FT-IR analysis of the precipitated material, as shown in the Appendix, Table A4 (spectrum 92), indicated that this material was predominantly aryl carboxylic acid, with a strong carbonyl signal at 1684cm^{-1}, in addition to carboxylate-carbonyl asymmetric and symmetric stretching frequencies at 1614/1577cm^{-1} and 1419cm^{-1}, respectively. Hence, both the reaction precipitate and the acid insoluble material are likely to be isophthalic acid formed during the reaction by hydrolysis of the acid chloride.

Therefore, the rate of ester formation appears to be negligible compared to the high rates of acid chloride hydrolysis and certainly far less rapid than the rate of the reaction between acid chloride and amine groups. It can be concluded, that reactions involving hydroxyl groups and the potential formation of cross-linked chains in hydroxyl containing polyamides, such as those based on hydroxylysine, are unlikely to occur.

4.6.3. Anhydrides.

4.6.3.1. Malonic acid.

Malonic acid was used in place of a diamine, aminol or diol to assess the interfacial reactivity of aliphatic carboxyl groups towards acid chlorides in the absence of a competing amine group. This was carried out to observe the suitability of the interfacial route for the synthesis of polyanhydrides and also the likelihood of cross-linking between polyamide chains bearing pendant carboxylic acid groups. The reaction conditions were identical to those noted above for the reaction between serine and isophthaloyl chloride, with the exception, that 0.25M malonic acid was used in place of serine. Only a small quantity of translucent material was formed, which collected on the surface of the aqueous phase upon termination of the reaction. The carbon tetrachloride phase also formed a separate layer. It is, therefore, unlikely that any reaction occurred between the acid chloride and the diacid which effectively precludes the possibility of cross-linking between pendant carboxylic acid groups located on adjacent chains in the amino acid-based poly(isophthalamides) previously described.

4.7. Acid Chloride-Based Block Polymers.

4.7.1. Development of Synthetic Strategy.

The surface chemical studies conducted on 0.1% aqueous solutions of lysine, ornithine, and diaminopropionic acid-based poly(isophthalamides), or in the case of tyrosine, isophthalamides, indicated that solutions of these materials did not markedly change their surface properties in response to changes in pH. These studies are described in detail in chapter 6. The inability of these molecules to form surface active amphipathic structures may, in part, result from the alternating nature of the poly(isophthalamides), where each hydrophobic isophthaloyl group is separated by a hydrophilic amino acid residue. This arrangement effectively limits the ability of the polymer to adopt a conformation in which the two molecular types are separated into distinct domains. In addition, the incorporation of a benzene ring structure into the polymer backbone, with its partial double bond character, will confer a degree of rigidity on the polymer chain and will tend to limit flexibility within the polymer

backbone. This lack of backbone flexibility may also be a contributory factor in preventing domain formation.

To overcome these perceived structural limitations two alternative synthetic strategies were adopted:

i) To replace the aromatic isophthaloyl grouping by synthesizing polymers with aliphatic acid chlorides.

ii) To incorporate hydrophilic blocks between the hydrophobic groups. Such structures may behave in a similar manner to other block copolymers that contain distinct hydrophobic and hydrophilic blocks, e.g. polyoxyethylene-poly-oxypropylene-based copolymers such as the Pluronic^{TM} series, that are known to be highly surface active.[164]

4.7.2. Alternating Polyamides.

4.7.2.1. Aliphatic Polymers Based on Succinyl Chloride.

The first synthetic strategy involved interfacially reacting succinyl chloride with the amino acid, diaminosuccinic acid and/or lysine ethyl ester, to potentially, produce an alternating terpolymer containing diamines with either anionic (carboxylic acid) or hydrophobic (ethyl ester) side chains. Diaminosuccinic acid was chosen because, it contained two carboxylic acid groups, and would therefore, confer a higher degree of charge density on any resultant polymer. This may render the polymer conformation more sensitive to changes in pH, since a high charge density within a polyelectrolyte leads to repulsion between adjacent charged groups and forms a rigid or rod-like molecular conformation. However, in a polyelectrolyte with a low charge density the spatial separation of the charged groups effectively prevents charge repulsion. In such instances, the macromolecular conformation is relatively insensitive to changes in pH.

meso-2,3-Diaminosuccinic acid (0.1M) and lysine ethyl ester (0.1M) were dissolved in 50mls of aqueous solution, containing sodium carbonate (0.76M), and interfacially reacted with succinyl chloride (0.25M), dissolved in 50mls of carbon tetrachloride. A flocculated precipitate of approximately 20mls in volume formed on blending and dissolved in the excess aqueous solution used for quenching the reaction. This material was reprecipitated upon, subsequent, acidification of the quenched

aqueous phase, indicating the presence of acidic groups. The acid insoluble material was then extracted into excess methanol.

FT-IR analysis failed to provide any evidence of amide formation, probably as a result of the rapid hydrolysis of acid chloride prior to any reaction with the diamines. The failure to form polymeric material may also, in part, be due to the formation of a six membered imide ring by a direct reaction between diamino succinic acid and succinyl chloride. To test this hypothesis a further reaction was undertaken using diaminosuccinic acid and acid chloride, without the presence of LETES.

Diaminosuccinic acid (0.1M), dissolved in 50mls of aqueous sodium carbonate (0.76M) solution, was interfacially reacted with succinyl chloride (1.2M), dissolved in 70mls carbon tetrachloride. The organic phase was added in three aliquots to give a final acid chloride concentration of 0.3, 0.6 and 1.2M, respectively, in order to maintain the acid chloride in excess. Although blending was continued for 30 minutes, only small amounts of solid material were formed and these collected at the surface of the aqueous phase and dissolved upon quenching the reaction mixture with excess water. No material was precipitated on acidification of the quenched aqueous phase, suggesting an absence of carboxylic acid groups and a failure to form any polyanionic material. Hence, in the above reaction the precipitated material is unlikely to contain any diaminosuccinic acid residues. This result was probably caused by the high charge density of the diaminosuccinic acid, which acts to limit the rate of ingress of this amino acid into the organic phase compared to that of LETES free base which is freely soluble in organic media.

4.7.3. Isophthaloyl Chloride-Based 'Block' Copolymers.

4.7.3.1. Aromatic Polymers Based on Isophthaloyl Chloride.

As a result of the failure of succinyl chloride to form aliphatic polyamides that contained both hydrophobic and hydrophilic amino acid residues, it was decided to revert to using isophthaloyl chloride, since our previous results had shown this to be far less prone to hydrolysis or imide formation. In addition to diaminopropionic acid, LETES was also incorporated into the reaction mixture in an attempt to confer increased flexibility into any resulting polymer backbone and partially overcome the structural rigidity conferred by the isophthaloyl grouping. It was hoped that this strategy would

favour the formation of hydrophobic microdomains and result in surface active properties.

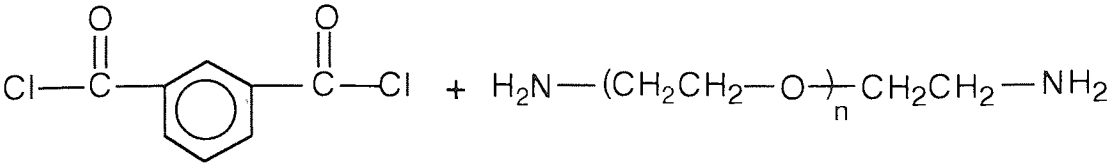
DL-Diaminopropionic acid (0.125M), dissolved in 2mls aqueous sodium carbonate (0.75M) solution was interfacially reacted with isophthaloyl chloride (0.3M), dissolved 2mls of hexane. Lysine ethyl ester (0.125M), also dissolved in 2mls of aqueous sodium carbonate (0.75M), was added to the reaction mixture after the reaction had proceeded for fifteen seconds. It was hoped that the diaminopropionic acid would form discrete hydrophilic blocks, while the LETES would form hydrophobic blocks. Hexane was used as an alternative organic phase to minimise hydrolysis of the acid chloride, by raising the partition coefficient against the aqueous phase and so favouring retention of acid chloride in the former phase. A pale yellow precipitate formed on blending and collected at the surface on termination of the reaction. The precipitated material was insoluble in water at neutral pH, hexane and methanol.

FT-IR analysis, as shown in the Appendix, Table A5 (spectra 96), indicated the presence of amide I and II bands, a carbonyl stretch within a carboxylic acid grouping and a C-O stretch at 1229cm^{-1}, characteristic of an ester or amino acid carboxylate grouping. Both C-H stretching and deformation frequencies, indicative of aliphatic groupings, were also present. In addition, there was some evidence of residual acid chloride with a small signal at 1791cm^{-1}. These results suggested that at least one, and possibly both, diamines were incorporated into an amide-based structure, which exhibited carboxylic acid groups (possibly in a pendant position). However, after extraction the amount of material available was insufficient to conduct GPC analysis, and as a result, the molecular weight of this material was not resolved.

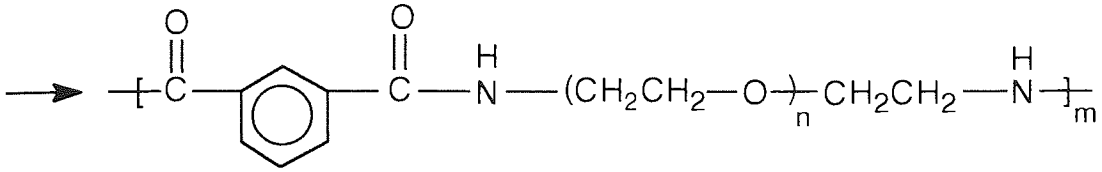
4.7.3.2 Block Copolymers Based on Poly(oxyethylene).

The second synthetic strategy adopted, involved incorporating polyoxyethylene blocks between hydrophobic isophthaloyl groups. It was hoped to achieve this by interfacially reacting polyoxyethylene bis(amine) [POE-bis amine], with a molecular weight of 3,350, with isophthaloyl chloride according to the reaction scheme shown in Figure 4.6. The highly flexible nature of the polyethylene oxide backbone should, potentially, enable the hydrophobic isophthaloyl groups to be forced out of the aqueous environment to form separate microdomains, and thereby, confer surface active properties upon any polymer molecule formed.

Figure 4.6. Proposed reaction scheme for synthesis of poly(POE isophthalamide) 'block' copolymer.



Isophthaloyl chloride Polyoxyethylene bis(amine)



Poly(POE isophthalamide)

Poly[oxyethylene bis(amine)] (0.003M) dissolved in 50mls of aqueous sodium carbonate (0.18M) solution was reacted with isophthaloyl chloride (0.06M), dissolved in 50mls of hexane. Previous studies had found that polyethylene glycol 400 (a related polyether) was insoluble in hexane. Hence, by using this solvent the POE diamine could be confined to the aqueous phase, so limiting its loss into the organic phase. No visible material was formed during the reaction, despite raising the acid chloride concentration to 0.18M, by two further additions of 0.0033 moles of acid chloride. On termination of the reaction and on quenching with water, a solid white material formed at the surface. This material was soluble in both methanol and in aqueous solutions above pH 11.5 and was probably isophthalic acid.

In subsequent experiments, the hexane phase was replaced with chloroform to increase the penetration of the diamine into the organic phase and higher concentrations of both isophthaloyl chloride (0.138M) and sodium carbonate (0.38M) were used. Under these conditions a dense, filmy-white, precipitate formed in the organic phase during the interfacial reaction. FT-IR analysis, as shown in the Appendix, Table A5 (spectra 117), indicated that the spectrum of this material was not substantially different to that of the unreacted POE bis(amine) (spectrum 109), with two prominent resonant frequencies around 1087cm^{-1}, characteristic of the C-O-C stretching frequency of an aliphatic ether, and two resonances at 2915cm^{-1} and 2846cm^{-1}, characteristic of the C-H stretching frequencies within aliphatic groups. Additional resonances were also

exhibited at 1652 and 1570cm^{-1}, suggesting the presence of carboxylic acid and carboxylate groups, these probably arose from the presence of isophthalic acid. The latter material was precipitated into the aqueous phase by acidification. It should be noted that the unreacted POE bis(amine) starting material also appeared to contain carboxylic acid groups.

As a result of this experiment, a further study was undertaken where the partition coefficient between aqueous and organic phases was raised to retain the POE bis(amine) in the aqueous phase. Higher concentrations of isophthaloyl chloride (0.276M) and sodium carbonate (0.84M) were also used to overcome the effects of acid chloride hydrolysis. Under these conditions no material precipitated during the reaction but a precipitate was recovered upon acidification of the aqueous phase and this material was probably isophthalic acid.

FT-IR analysis was conducted on a sample of the dried aqueous phase, after acidification, since any POE-based polyamide may have been water soluble. The results, as shown in the Appendix, Table A5 (spectra 114 and 109), demonstrated that the spectrum obtained from the aqueous phase (spectrum 114) was similar to the that of the POE bis(amine) starting material (spectrum 109), with two prominent resonant frequencies around 1100cm^{-1}, characteristic of the C-O-C stretching frequency of an aliphatic ether, combined with a C-H stretching resonance at 2900cm^{-1} and 1463cm^{-1}, characteristic of aliphatic groups. However, there were also two weak signals for amide I and II bands, suggesting that a limited amidation may have occurred and produced small amounts of oligomeric material. Future experiments should repeat this polymerization using a shorter chain POE bis(amine), e.g. polyethylene glycol bis(amine) with a molecular weight of 300 or 400, where the amine end group density will be higher and so more likely to react with the acid chloride groups.

4.7.3.3. Block Terpolymers Based on Poly(oxyethylene).

To overcome some of the problems encountered in synthesizing the simple copolymer, POE bis(amine) isophthalamide, it was decided to synthesize ornithine isophthalamide and 'dope' the reaction mixture with POE bis(amine). In this way smaller amounts of POE bis(amine) may, potentially, be incorporated into poly(ornithine isophthalamide) and this would render the resulting terpolymer sensitive to pH, and therefore, extractable in acidic conditions by precipitation.

Calculations suggested that by using 0.00015 moles of POE bis(amine) and 0.01 moles of ornithine then, by statistical chance, the ornithine would be likely to react with isophthaloyl chloride seven times as often as the POE bis(amine). This would result in formation of a block of isophthalamide containing 64 ornithine residues, at which point, the amine end group concentration of the poly(ornithine isophthalamide) would be equal to that of the POE bis(amine). This would give a theoretical molecular weight for the poly(ornithine isophthalamide) of 16,000. Thus, the reaction would proceed almost to completion before POE bis(amine) and poly(ornithine isophthalamide) would have an equal chance of incorporation into any resulting terpolymer. In this way, fairly large blocks of the respective polymers may be incorporated into the final terpolymer.

The experiments described in section 4.5.4.4 had shown the feasibility of using hexane as the organic phase, in place of carbon tetrachloride, for the synthesis of poly(ornithine isophthalamide). This acted to minimise acid chloride hydrolysis by retaining the acid chloride in the organic phase. Using similar conditions to those described in 4.5.4.4, POE bis(amine) (0.0003M) was added to the reaction mixture. A precipitate formed during the blending and a further 20mls of both water and hexane were added to the reaction, to solubilize the coagulated mass. On termination of the reaction, acidification of the aqueous phase resulted in the formation of further precipitated material, which was extracted into excess methanol.

FT-IR analysis of the methanol insoluble and soluble material, as shown in the Appendix, Table A5 (spectra 108 and 118), indicated a similar spectral pattern in both samples with the presence of amide I, II and III bands and a carboxylic acid carbonyl group resonance. The latter was stronger in the methanol soluble material possibly as a result of the lower molecular weight and higher carboxylic acid end group density. Both samples also exhibited C-H stretching and asymmetric bending frequencies indicative of aliphatic groupings, in addition to primary amine groups. However the lack of any strong C-O-C ether resonances suggested that POE had not been incorporated into the precipitated material.

Further evidence suggesting a lack of incorporation of the POE bis(amine) into the extracted polymer was provided by FT-IR analysis of the acidified aqueous filtrate, see Appendix, Table A5 (spectrum 116), where a prominent band at 1084cm^{-1}, characteristic of a C-O-C ether stretching frequency or a carboxylic acid, is found along with a primary amine bending resonance at 1602cm^{-1}. These data strongly suggest that most of the POE bis(amine) remained unreacted in the aqueous phase. The spectrum is, however, dominated by asymmetrical and symmetrical stretching frequencies, at 1564 and 1387cm^{-1}, respectively, characteristic of a carboxylic acid and probably represents hydrolysed isophthaloyl chloride present as isophthalic acid.

In conclusion, it would appear that a small amount of POE bis(amine) is incorporated into poly(ornithine/isophthalamide) by this synthetic route, but most of the starting material probably remained unreacted in the aqueous phase or in the case of isophthaloyl chloride became hydrolysed.

4.8. **Synthesis Based on Terephthaloyl Chloride.**

The previous experiments described in this chapter demonstrated that interfacial polymerization did not occur between lysine and phthaloyl chloride to any appreciable extent. Probably as a result of reaction end-capping caused by the formation of phthalamide (imide) groups. The use of isophthaloyl chloride prevented this effect and resulted in the successful formation of lysine based poly(isophthalamides). To complete the investigations of the effect of isomeric form upon the reactivity of aromatic acid chlorides, terephthaloyl chloride was reacted with lysine in the presence of a high concentration of sodium carbonate.

Terephthaloyl chloride is insoluble in chloroform, carbon tetrachloride and cyclohexane and could only be partially dissolved by using excess hexane. Hence, lysine (0.2M), dissolved in 100mls of aqueous sodium carbonate (0.472M) solution was reacted with terephthaloyl chloride (0.14M), dissolved in excess hexane (160mls). A yellow coloured, flocculated, material rapidly formed on blending and required the addition of a further 50mls of hexane and 150mls of water to solubilize the reaction mixture. On termination of the reaction, acidification of the aqueous phase caused a substantial quantity of unctuous material to be precipitated, resulting in a product yield of 3.67g. All of the extracted material dissolved in methanol.

FT-IR analysis of this material, showed an absence of amide I and II bands, although a mixed carbonyl ester/carboxylic band was present at 1728cm^{-1} suggesting that the material extracted from the aqueous phase was primarily a mixture of lysine and terephthalic acid. This is confirmed by the solubility profile of the extracted material which was identical to that of terephthalic acid, i.e., soluble in water and alcohols. It can be concluded, that lysine does not polymerize with terephthaloyl chloride when reacted interfacially. Probably for the reasons previously outlined for the other aromatic acid chlorides, namely, because of the poor solubility of lysine in the hydrophobic organic phase, compared to its high solubility in the alkaline aqueous phase, combined with a rapid hydrolysis of the acid chloride in aqueous alkaline conditions.

CHAPTER 5

INTERFACIAL POLYMERIZATION II

ALIPHATIC POLYAMIDES

5.1. Introduction and Synthetic Rationale.

The interfacial studies on 0.1% solutions of poly(isophthalamides), as described in chapter 6, demonstrated that a reduction in solution pH did not induce formation of a surface active molecule but caused an increase in the interfacial tension, possibly as a result of the formation of intramolecular micelles. This result may have occurred as a consequence of the high ratio of hydrophobicity to hydrophilicity within these polymers, i.e., one carboxylic acid pendant group per hydrophobic isophthaloyl residue. In an attempt to overcome this 1:1 ratio, which is an intrinsic limitation of the poly(isophthalamide) structure, the aromatic acid chlorides were replaced with their aliphatic counterparts.

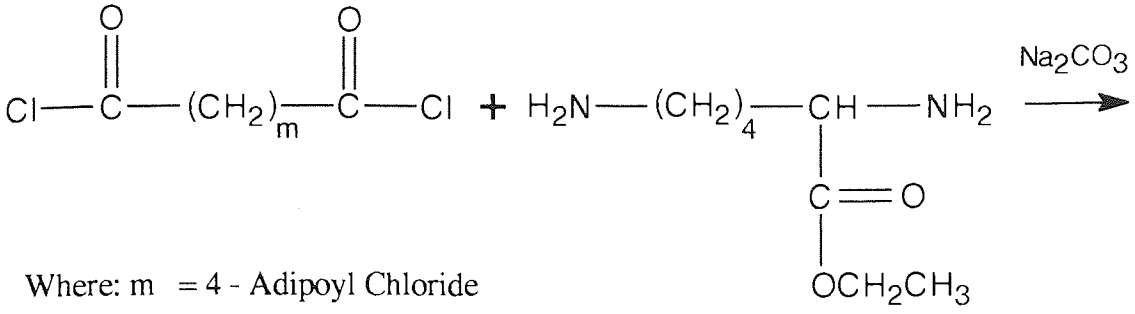
The aliphatic acid chlorides have an additional advantage, that when incorporated into a polyamide chain they are likely to be degraded on contact with biological fluids into their corresponding diacids, e.g. adipic acid and glutaric acid. Such organic acids are normally present in body fluids, such as the blood[165] and are metabolised by the fatty acid ω-oxidation pathway. Adipic acid is also used as a food additive (E355) and is present in food products such as beet juice. As a result, the release of adipic or glutaric acid upon degradation of poly(adipamides) or poly(glutaramides), respectively, would fulfil the original specification; that all degradation products should either be a constituent of normal metabolism or harmless, non-toxic products.

The studies of Kyte *et al.*[140] on the relative hydrophobicity of amino acids (termed hydropathy) show that the order of hydrophobicity of amino acid side chain residues is; isoleucine>valine>leucine>phenylalanine. The increased hydropathy exhibited by leucine, in comparison to phenylalanine, suggests that the ethyl ester side chain of LETES may induce the same hydrophobic moment as the aromatic ring of isophthaloyl chloride. However, the advantage of using LETES to contribute the hydrophobic moiety within a polyamide, is the lack of any constraining influence imposed upon rotation of the polymer backbone. This rotational freedom may enable formation of microdomains and result in surface active behaviour.

A series of initial experiments were conducted to test the feasibility of this synthetic strategy, by reacting simple amino acids, e.g. ornithine, lysine, and 5-hydroxytryptophan with aliphatic acid chlorides, in an attempt to form aliphatic polyelectrolytes with varying charge density. Subsequent experiments made use of LETES to contribute a hydrophobic moiety to the polymer structure. Removal of the pendant ester groups by selective hydrolysis was then used as a method of controlling the ratio of hydrophobic ester groups, to charged carboxylate groups, a typical reaction scheme is shown in Figure 5.1.

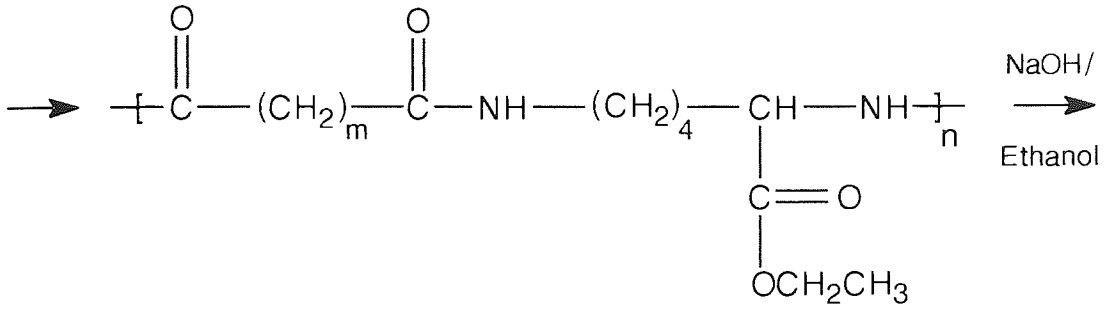
5.2. **Synthetic Procedure.**

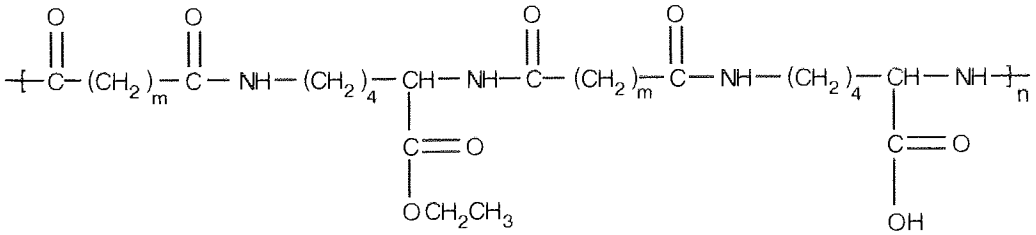
In an attempt to minimise hydrolysis of the acid chloride, sodium carbonate was used in place of sodium hydroxide and the reactions undertaken according to the procedure described in sections 4.5.1.2 & 4.5.1.3 and to the reaction scheme illustrated in Figure 5.1.



Where: m = 4 - Adipoyl Chloride

= 3 - Glutaryl Chloride





Products of Hydrolysis:

Where: m = 4 - Adipic acid + Lysine + Ethanol

= 3 - Glutaric acid + Lysine + Ethanol

Figure 5.1. Synthesis of aliphatic polyamides based on lysine ethyl ester.

5.2.1. Defining the Synthetic Methodology.

5.2.1.1. Effect of Acceptor Concentration.

L-Lysine was used as a model amino acid in defining the methodology needed to synthesize poly(adipamides) using the interfacial technique.

Lysine (0.2M) dissolved in 50mls of aqueous sodium carbonate (0.76M) was interfacially reacted with adipoyl chloride (0.5M), dissolved in 50mls carbon tetrachloride. The amount of acceptor used was found to be insufficient to maintain alkaline conditions. This led to the formation of only small amounts of resinous material and FT-IR spectroscopy indicated that the predominant product was adipic acid, which was readily solubilized upon raising the pH of the aqueous phase.

The concentration of carbonate acceptor was then increased to 1.14M, in order to maintain alkaline conditions throughout the reaction, and so maximise the rate of polymerization. Under these conditions, twice the volume of precipitated material was formed during the interfacial reaction and, subsequently, collected upon acidification of the quenched aqueous phase. However, insufficient material was produced for methanol extraction to be considered worthwhile. The lower organic phase was removed and mixed with excess hexane to precipitate any dissolved polymer. This procedure did not result in any further material being recovered. FTIR analysis of material derived from the aqueous phase, reported in the Appendix, Table A6 (spectrum 120), clearly showed the presence of amide I and II bands, carboxylic acid, carboxylate, aliphatic, amine and carbonate groups. With a resonance at 1280cm^{-1}, characteristic of an O-H deformation within a carboxylic acid.

Raising the concentration of carbonate acceptor to 1.88M and doubling the quantity of lysine present from 0.01 to 0.02 moles, i.e., by using 60mls of an aqueous solution of lysine (0.33M) containing sodium carbonate, once again, produced only a small quantity of material upon interfacial reaction with adipoyl chloride (0.625M), dissolved in 40mls carbon tetrachloride. Resulting in a 3% (0.18g) product yield.

5.2.1.2. Effect of Organic Phase Polarity.

To increase the solubility of lysine in the organic phase, and thereby, increase its rate of ingress to the locus of polymerization, the carbon tetrachloride organic phase was replaced with chloroform. Under these reaction conditions only a small amount of precipitated material formed upon interfacial reaction and no further material was precipitated on subsequent acidification of the aqueous phase. As an alternative approach, the chloroform phase was replaced with hexane, to raise the partition coefficient of the acid chloride and so minimise loss of the latter into the aqueous phase. When the above experiment was repeated under these conditions only small quantities of material were produced upon post-reaction acidification of the aqueous phase. This material proved to be insoluble in both methanol and DMF. FT-IR analysis of the material collected, as reported in the Appendix, Table A6 (spectra 122), indicated a strong carboxylic acid carbonyl stretch and O-H deformation, along with strong aliphatic resonances, suggesting that the material produced was largely composed of adipic acid with perhaps some residual lysine. This was confirmed by the presence of a resonance at 1242cm^{-1}, characteristic of a C-O stretch within an amino acid.

The partition coefficient between the two solvent phases could also be changed through raising the polarity of the aqueous phase by incorporating sodium chloride, in addition to the 1.88M sodium carbonate already present as the acid acceptor. This would have the effect of 'salting out' the lysine into the organic phase. When this procedure was adopted it was found that the aqueous solution required warming in order to solubilize the salts and this resulted in precipitation of the reactants, possibly due to the formation of a lysine salt with low solubility, consequently, this approach was not pursued.

5.2.1.3. Effect of Acid Chloride Chain Length.

The influence of chain length on acid chloride reactivity was assessed by using the shorter chain diacid chloride, succinyl chloride, in place of adipoyl chloride. L-Lysine (0.3M) dissolved in 50mls of aqueous sodium carbonate (1.46M) solution was reacted interfacially with succinyl chloride (0.425M), dissolved in 40mls carbon tetrachloride. This reaction produced only a small quantity of yellow/brown coloured material which was soluble in alkaline aqueous conditions. Indicating the presence of acidic groups and probably representing succinic acid.

The poor solubility of lysine in the organic phase, when in its charged state (in alkaline conditions), probably accounted for the failure to produce substantial quantities of polymer-like material when this amino acid was reacted interfacially with aliphatic acid chlorides. It is likely that the rate of ingress of the amino acid into the organic phase is far slower than the rate of dissolution, and subsequent hydrolysis, of the short chain aliphatic acid chlorides into the alkaline aqueous phase. To further test this hypothesis both ornithine and 5-hydroxytryptophan were reacted with adipoyl chloride; the following results were obtained:

a) L-Ornithine, (0.2M) dissolved in aqueous sodium carbonate (0.76M) solution was reacted interfacially with adipoyl chloride (0.25M), dissolved in 50mls of carbon tetrachloride. This reaction failed to produce any substantial quantities of material. However, on termination of mixing the carbon tetrachloride separated into a distinct layer, representing some 49.6% of the original volume used. This phenomenon was not observed when using isophthaloyl chloride, probably because, the adipic acid formed during the reaction is not amphipathic and, unlike isophthalic or benzoic acids, does not behave as a solubilizing hydrotope. The latter acid has been reported[166] to form complexes with aqueous insoluble materials and partially solubilize them.

b) 5-Hydroxytryptophan, (0.075M) dissolved in 40mls aqueous sodium carbonate (0.275) solution was reacted interfacially with adipoyl chloride (0.094M), dissolved in 40mls of carbon tetrachloride. Once again, only a very small quantity of insoluble material was produced upon acidification of the aqueous phase and showed a marked discolouration upon standing in air.

5.2.1.4. Effect of Amino Acid Esterification upon Interfacial Reactivity.

The effect of using an amino acid ester to increase the solubility of the diamine in the organic phase, and thereby, maximise the rate at which the diamine reacted with the acid chloride was investigated by using L-lysine ethyl ester (LETES) in place of lysine. In addition, the ethyl ester side groups would contribute hydrophobic side chains to any resultant polymer formed, as described in section 5.1.

Lysine ethyl ester, (0.2M) dissolved in 50mls aqueous sodium carbonate (0.94M) solution was reacted interfacially with adipoyl chloride (0.25M), dissolved in 50mls carbon tetrachloride. A grey resinous precipitate was formed which collected in the separated carbon tetrachloride phase. No additional material was precipitated upon extraction of the organic phase with excess hexane or upon acidification of the quenched aqueous phase. The recovered resin was soluble in methanol and appeared to absorb machine oil, which it readily exchanged with hexane, although the resin remained insoluble in hexane itself.

FT-IR analysis of the collected resin, as reported in the Appendix, Table A6 (spectra 125 & 126), indicated the presence of amide I and II bands and also a carbonyl carboxylic acid, both of which were absent from the ester starting material (spectrum 124). The appearance of these bands was concomitant with the disappearance of the primary and quaternary amine deformation bands at $1601cm^{-1}$ an $1507cm^{-1}$, respectively, and of the ester carbonyl band at $1734cm^{-1}$ and C-C vibration at $1015cm^{-1}$. After extraction in excess hexane the carbon tetrachloride phase (spectrum 126) seemed to contain largely ester and carboxylic acid groups, probably present as unreacted LETES starting material and adipic acid. Spectral data from both the resin and reactant phases showed strong aliphatic stretching and deformation frequencies, and also hydrogen bonding as expected for an aliphatic carboxylic acid and diamine.

GPC analysis, see Table 5.1, sample code LYA-1 (5379), shows M_W of 126,000 and the M_n of 13,750 and confirms the formation of a high molecular weight polyamide.

Table 5.1. GPC molecular size analysis: Aliphatic polyamides.*

Sample Code	Sample No.	M_W	M_n	Polydispersity
LYA-1	5379	126,000	13,750	9.15
LYD-5	5380	27,800	11,050	2.50
LYS-G	5381	26,250	10,350	2.55

* All samples were analysed using DMF as the solvent medium. Each value shown is the mean of two determinations. Values of M_W and M_n are shown rounded to the nearest 50. Poly(ethylene oxide) and poly(ethylene glycol) were used to calibrate the GPC system and all results are expressed as the PEO/PEG equivalent molecular masses. All GPC molecular weight determinations were conducted by RAPRA Technology Ltd.

In an attempt to obtain an increased yield of polymer the reaction was repeated with approximately twice the quantity of reactants; doubling the quantity of lysine ethyl ester to 0.02 moles (0.33M), in 60mls of aqueous solution, and adipoyl chloride to 0.022 moles (0.55M), in 40mls of dichloromethane. The concentration of sodium carbonate required (1.88M), i.e., 19.9%, approached the limit of its solubility in aqueous solution. It was, therefore, replaced with sodium hydroxide (1.73M). Under these conditions no resinous material precipitated from the solution during the interfacial reaction, either as a result of the insufficient quantity of base used in the reaction, or due to excessive hydrolysis of the acid chloride in the strongly basic starting conditions. In addition, no further material formed upon acidification of the aqueous phase to pH 1.15.

FT-IR analysis of the post-reaction aqueous phase, as reported in the Appendix, Table A6 (spectrum 140), revealed the presence of amide I and II bands but these were partially obscured by a strong asymmetric stretching frequency of a carboxylate group ($1589cm^{-1}$). The presence of carboxylate was also supported by a strong signal at $1400cm^{-1}$, characteristic of the carboxylate symmetrical stretching frequency. The carboxylate may be present either as adipic acid or as lysine from hydrolysed LETES. Evidence for the former is stronger because, of the absence of any ester bonds. Resonant frequencies indicative of aliphatic and amine groups were also present in this phase, which would tend to support the presence of lysine.

It is, therefore, probable that the sodium hydroxide acts as such a strong base that it substantially hydrolyses the esterified starting material, limiting the amount of ester available to penetrate the organic phase and undergo polymerization. Any polyamide material that may have been formed would have rapidly become saponified in the strongly basic conditions and then solubilized in the aqueous phase. In a further experiment the concentration of sodium hydroxide was raised to 2.08M, in an attempt to maintain alkaline conditions throughout the reaction. In addition, chloroform was replaced with hexane to limit acid chloride loss into the aqueous phase. Under these conditions only very small quantities of material were formed.

A subsequent experiment used twice the quantity of reactants as initially used, i.e., LETES (0.33M) in 60mls aqueous solution and adipoyl chloride (0.625M) in 40mls carbon tetrachloride. However, in this experiment the sodium hydroxide was replaced with 1.57M sodium carbonate and this resulted in a greatly increased product yield [63.4% (3.64g)]. Under these reaction conditions a hydrophobic material was produced, which collected in the separated organic phase, and formed a resinous mass when the organic phase was mixed with excess hexane. This material dissolved in

methanol and formed resilient films upon drying. FT-IR analysis, as reported in the Appendix, Table A6 (spectra 129 and 127), demonstrated the presence of amide I and II bands, ester carbonyl and C-O-C resonances, and aliphatic C-H stretching, deformation and scissoring vibrations. These data suggest the formation of an amide with the presence of ester groups. No amide was present in the hexane supernatant.

5.3. Side Chain Modification of Poly(lysine ethyl ester adipamide).

5.3.1. Formation of a Potentially Degradable Hypercoiling Polymer.

Partial de-esterification of poly(lysine ethyl ester adipamide) [PLETESA] produced using the high concentration carbonate-based technique, described in section 5.2.1.4. could, potentially, form a polymer which contained both pendant ester and carboxylate groups, that is, a mixture of hydrophobic and charged groups. Such functional side chains may confer associative or hypercoiling properties on the polymer. By virtue of the amide backbone and ethyl ester side chains it is hoped that this polymer would degrade by hydrolysis into lysine, ethanol and adipic acid when in contact with biological fluids. These three breakdown products are all components of natural metabolic processes and either occur naturally in man, or are tolerated in relatively high doses, without toxic side effects.

Beaumais[5] has shown that the ethyl ester side chains of a poly(sulphonamide) produced from LETES and disulphonyl chloride could be removed by saponification in strongly alkaline conditions. Similarly, Katsarava[39] was able to saponify PLETESA and remove 97% of the ester groups by stirring the polymer in 0.025 M (5%) ethanolic sodium hydroxide. However, neither author describes partially saponified or de-esterified polymer, where a significant degree, i.e., more than a few percent, of ester groups are retained. Hence, these authors do not envisage the aliphatic LETES-based polyamides described herein. Additionally, the amphipathic functionality and potential significance of partially saponified PLETESA is not recognized, although this may be its most valuable property, at least in terms of its application to human medicine.

5.3.2. Partial Saponification of PLETESA: Experimental Procedure.

A 2% solution of PLETESA was prepared in approximately 2% ethanolic sodium hydroxide (containing 10% water). Strands of resinous material were observed to become deposited over time, presumably as a result of hydrolysis of the hydrophobic ester side chains and formation of carboxylate groups. The accompanying change in polymer polarity resulted in a reduction of solubility and caused precipitation. Acidification of the solution resulted in further precipitation as the pendant carboxylate ions became neutralized. It was noted that when this precipitate was resolubilized in reagent grade ethanol it could not be reprecipitated by addition of excess aqueous solution at neutral pH.

One possible explanation of these observations, is that as the polymer chain becomes saponified its non-polar character is progressively lost, until hydrophobic interactions with ethanol are no longer sufficient to retain the polymer in solution, resulting in precipitation. Whereas, acidification of the polymer causes a conversion of polar carboxylate groups to, essentially non-polar, carboxylic acid groups, causing a loss of solubility due to the prevention of ionic interactions between the polymer and the aqueous phase of the solvent system. Since the precipitated polymer is largely hydrophobic it proved to be soluble in approximately 100% ethanol. These findings suggest that the hydrophobic/hydrophilic character of saponified PLETESA is very finely balanced between the solvation values, or so called Y values, exhibited by 100% ethanol (Y = -2.033), 90% ethanol (Y = -0.747) and water (Y = 3.493)[167] and is extremely sensitive to both solution pH and solvent polarity, either of which can be used to modify its conformation.

5.3.3. Timed Saponification.

The rate of saponification of PLETESA in ethanolic sodium hydroxide was monitored by aliquot sampling and FT-IR analysis of the ethanolic solvent over a timed period and according to the following procedure:

0.54g of PLETESA-base polymer was dissolved in 50g of ethanol containing 0.04% wt/wt sodium hydroxide (0.01M). The degree of hydrolysis was monitored by aliquot sampling and FT-IR spectroscopy after 15, 30, 60 minutes and overnight (approx. 12hrs.). The spectral data of the ethanolic aliquots, shown in the Appendix, Table A6 (spectra 130-133), were identical to those of the base polymer with no

reduction in the height the ester peak. Hence, no saponification appeared to have occurred under the reaction conditions over the time period monitored.

The level of sodium hydroxide, was therefore, increased to 0.2% (0.05M) and the degree of hydrolysis monitored by sampling after 30 minutes, 1, 2, 4.5 hours and overnight. The FT-IR spectral data obtained from the aliquots, as shown in the Appendix, Table A7 (spectra 134-137 and 139), indicated a gradual disappearance of the ester-carbonyl resonance ($1734cm^{-1}$) with time, concomitant with a broadening of the carbonyl carboxylate and primary amine bending frequencies, the latter two form a combined band around $1620cm^{-1}$. Saponification was virtually complete within 4.5 hours. The loss of the ester band and the appearance of an amine band was also accompanied by a loss of the amide II band at $1545cm^{-1}$, suggesting that hydrolysis of the amide backbone was also occurring to some extent. A resinous material was precipitated from solution and became clearly visible after 4.5 hrs hydrolysis. This material was likely to be saponified, ethanol insoluble, poly(lysine adipamide). Hence, the changes identified by spectrophotometric analysis mirrored those observed in the physical properties.

GPC analysis of the resinous precipitate also reflected the hydrolytic cleavage of the polyamide backbone, see Table 5.1, sample code LYD-5 (5380), where a M_W of 27,800 and an M_n of 11,050 is reported. These values are approximately 25% of those of the fully esterified PLETESA-base polymer.

Saponification of PLETESA resulted in a 65.9% yield of polymeric material. The latter was found to be soluble in alkaline aqueous solution, methanol and DMF. This solubility profile is that expected for a polyamide bearing pendant carboxylic acid groups. This structure was also confirmed by FT-IR analysis of the insoluble saponified resin, as shown in the Appendix, Table A7 (spectra 138 & 142), by strong resonances at $1589cm^{-1}$ and $1400cm^{-1}$ characteristic of the carbonyl asymmetric and symmetric stretches within carboxylate groups. Ester carbonyl groups were also present suggesting that only partial saponification occurred. Acidification of the resin in alcoholic solution resulted in a disappearance of the carboxylate carbonyl asymmetric stretch and a marked reduction of the symmetric stretch, concomitant with the appearance of a strong C-O stretch and an O-H deformation characteristic of a carboxylic acid, i.e., at 1021cm-1 and $1261cm^{-1}$, respectively. Providing further confirmatory evidence that saponification had occurred.

5.3.4. Variation of Solvent Polarity: Rationale.

The differential solubility exhibited by saponified PLETESA was used as a method of extracting polymeric material with varying degrees of esterification. This was achieved by conducting the saponification in alcoholic 0.0625M sodium hydroxide. By raising the percentage of methanol, in an ethanol/methanol solvent mixture, the solvation number and the polarity of the solvent could be increased. In a 100% ethanolic solution hydrolytic cleavage of the hydrophobic ethyl ester pendant groups was expected to result in a loss of solubility and precipitation of the polymer. However, as the solvent polarity was increased, i.e., contained a greater percentage of methanol, its ability to solvate polar material was also expected to increase. Hence, a greater proportion of the side chain ester groups would, potentially, be hydrolysed to carboxylic acid groups before the polymer became insoluble. As a result, the resinous precipitate collected would be expected to show a greater degree of saponification as the percentage of methanol was increased.

5.3.5. Experimental Procedure.

0.5g of PLETESA was hydrolysed in 0.0625M ethanolic/methanolic sodium hydroxide (0.25%) containing 0, 5, 10 and 25% methanol, respectively. The amounts of resinous (dry weight) material recovered after two days saponification at room temperature are shown in Table 5.2.

The concentration of sodium hydroxide was doubled to 0.5% in all solutions after stirring overnight, in order to precipitate de-esterified polymer from solution. The latter, collected as hard, waxy, film-forming resins. The modified polymer was dissolved in aqueous solution along with any soluble monomeric breakdown products. The polymer was then separated by reprecipitation as the hydrochloride salt on neutralization with hydrochloric acid. This procedure stabilized the polymer and prevented the formation of cyclic amides which are known to form in amino acid esters.[168]

Table 5.2. Effect of solvent polarity on PLETESA saponification.

% Ethanol	% Methanol	Resin recovered (g)	% Recovery (of start resin)	Solution appearance
100	0	0.221	44.2	Precipitate
95	5	0.213	42.6	Turbid Soln.
90	10	0.140	28.0	Turbid Soln.
75	25	0.251	50.2	Turbid Soln.

FT-IR analysis of the ethanolic solvent phase after removal of the resin, Appendix, Table A7 (spectra 38 and 39), indicated that solvent from 100% and 75% ethanol containing reactions were largely composed of carboxylic acid salts, exhibiting the characteristic carbonyl asymmetric and symmetric stretching frequencies at 1563 and 1400cm^{-1}, respectively, combined with strong CH aliphatic stretching and deformation resonances and a primary amine deformation at 1614 cm^{-1}. These possibly represent the release of adipic acid and lysine during partial hydrolysis of the backbone of PLETESA. In contrast to the solvent phase, the resin extracted from the 10% methanol solvent system (spectrum 165) exhibited strong amide I and II bands, indicating that the polyamide backbone was largely intact. A mixed ester/carboxylic acid carbonyl resonance at 1722cm^{-1} was also apparent suggesting that a significant number of side chain groups remained esterified.

^{13}C-NMR analysis was used to quantify the proportion of ester groups remaining in the samples of resin recovered. This data was used to assess the efficacy of saponification. The results are reported in chapter 7, section 7.2.

5.4. Low Temperature Synthesis of Poly (lysine ethyl ester adipamide).

5.4.1. Introduction and Rationale.

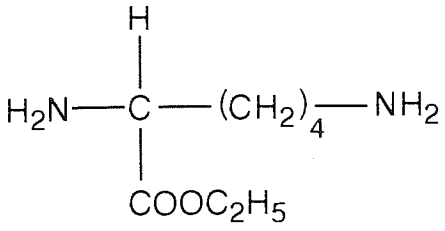
Katsarava et al.[39] have described a series of lysine ethyl ester (LETES) based polymers, synthesized by reacting the bis(trimethylsilyl) derivative of LETES with aliphatic and aromatic acid chlorides by a process of solution polymerization. These authors also describe a low temperature solution polymerization of a polyurea, based on LETES.[40] This approach was adopted to facilitate 'head-tail', i.e., HT bonding, between the amine substituted at the α-carbon atom (head amine - designated H) located

on one LETES group and the amine group substituted on the ε-carbon atom (tail amine - designated T) located on a second molecule of LETES, linked by reaction with a diacid chloride. This gives rise to the production of four possible triad (amino acid - acid chloride - amino acid) sequence distributions, i.e., HTTH, HTHT or THTH and THHT, as shown in Figure 5.2. The most favoured reaction between a diamino acid and an acid chloride at ambient reaction temperatures occurs between the amine substituted at the ε-carbon atom and the acid chloride groups, since the latter amine is a more basic group than the amine group substituted at the α-carbon atom. Hence, the relative rates of reaction of the amine groups with acid chloride are as follows :

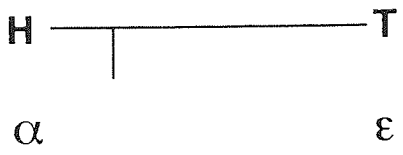
$$k\varepsilon\varepsilon > k\varepsilon\alpha = k\alpha\varepsilon > k\alpha\alpha$$

At low temperatures hydrogen bonding between amide, amine and carbonyl groups becomes far more important in defining the position of the reacting species and random, kinetically driven, effects become less important and favour the formation of HT triads. The polyureas comprised of HT triads appear to exhibit maximal intra-chain hydrogen bonding and tend to adopt helical conformations when dissolved in polar solvents.[34] By making use of this effect, Katsarava[40] was able to synthesize poly(lysine methyl ester adipamide) with a defined microstructure. Although the author reports that ^{13}C-NMR was not useful in gaining information related to the triad structure and could only be applied successfully to define the triad structure of poly (lysine terephthalamide).

Lysine

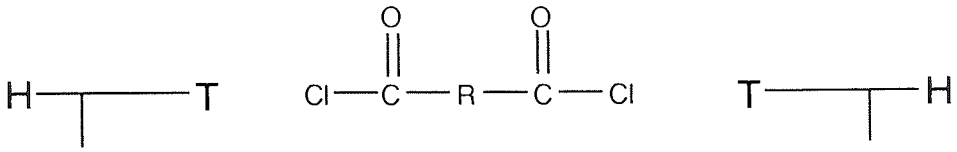


Diamine Nomenclature

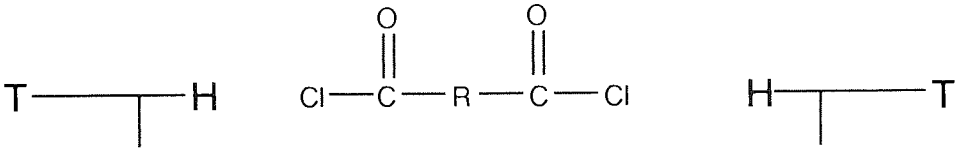


Possible Reaction Triads :

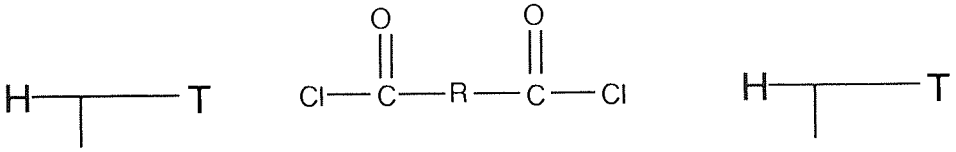
a) T T



b) H H



c) T H



d) HT

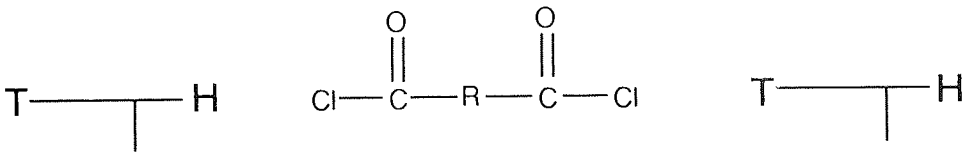


Figure 5.2. Possible reaction triads of LETES and diacid chlorides.

The concepts of low temperature synthesis described by Katsarava *et al.* were applied to the synthesis of PLETESA by using the interfacial technique described herein. The objective of this work was to produce a polyamide with a defined sequence distribution and possibly a secondary structure, e.g. a helical coil. Such a structure would potentially enable hydrophobic guest molecules to be entrapped and carried within the hydrophobic helical core, while the 'loaded' polyamide remained dissolved in aqueous solution. The use of such a biodegradable polymer may offer considerable advantages in drug therapy by enabling sustained delivery of the drug molecules as the polymer became enzymatically degraded.

5.4.1.1. Low Temperature Synthesis of PLETESA: Sodium Carbonate-Based Technique.

Lysine ethyl ester dihydrochloride (0.4M) was dissolved in 43.3mls of saturated sodium carbonate solution (approximately 2.0M), cooled to 3°C and interfacially reacted with adipoyl chloride (0.83M), dissolved in 30mls of carbon tetrachloride, cooled to -10°C. Powdered dry ice was added throughout the reaction so as to maintain a low temperature. A resinous mass was rapidly formed on blending and remained coagulated throughout the blending period. The resin was directly removed from the reaction vessel to give a product yield of 5.12%. Further resinous material was also extracted from the organic phase by precipitation with excess hexane, giving an additional yield of 1.25%. The resinous material collected was found to be soluble in methanol. The lack of solubility of the resin in the reaction mixture contrasted, markedly, with the material previously produced at room temperature, which largely dissolved in the organic phase. Such differences in the physical characteristics of the resin may have been the result of the low temperatures and reduced solubility. Alternatively, they may have resulted from differences in intra-molecular bonding or association within the polymer products.

Despite the apparent excess of sodium carbonate used, the reaction mixture was acidic on termination of blending. This was almost certainly due to the loss of base by crystallization under the low temperature conditions and may have accounted for the low product yield obtained.

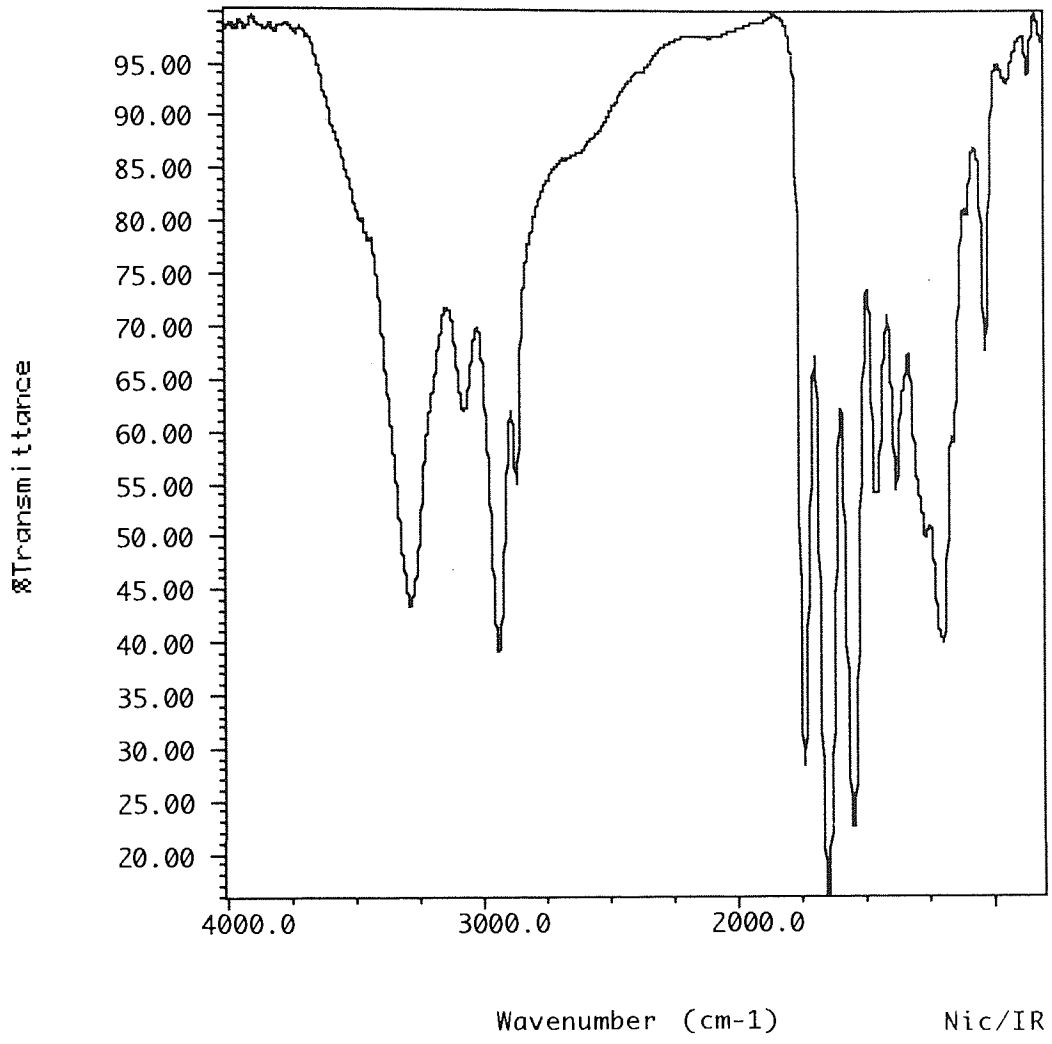


Figure 5.3. FTIR spectrum of PLETESA - base polymer. Low temperature synthesized - high [Na_2CO_3] technique (spectrum 161).

The FT-IR spectrum of the precipitated resin is shown in Figure 5.3, and the spectral data is shown in the Appendix, Table A7 (spectrum 161). The spectral data indicates a very strong resonance for the amide I and II bands, the ester carbonyl group and asymmetric C-O-C stretch, as well a methyl symmetrical deformation at 1375cm^{-1} characteristic of a ester grouping. The FT-IR data from the organic phase (spectrum 162), after addition of excess hexane, indicates the presence a mixed carbonyl band at 1728cm^{-1}, representing contributions from both ester and carboxylic acid groups. The presence of unreacted ester in the hexane phase is confirmed by the strong C-O-C resonance at 1279cm^{-1} and a methyl ester resonance at 1375cm^{-1}. Aliphatic groups are also apparent. These results suggest that the organic phase contains predominantly hydrolysed adipic acid and unreacted LETES.

A portion of the synthesized resin (0.293g) was saponified in 30mls of ethanolic sodium hydroxide (0.0625M) and yielded 0.159g (54.3%) of a precipitated material. FT-IR analysis of this material, as shown in the Appendix, Table A7 (spectrum 173), indicates a loss of the ester bond and an appearance of a carbonyl carboxylic acid resonance at 1715cm^{-1} and C-O stretch at 1084cm^{-1}, without any loss of the amide I and II bands. These results suggest that the ethyl ester side chains of the polymer had become almost completely saponified, whilst a proportion of the amide groups within the polymer backbone remained intact.

5.4.1.2. Low Temperature Synthesis of PLETESA: Potassium Carbonate Based Technique.

To increase the polymer yield and maintain alkalinity throughout the reaction period the interfacial reaction was repeated using potassium, rather than sodium, carbonate because of its greater aqueous solubility (approximately three fold). Further methodological improvements included pre-drying the carbon tetrachloride by addition of 0.05% thionyl chloride, so a to minimise acid chloride loss by hydrolysis in the organic phase.

LETES (0.286M) dissolved in 70mls of saturated potassium carbonate solution (4.86M) at 11.4°C was reacted interfacially with adipoyl chloride (0.625M), dissolved in 40mls of dried carbon tetrachloride at 0°C. A light brown resinous precipitate formed and was insoluble in hexane, but soluble in methanol and ethanol. However, on extraction into methanol, a small amount (0.025g) of a white, methanol insoluble (but swellable) material also became apparent. This material was also insoluble in DMF and saturated lithium bromide solution and bore a close resemblance to the beta sheet

polyamide formed by denatured silk fibres. The total yield of both methanol soluble and insoluble material was 17.32% (0.984g dry weight), which was approximately three times that produced using sodium carbonate as the acid acceptor. This improvement may, in part, have arisen because the aqueous phase remained alkaline throughout the reaction, so favouring the reaction between diamine and diacid chloride.

FT-IR analysis of the resinous material, as reported in the Appendix, Table A8 (spectrum 166), showed a very strong resonance for the amide I , II and III bands at 1640, 1545 and 1254cm^{-1}, respectively, for the ester carbonyl group at 1734cm^{-1} and for the C-O-C asymmetric and symmetric stretching frequencies at 1190 and 1027cm^{-1}. In addition to aliphatic groups. A virtually identical spectrum was obtained on investigating the methanol insoluble fractions (spectra 167 and 170). Suggesting an identical polymer, but possibly one, with either a higher molecular weight or different secondary structure that may account for the different physical properties noted. The methanol insoluble, beta sheet-like material, also exhibited a strong amide III resonance at 1305cm^{-1} which may indicate the formation of a distinct secondary structure, since this resonance was rarely observed.

A portion of the methanol soluble resin (0.73g) was saponified in 72.8mls of ethanolic sodium hydroxide (0.0625M) and yielded 0.243g (34.7%) of precipitated material. FT-IR analysis of the precipitated resin after washing in ethanol, indicated a reduction of the ester bond signal strength as compared to the starting material and suggested that a limited saponification had occurred. The FT-IR spectrum, reported in the Appendix, Table A8 (spectrum 168), shows a shoulder around 1760cm^{-1} demonstrating that some residual ester groups remained, while the strong signal at 1709cm^{-1} suggests a preponderance of carboxylic acid groups. The bands at 1596 and 1406cm^{-1} also confirm the formation of carboxylate ions upon saponification. The resonance at 1596cm^{-1} disappeared upon acidification of the sample, the spectrum of which is show in Figure 5.4 and reported in Table A8 of the Appendix (spectrum 169). This effect is characteristic of carboxylate ions upon neutralization to carboxylic acid groups. Amide I and II bands are also evident at 1646 and 1545cm-1, respectively, suggesting that no appreciable backbone hydrolysis had occurred.

The ethanol phase after saponification was extracted and dried forming a white crystalline powder which was also analysed by FT-IR spectroscopy (spectrum 171) and found to exhibit a virtually identical spectrum to that of the resinous material. A similar spectral change also occurred upon acidification (spectrum 172), once again, indicating the presence of carboxylic acid groups. Hence, the ethanol phase appeared to contain saponified PLETESA, but probably of lower molecular weight.

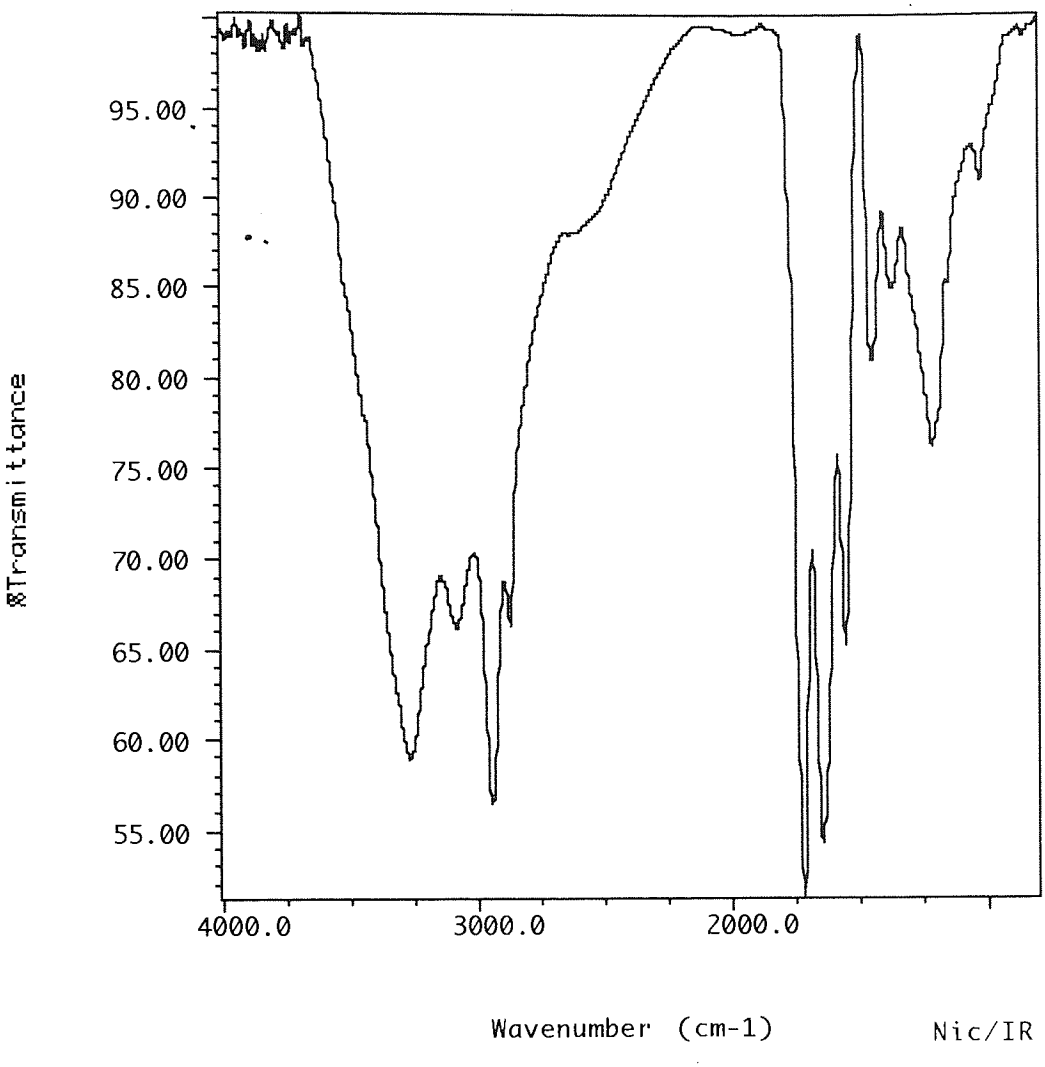


Figure 5.4. FTIR spectrum of PLETESA - saponified in 100% ethanol. Low temperature synthesized - high [K_2CO_3] technique. Acidified sample (spectrum 169).

5.5. Lysine Ethyl Ester/Lysine Adipamide Terpolymer.

The synthetic technique which used higher concentrations of reactants in a high salt environment was also employed to synthesize a polymer from both LETES, lysine and adipoyl chloride starting materials. Such a terpolymer may possess amphipathic properties, where the ethyl ester groups contribute hydrophobic moieties and lysine contributes charged carboxylate pendant groups. A high concentration of sodium chloride was also used, in order, to 'salt out' lysine from the aqueous solution into the organic phase and, at the same time, limit the solubility of adipoyl chloride in the aqueous phase.

An initial experiment using only lysine was conducted to assess the feasibility of the concept, where lysine (0.33M) dissolved in 60mls of aqueous sodium carbonate (0.79M) solution containing sodium chloride (3.42M) was reacted interfacially with excess adipoyl chloride (0.625M) dissolved in 40mls carbon tetrachloride. No material precipitated during the reaction. However, on termination of the reaction, the reaction mixture was found to be acidic. When the aqueous phase was mixed with excess water and acidified only a slight turbidity was noted, suggesting that no polyanionic material had been formed.

FT-IR spectral analysis of the aqueous phase, as shown in the Appendix, Table A6 (spectrum 164), indicated the presence of the amide I and II bands, a strong carboxylic acid carbonyl resonance at 1697cm^{-1}, along with C-O and O-H stretching and deformation resonances at 1280 and 1191cm^{-1}, respectively. Aliphatic groupings were also apparent. These results suggest that the principal reaction product is adipic acid, formed by acid chloride hydrolysis, in addition to small amounts of, possibly, oligomeric adipamide.

In conclusion, it does not appear to be possible to synthesize poly(lysine adipamide) by the interfacial route, irrespective of the type or amount of acid acceptor used or the polarity of the aqueous phase. This is almost certainly due to the poor solubility of lysine in the carbon tetrachloride and its low rate of ingress to the locus of polymerization.

5.6. Lysine Ethyl Ester-Based Polyamides: Variation of Aliphatic Acid Chloride.

The effect of aliphatic chain length and saturation upon the reactivity of acid chlorides towards LETES was investigated by replacing adipoyl chloride with succinyl, glutaryl and fumaryl chlorides, respectively:

5.6.1 Succinyl Chloride.

Succinyl chloride (0.625M) dissolved in 40mls of carbon tetrachloride was reacted interfacially with LETES (0.33M), dissolved in 60mls of aqueous sodium carbonate (1.57M) solution. On blending a fine white precipitate formed which was allowed to settle out into the lower organic phase and was then mixed with excess hexane. However, no precipitated material was recovered. The remaining aqueous phase was acidified, resulting in the formation of a flocculated precipitate which was then extracted into hexane, once again, no resinous precipitate formed.

FT-IR analysis of the organic phase, as shown in the Appendix, Table A8 (spectrum 145), indicated a weak amide I band, but a strong amide II band and a strong mixed carbonyl resonance at 1728cm^{-1}, characteristic of a mixture of ester and carboxylic acid groups, in addition to a strong C-O aliphatic ester or carboxylic acid signal at 1280cm^{-1}, and a carboxylic O-H deformation resonance at 1122cm^{-1}. These results suggest that the predominant components present in this phase were unreacted LETES, succinic acid and perhaps small amounts of oligomeric LETES succinamide, or possibly cyclic amides (imides). The spectrum obtained from the aqueous phase (spectrum 144) appeared to be somewhat similar but displayed an amide III band at 1261cm^{-1}, again suggesting the formation of low molecular weight, water soluble, oligomer. Asymmetric and symmetric stretches of aliphatic ester groups were also present.

These results suggest that a substantial amount of acid chloride undergoes hydrolysis to form succinic acid, possibly as a result the high aqueous solubility of the acid chloride and the remaining material probably forms a cyclic imide which end-caps the reaction and prevents polymerization.

5.6.2 Glutaryl Chloride.

Glutaryl Chloride (0.31M) dissolved in 40mls of carbon tetrachloride was reacted interfacially with LETES (0.17M), dissolved in 60mls of aqueous sodium carbonate (1.57M) solution. Blending resulted in the formation of an oily material which separated into the organic phase and was washed with excess hexane. On drying a brittle, film-like, material was produced which was soluble in both methanol and DMF.

FT-IR analysis of the resinous precipitate, as reported in the Appendix, Table A8 (spectrum 149), showed the characteristic amide I & II bands, ester carbonyl and aliphatic ester C-O-C stretching resonances. The hexane washings (spectrum 160) of the resin contained primarily unreacted ester and hydrolysed acid chloride. GPC analysis of the resinous material, as shown in Table 5.1, sample code LYG-6 (5381), indicated a M_W of 26,250 and M_n of 10,350, confirming the formation of a high molecular weight polyamide. It can be concluded, that poly(LETES glutaramide) was produced by the interfacial technique. This is a novel finding.

5.6.3 Fumaryl Chloride.

Fumaryl Chloride (0.275M) dissolved in 40mls of carbon tetrachloride was reacted with LETES (0.17M), dissolved in 60mls of aqueous sodium carbonate (1.57M) solution. Blending resulted in instantaneous formation of a precipitate that coagulated and required the addition of a further 40mls of each solvent so solubilize the reaction mixture. On termination of the reaction the lower organic phase was separated and mixed with excess hexane, from which an aqueous soluble, methanol insoluble, material was recovered.

FT-IR analysis of a dried sample of the aqueous soluble material, as shown in the Appendix, Table A8 (spectrum 150), indicated that it contained predominantly ester, carboxylate, and alkane groups; representing unreacted LETES and hydrolysed acid chloride. In addition, the carbon-carbon double bond stretching frequency at 1627cm^{-1} indicates the presence of an alkene, probably present as fumaric acid. These results suggest that poly(LETES fumaramide) is not produced by the interfacial technique, probably as a result of rapid hydrolysis of the acid chloride in the aqueous phase.

CHAPTER 6

PHYSICAL PROPERTIES

OF

MONOMERS & POLYMERS.

6.1. **Potentiometric Titration.**

6.1.1. Introduction and Rationale.

Potentiometric titrations were conducted on aqueous solutions of the amino acids and amino acid derivatives used as monomers in the synthesis of poly(isophthalamides) and poly(adipamides). Similar titrations were also conducted on solutions of the poly(isophthalamides) and poly(adipamides) themselves, to assess the strength of the acidic pendant groups, i.e., their pK_a values. This gave some indication of the environment of the carboxylate groups and, by inference, revealed information regarding the possible secondary structure. In particular, a shift in apparent pK_a values upon acidification would indicate the formation of hydrophobic microdomains such as those that occur in the hypercoiling vinyl-based polymers described in chapter 2.

6.1.2. Experimental Procedures.

All titrations were conducted using either 0.1% and/or 0.5% aqueous solutions of the monomer materials, and 0.1% aqueous solutions of the polymeric materials. All amino acids and amino acid derivatives were supplied by the Sigma Chemical Co. and used without further purification. The samples of polymers used were those described in chapters 4 and 5 of this thesis. The poly (isophthalamides) in free acid form were found to be insoluble in water and were initially solubilized by adjusting an aqueous suspension of the polymer to strongly alkaline conditions, e.g. pH 12.42 - 11.94. A similar procedure was adopted for the two poly(adipamide) samples and these were adjusted to 12.36 & 12.23, respectively. Hydrochloric acid (0.02N - 0.04N) was always used as the titrant and additions were made in 1, 2 or 5ml aliquots. All pH measurements were made with a Mettler Delta 340 pH meter, using a BDH Gelplas semi-micro combination electrode, and results presented graphically as pH vs. cumulative moles of hydrochloric acid added, or as the second derivative value, i.e., $\Delta pH/\Delta V$ (where V = volume of hydrochloric acid added).

6.1.3. Amino Acid Staring Monomers.

The results of the potentiometric studies conducted on 0.1% and 0.5% L-ornithine hydrochloride are shown in Table 6.1 and an example of the titration curves obtained is shown graphically in Figure 6.1 and with the corresponding derivative values (of the

0.1% solution) shown in Figure 6.2. The derivative values, such as those shown for 0.1% L-ornithine in Figure 6.2, were used to indicate the equivalence point, and the half equivalence point value was used to determine the respective pK values, which are also listed in Table 6.1. A similar analytical procedure was followed with L-lysine hydrochloride and lysine ethyl ester dihydrochloride. The pK values obtained for L-lysine correspond fairly closely to published values,[169] where pK values of 10.73 and 1.94 are reported, corresponding to the amine and carboxylic acid groups substituted at the α-carbon atom, respectively, with the isoelectric point at pH 5.64. Similar values were recorded with ornithine, and interestingly, with LETES, which suggests that some of the ester groups had become hydrolysed in the strongly basic conditions used during titration. This latter finding was further supported by the higher isoelectric point and pK_1 value noted for LETES, which would suggest a higher proportion of amine groups than that found in lysine.

Table 6.1. Titration data of amino acids and derivatives.

Amino Acid/Deriv.	pK_1 - αCOOH	pK_2 - αNH_3^+	pH_I - Isoelectric Point.
Ornithine HCl 0.1%	2.10	10.0	5.84
Lysine HCl 0.5%	1.94	10.73	5.64
LETES Di HCl 0.1%	2.48 *	11.59	6.47

* Presence of pK_1 in LETES suggests that a partial hydrolysis occurred in alkaline conditions with the formation of carboxylate groups.

6.1.4. Poly(isophthalamides).

The results of the potentiometric studies conducted on 0.1% poly(isophthalamide) solutions based on L-ornithine, DL-diaminopropionic acid, δ-hydroxylysine and L-lysine are graphically presented in Figure 6.3 and shown individually in Figure 6.4 (a-d). The pK_a values derived from the equivalence values shown in Figure 6.4 (a-d) are given in Table 6.2.

Figure 6.1. Titration of 0.1% & 0.5% L-ornithine.

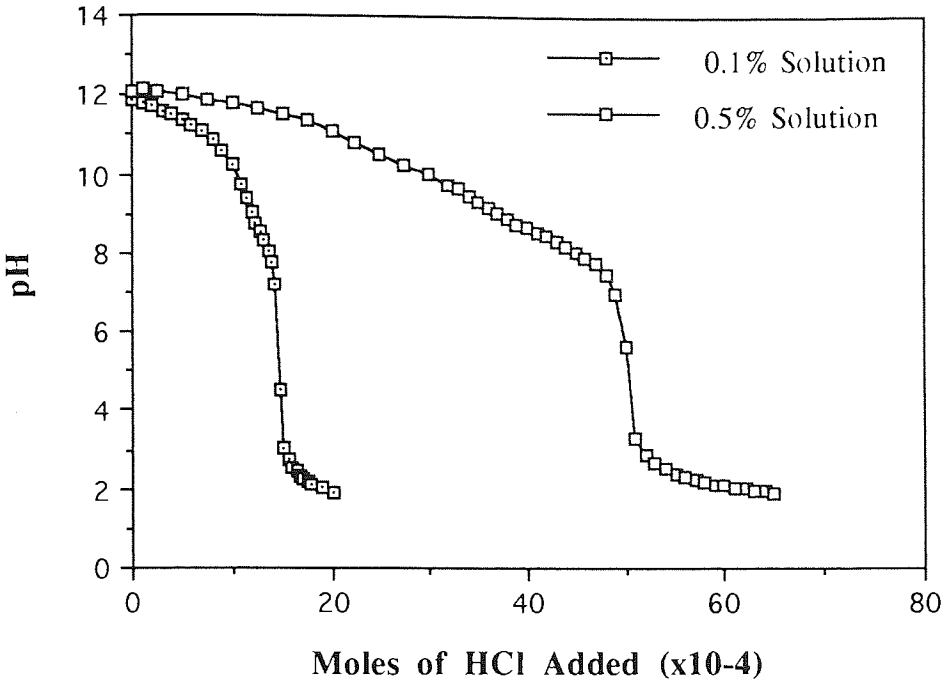


Figure 6.2. Titration of 0.1% L-ornithine showing 2nd derivative values.

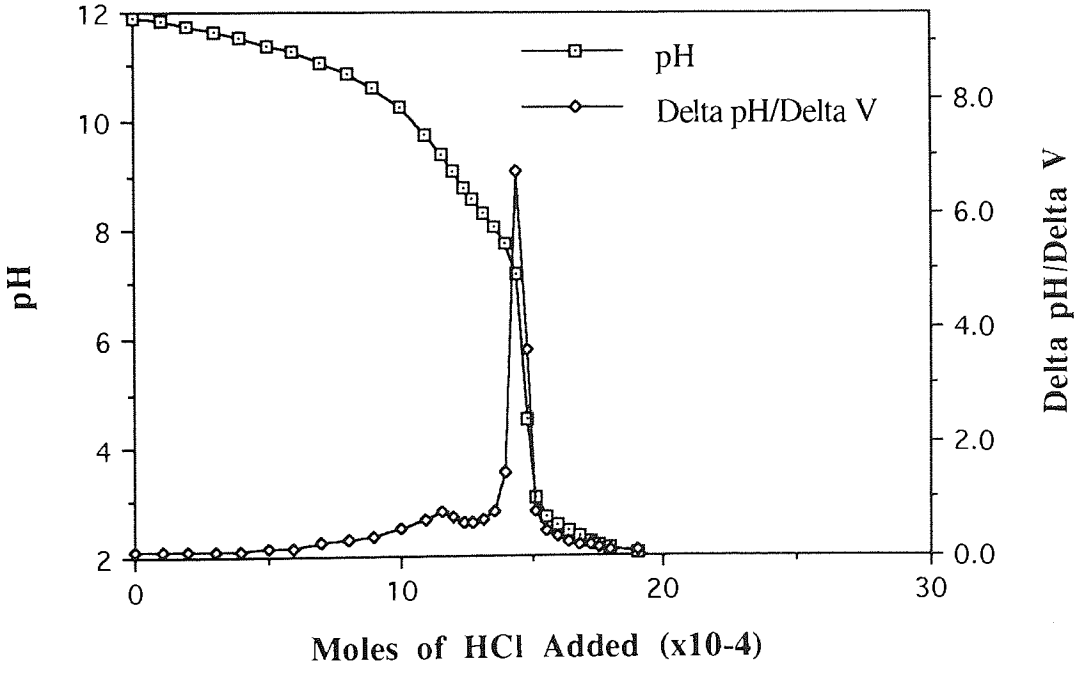
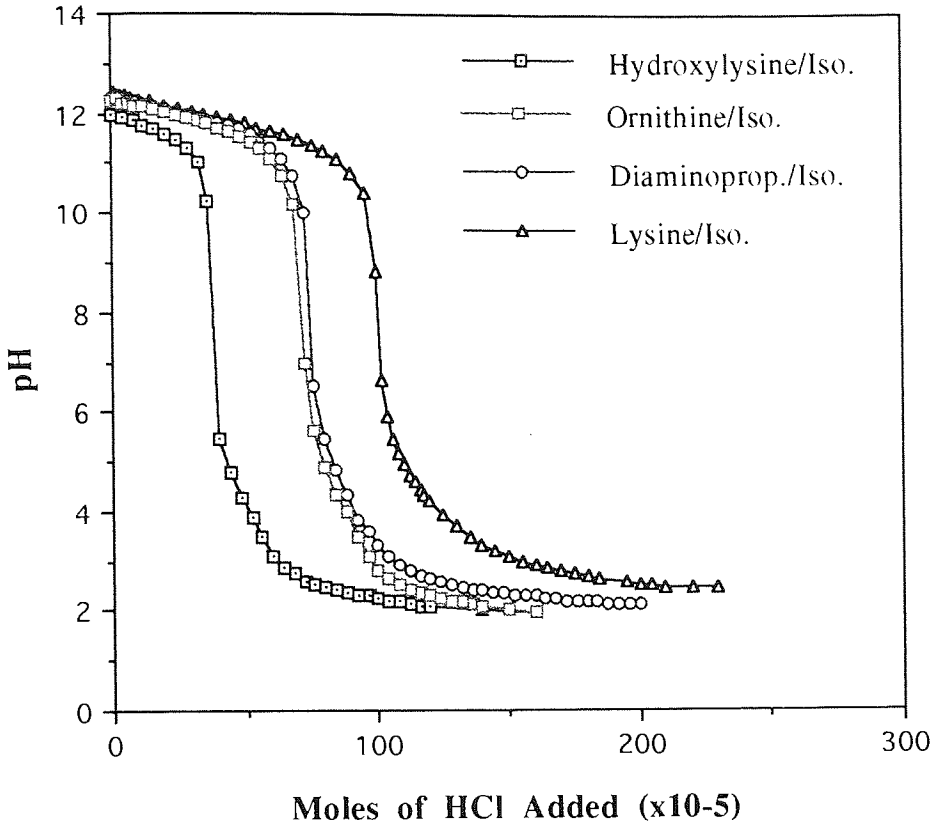


Figure 6.3. Titration of 0.1% poly(isophthalamides).



As can be observed in Figure 6.3, the inflection that was observed around pH 10.5, in the monomeric starting materials, resulting from the presence of amine groups, is absent from the polymer titration curves, as would expected for a polyamide. There appear to be two distinct inflection points, and hence, two pK_a values, in all of the poly(isophthalamide) titration curves; one around pH 2 and a second around pH 4. The presence of two pK_a values would suggest that the poly(isophthalamides) under investigation are polydiacids. This may arise, because, the polymers adopt two conformations, one in the extended state when pendant carboxylic acid groups behave as stronger acids with lower pK_a values, and a second, upon collapse of the polymer chain, where the pendant carboxylic acid groups are contained within a hydrophobic domain, and are therefore, sterically hindered and so behave as weaker acids and exhibit a correspondingly higher pK_a value.

Figure 6.4. (a) Titration of 0.1% poly(diamino-propionic acid isophthalamide).

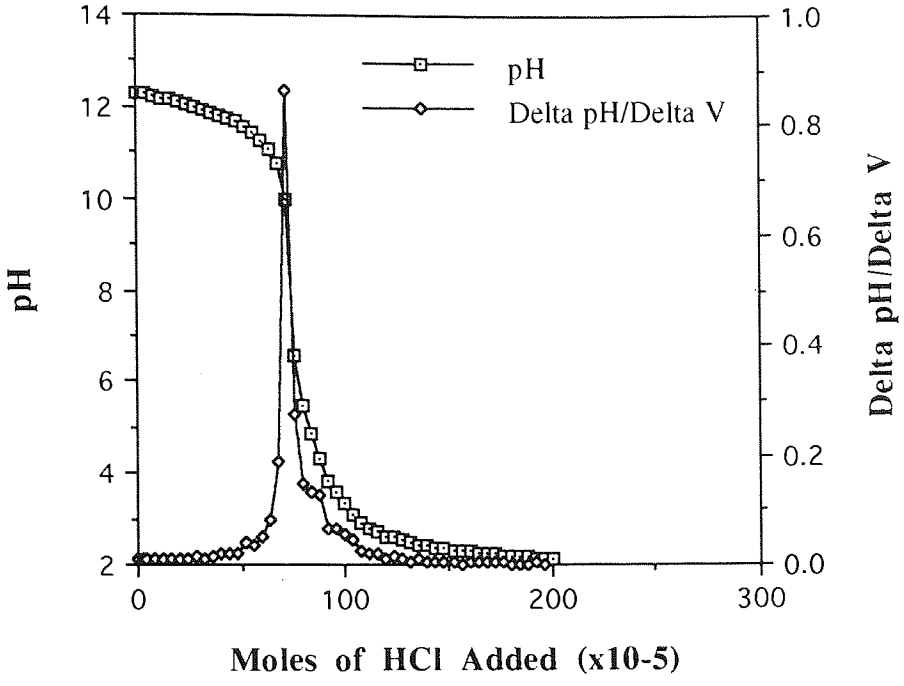


Figure 6.4. (b) Titration of 0.1% poly(hydroxy-lysine isophthalamide).

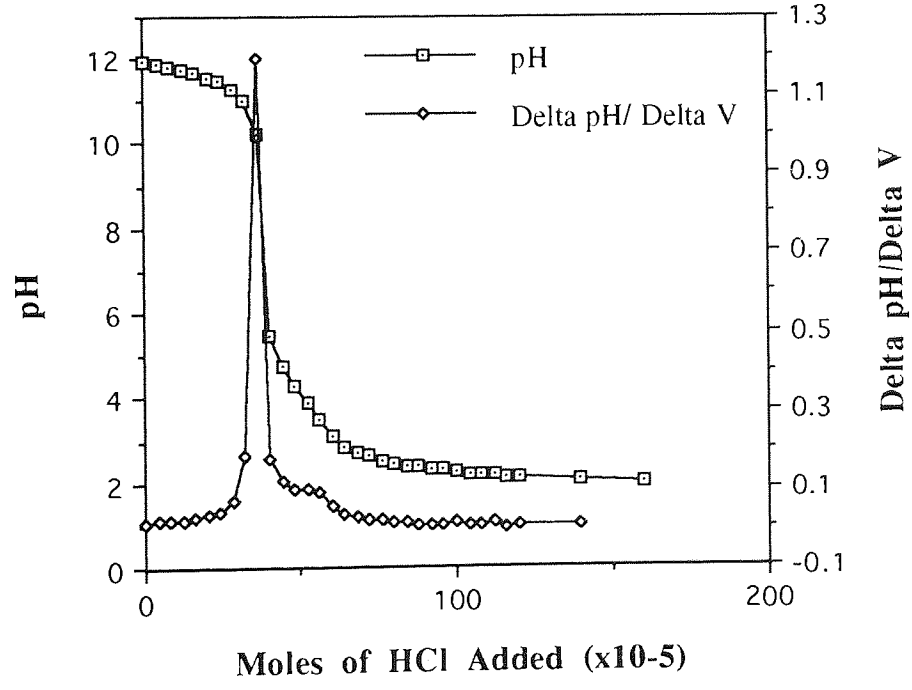


Figure 6.4. (c) Titration 0.1% poly(lysine isophthalamide).

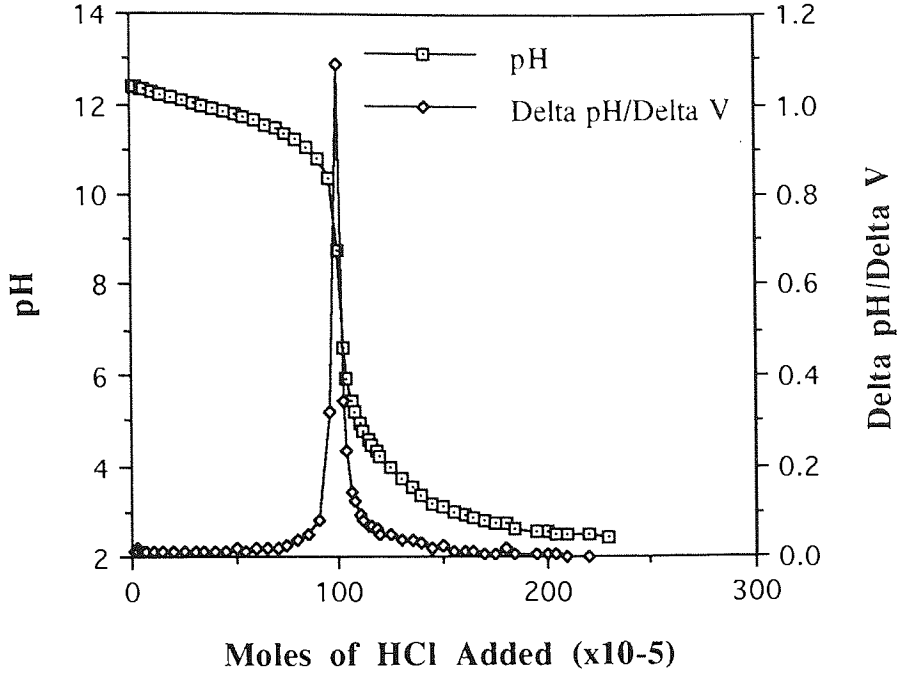


Figure 6.4. (d) Titration of 0.1% poly(ornithine isophthalamide).

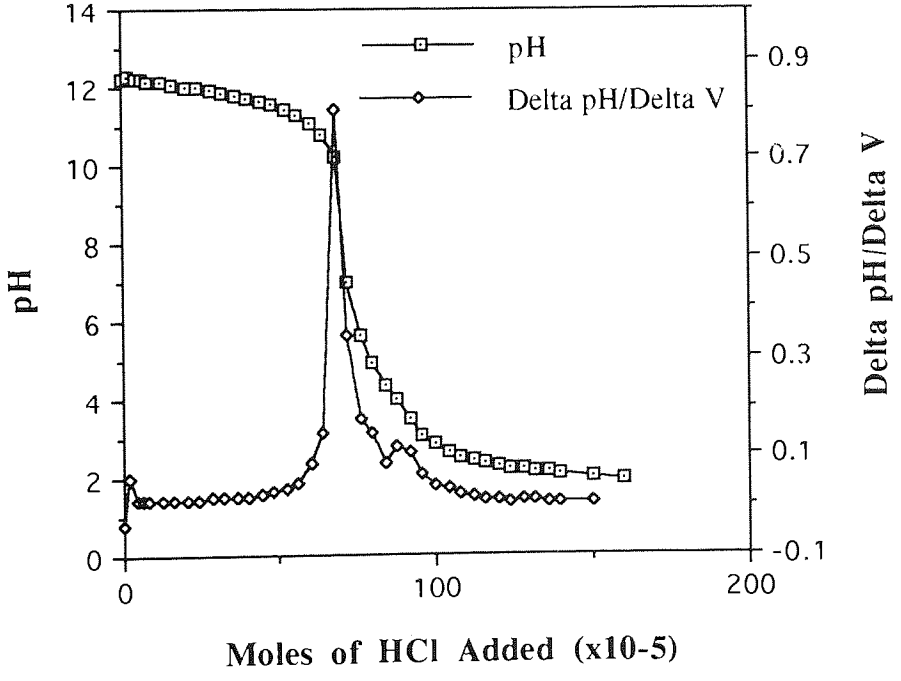


Table 6.2. Titration data of poly(isophthalamides).

Poly (Amino acid/ isophthalamides) 0.1%	Equivalence Point.	pK_a *	pK_a - COOH 2nd/end grp.
Ornithine isophthalamide	8.58	4.29	1.88
Diaminopropionic A. isophthalamide	8.28	4.14	1.73
Hydroxylysine isophthalamide	7.86	3.93	2.06
Lysine isophthalamide	7.71	3.86	2.04

* Half Equivalence Point method used for determination.

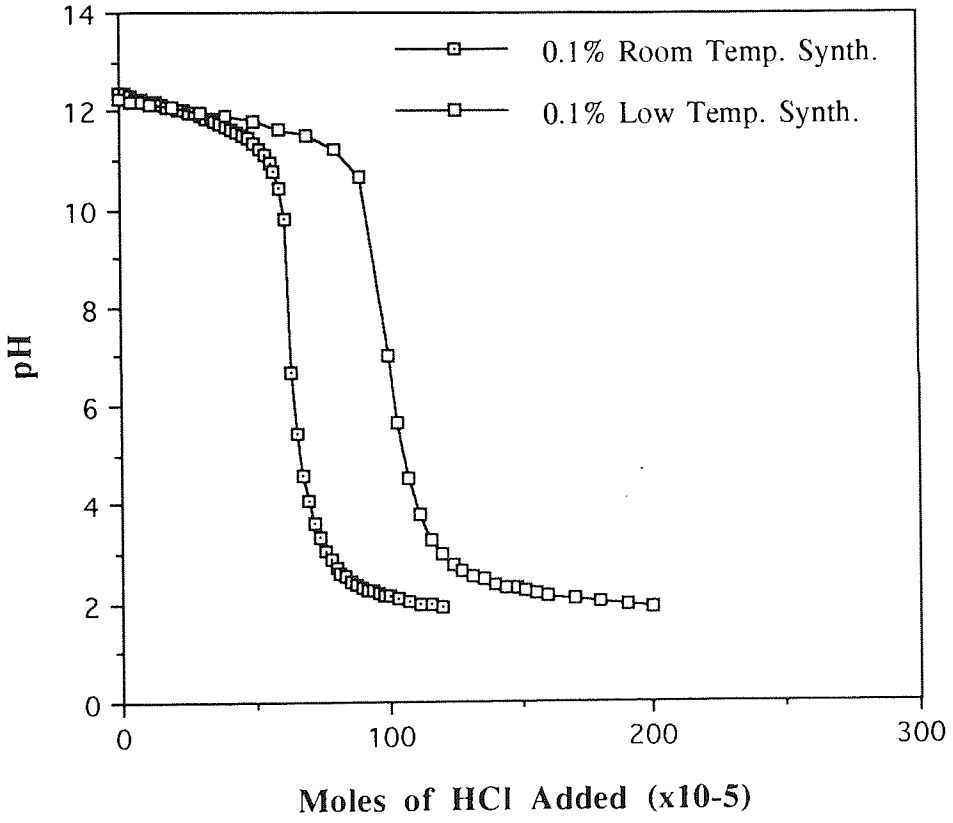
The results shown in Table 6.2 also indicate that those polymers based on ornithine and diaminopropionic acid behave as weaker acids than those based upon hydroxylysine and lysine. This is most probably because the shorter interchain distance between pendant carboxylic acid groups in the former polymers, results in a greater degree of steric hindrance and an increased inductive effect between the pendant carboxylic acid groups. The pK_a value recorded around 2 shows a reverse of this trend, where ornithine and diaminopropionic acid-based polymers appear to be stronger acids, while polymers based on lysine and hydroxylysine appear as weaker acids, suggesting that in the extended conformation the carboxylic acid groups of ornithine and diaminopropionic acid-based polymers are less sterically hindered, as would be expected from their higher charge density.

An alternative explanation of these results, is to assume that the pK_a values observed around 4 represent the carboxylic acid pendant groups, while the values obtained around 2 represent the terminal isophthalic acid residues, where the electron withdrawing effect of the benzene ring acts to form a stronger carboxylic acid than that of a pendant carboxylic acid in a polyamide chain.

6.1.5. Poly(adipamides).

The results of the potentiometric studies of 0.1% PLETESA solutions synthesized at room temperature and in low temperature conditions, and then partially saponified in 100% ethanol, are graphically presented in Figure 6.5 and shown individually in Figure 6.6 (a) and (b). The pK_a values derived from the equivalence values shown in Figure 6.6 are given in Table 6.3.

Figure 6.5. Titration of 0.1% poly(lysine ethyl ester adipamide) - saponified in 100% ethanol.



The titration profiles and pK_a values shown in Figure 6.5 are similar to those observed with the poly(isophthalamides), although the low temperature synthesized polymer is a weaker acid, perhaps because of the formation of a secondary structure such as a coil, which would have the dual effect of increasing the degree of both steric hindrance and inductive effects acting upon the carboxylate pendant groups.

Table 6.3. Titration data of poly(adipamides).

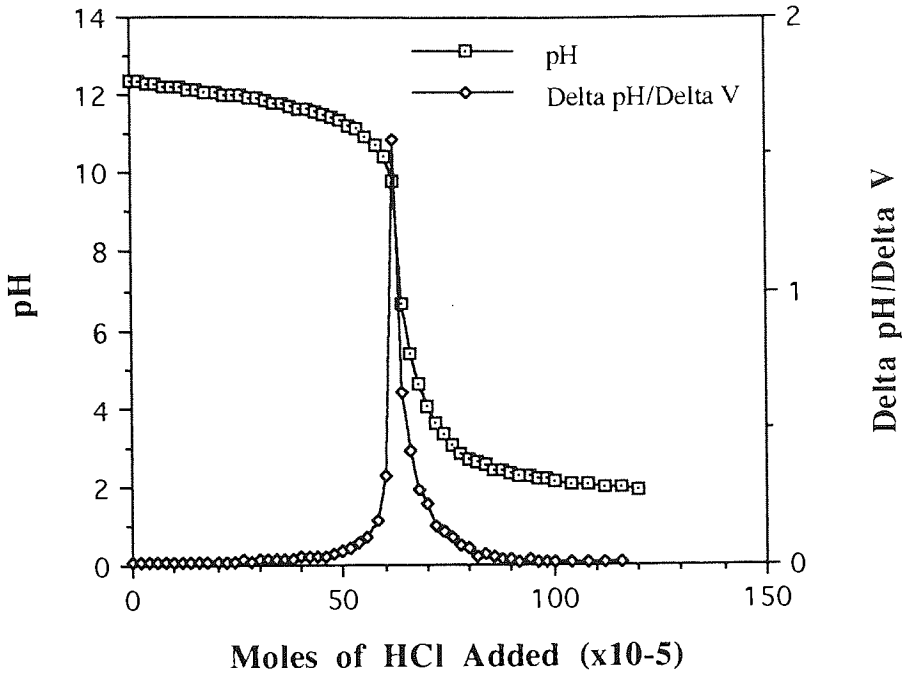
Poly(Lysine ethyl ester/adipamide) 0.1%	Equivalence Point.	pK_a *	pK_1- αCOOH 2nd/end grp.
Room Temp. Synthesis - Saponified	8.24	4.12	2.12
Low Temp. Synthesis - Saponified	8.85	4.43	2.15

* Half Equivalence Point method used for determination.

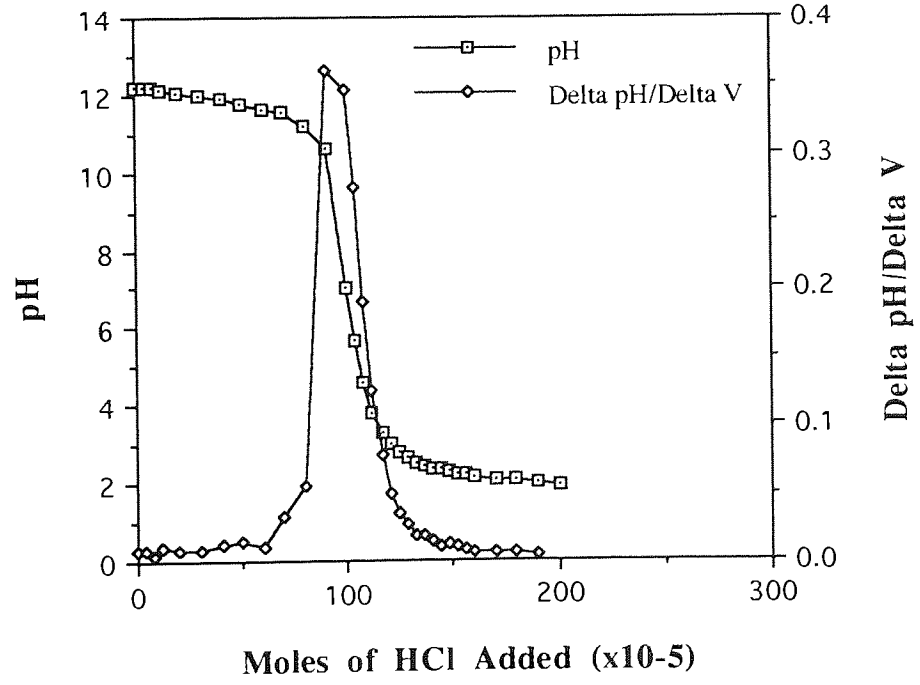
The second pK_a value recorded around 2 in the poly(isophthalamides) is virtually absent and the minor inflections noted probably represent the small number of terminal carboxylic acid groups substituted at the α-carbon atom, which could be expected to be present in polymers of high molecular weight, such as in those poly(adipamides) under investigation.

Figure 6.6. Titration of 0.1% PLETESA - saponified, showing ΔpH/Δvolume HCl acid added.

a) Room temperature synthesized.



b) Low temperature synthesized.



6.2. Measurement of Surface Tension.

6.2.1. Introduction and Rationale.

As described in chapter 2, surface tension has been used as a simple method to monitor the changes that occur in polymer conformation with variation of pH. In the series of studies described in this chapter, the orientation of the monomeric starting materials and the conformation of the polymeric products, in aqueous solution, will be probed by measuring surface activity while varying the pH of the test solution.

6.2.2. Experimental Procedures.

All surface tension measurements were carried out using a digital tensiometer (Whites Electrical Instruments, Malvern England. Model No. DB 2kS), and assessed by using the du Noüy ring technique. Approximately 10mls of sample was used for each determination. Care was taken to flame clean the platinum/iridium du Noüy ring, prior to each measurement to remove any contaminants. Particular care was taken when recording the surface tension to observe only the maximal value displayed by the instrument, further movement of the du Noüy ring was then stopped, in order to prevent the ring from breaking through the air/liquid interface. This procedure resulted in minimal disturbance to any lamella structures formed at the surface of the solution, and hence, enabled a more accurate measurement of surface forces to be obtained. Readings continued to be taken until consistent recordings were observed, this usually required taking four to six measurements for each sample. All results expressed are graphically shown with their corresponding standard errors about a mean value.

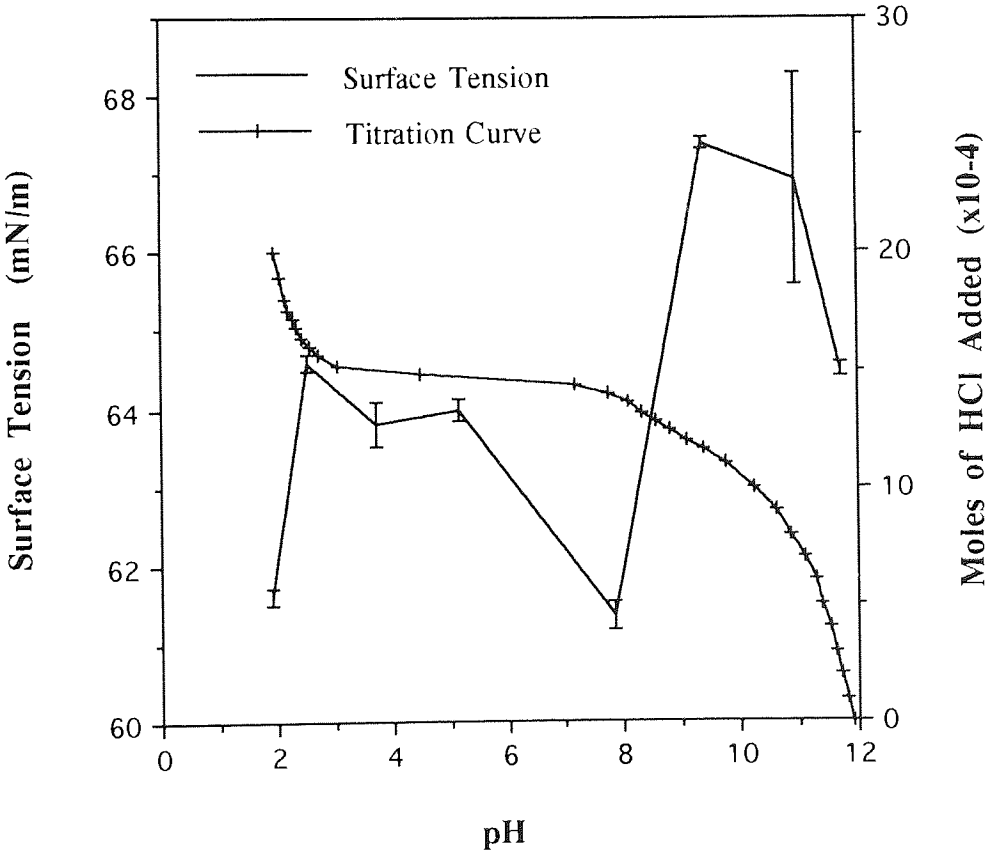
6.2.3. Starting Materials.

In order to assess the effect that residual starting materials, such as amino acids and their derivatives or hydrolysed acid chlorides, may have upon surface tension measurements conducted on the test polymer solutions, some initial control experiments were undertaken. A series of starting materials and potential hydrolytic breakdown products were chosen as model compounds to illustrate the likely surfactant effects of such contaminants. These studies sought to measure the surface tension of ornithine, lysine, lysine ethyl ester and a mixture of polypeptides obtained from denatured silk.

6.2.3.1. Ornithine & Isophthalic Acid.

It can be seen in Figure 6.7 that the change in surface tension of a 0.1% ornithine solution in response to changes in pH is minimal, and only varies between 68 and 61 mN/m. The 0.5% solution appeared to be more surface active, although, a rise in surface tension occurred, from 56 to 65 mN/m, as the pH was reduced from 11.7 to 1.9. It is unlikely, though, that ornithine would be present in such high concentrations as part of an extracted polymer product. Both solutions show a marked increase in surface activity at pH 8 and, as can also be seen in Figure 6.7 (where the titration curve is overlaid) the fall in surface activity corresponds precisely to the inflection point at pH 8.2 on the titration curve. This represents the pK_a of the amine group substituted at the α-carbon atom.

Figure 6.7. Titration and surface tension vs. solution pH - 0.1% L-ornithine hydrochloride.



Isophthalic acid (0.05%) became more surface active on acidification. As the pH was reduced from 12.5 to 7.4, the surface tension fell from 56 to 49 mN/m. This probably arose from a partial loss of charge, and therefore, a decrease in polarity, resulting in a change in the hydrophobic/hydrophilic balance and leading to the formation of a more hydrophobic molecule with a greater tendency to accumulate at the air/water interface.

6.2.3.2. Lysine & Lysine Ethyl Ester (LETES).

Lysine (0.5%) and LETES (0.1 & 0.5%) solutions showed remarkably similar surface tension/pH profiles to those reported with ornithine, as shown in Figures 6.8 and 6.9, respectively. A marked change in surface tension occurred as the pH was lowered between pH 12 and 2. Three distinct minima in surface activity occur with lysine, at pH 10.5, 6 and 4, and two minima occur with LETES. These represent the pK_a values for the amine groups substututed at the ε and α-carbon atoms and the carboxylic acid groups substituted at the α-carbon atoms, respectively. The effect due to the carboxylic acid substituted at the α-carbon atom is absent in LETES. The minima values also correspond to the pK_a values recorded for these materials as shown in Table 6.1 and are in close agreement with those reported in the literature for lysine of 10.53, 8.95 and 2.18.[169] This would suggest that charging of the carboxylic acid group substituted at the α-carbon atom of an amino acid enhances the surface activity. The maximal surface activity occurs at pH values where maximal charge exists on the molecule, e.g. around pH 11.5, 6 and 4, i.e., when the amine group substituted at the ε-carbon atom is charged, when the amine group substituted at the α-carbon atom is charged, combined with the isoelectric point, and finally, on charging of the carboxylic acid group substituted at the α-carbon atom.

Figure 6.8. Surface tension vs. solution pH of L-lysine - 0.5%.

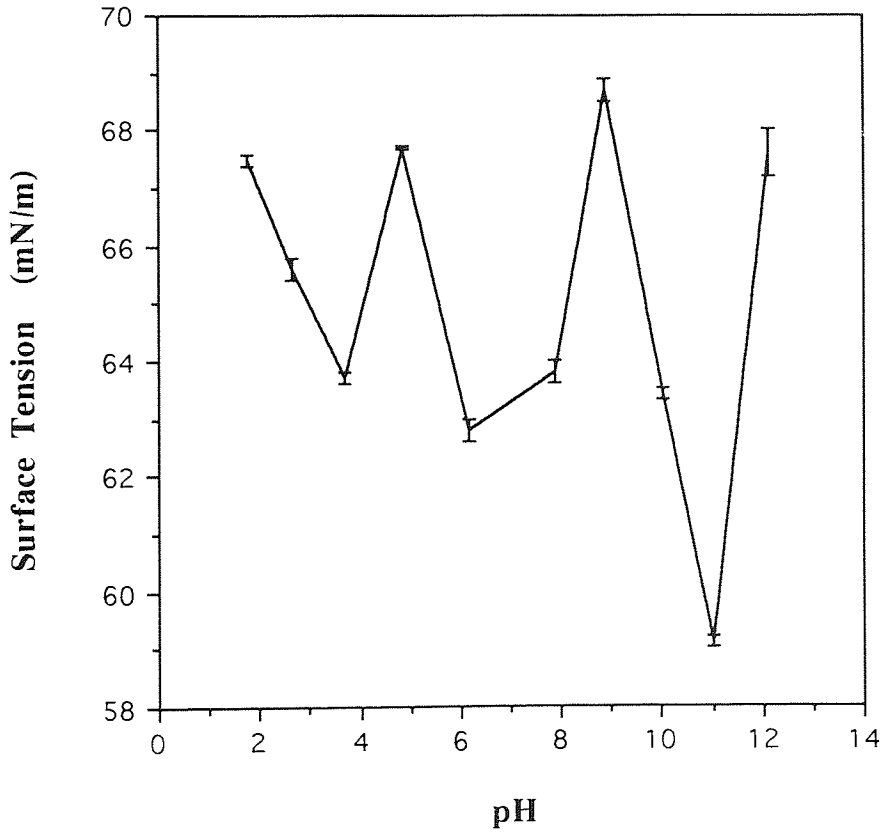
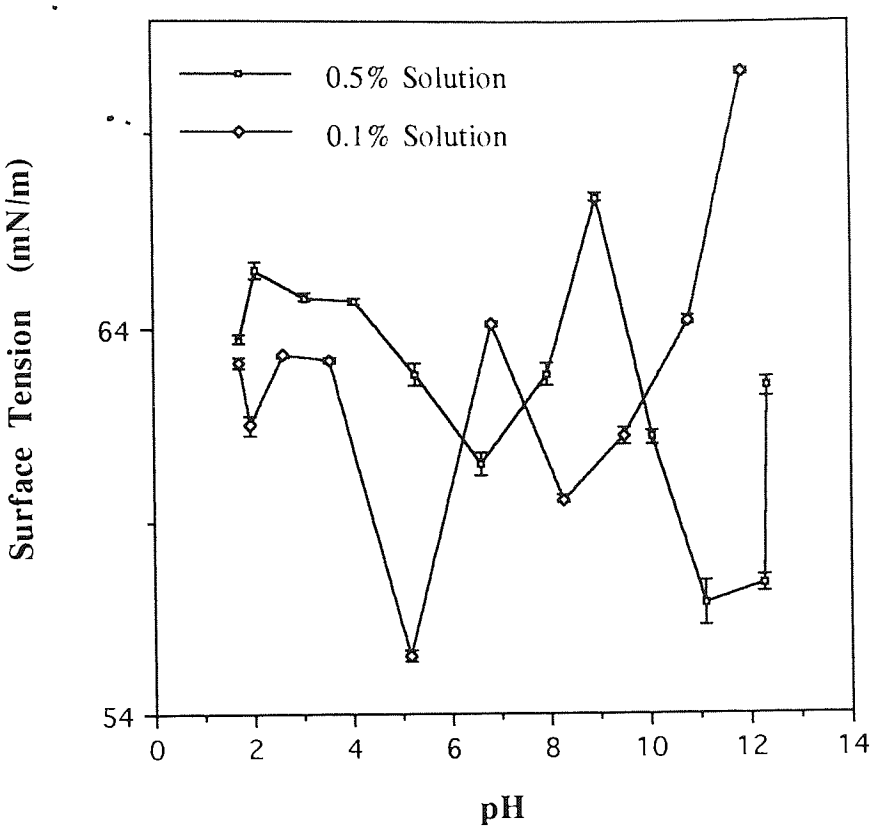


Figure 6.9. Surface tension vs. solution pH of lysine ethyl ester dihydrochloride - 0.5% and 0.1%.



There also appears to be a concentration dependency in the case of LETES, where the surface activity maxima appear to be shifted to higher values by approximately 2 pH units. This may, perhaps, be due to surface packing, whereby at high surface pressures, charge shielding effectively changes the pK_a of the molecules at the surface, and this has a greater effect at higher concentrations.

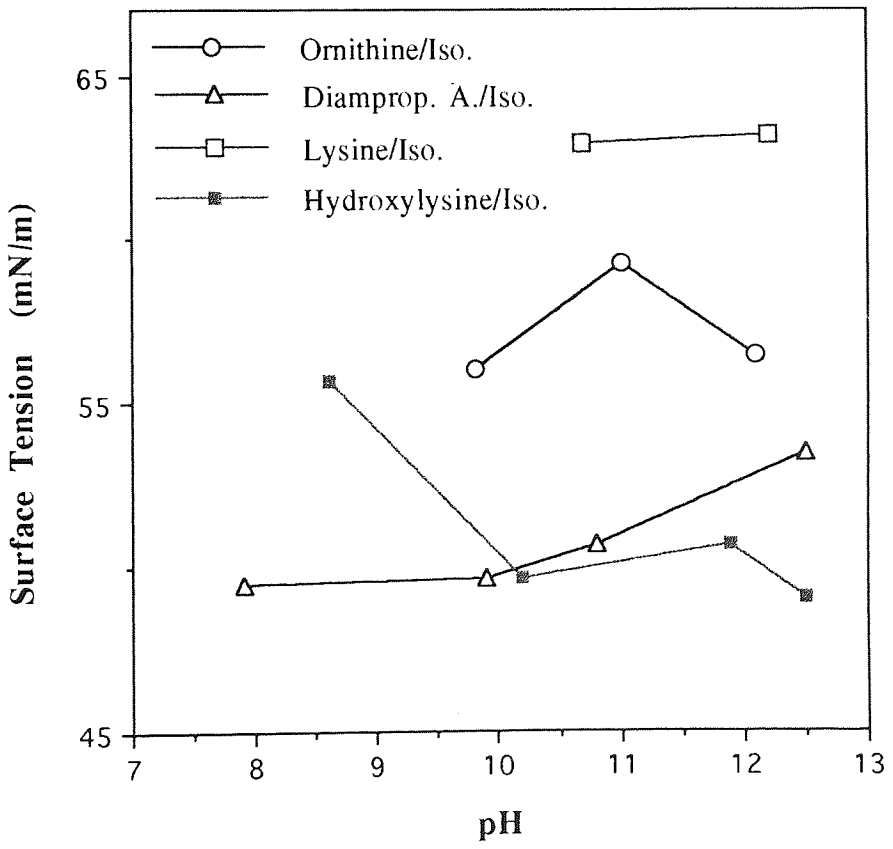
In conclusion, the surface tension results reported for the polyamides should be viewed with some caution, considering the surfactant properties of the starting materials and breakdown products. Although, it is unlikely in view of the combined aqueous/methanolic extraction procedure used in producing the polymeric materials, that any substantial amounts of starting material or hydrolytic products would remain in the polymer samples.

6.2.4. Polymers.

6.2.4.1. Poly(isophthalamides).

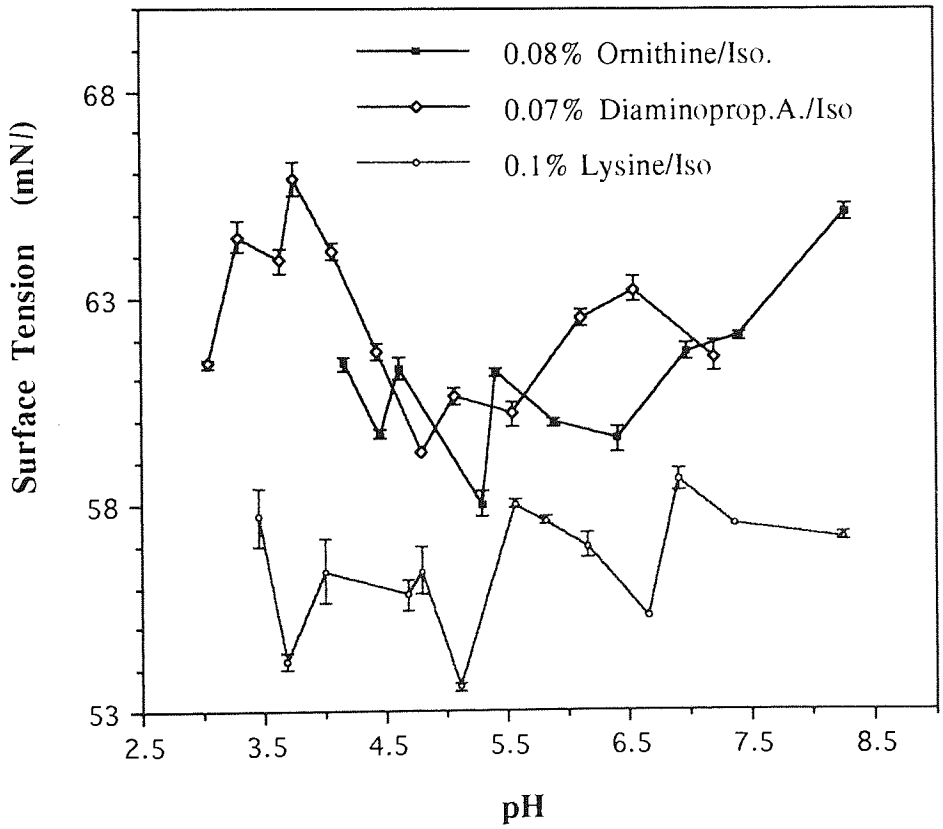
Surface tension vs. pH studies were conducted on 0.5% solutions of poly(isophthalamides) based on ornithine, lysine, hydroxylysine and diaminopropionic acid, respectively. The results are graphically presented in Figures 6.10 and 6.11. Initial studies concentrated on observations in alkaline conditions, i.e., from pH 7.9 to 12.5, since it was felt that the poly(isophthalamides) would show conformational changes as their pendant carboxylic acid groups became neutralized. Potentiometric studies indicated that this would occur around the equivalence point at pH 8. Figure 6.10 clearly demonstrates, that unlike the starting materials and the acid chloride hydrolytic products, the poly(isophthalamides) show little variation in surface activity in response to changes in pH in highly alkaline conditions. Thus, suggesting, that conformational changes are not occurring within the structure of the polymers and also indicating that unreacted starting materials are not present to any significant degree.

Figure 6.10. Surface tension vs. solution pH - poly(isophthalamides) - 0.5%.



Reducing the pH towards the principal pK_a value exhibited by the polymers caused a reduction in surface tension as can be seen in Figure 6.11, a minimal value was observed in the pH 4.5-5.5 range, coincident with the respective pK_a value. In all three polymers tested the surface tension rose in the pH range from 4.5 to 3.5, immediately prior to the polymers precipitating out of solution. From these observations it can be deduced that as charge is lost, upon acidification, the extended polymer chain collapses to form hydrophobic microdomains interspersed with charged groups. This amphipathic character renders the polymers surface active and causes a fall in surface tension. It should also be noted from Figure 6.11 that there is some evidence for a transient rise in surface tension, i.e., a fall in surface activity, prior to neutralization, which occurs over a range of some 1.5-2 pH units from pH 5 to pH 7. This effect could be explained by the formation of intramolecular micelles as proposed by Jorgensen[86] and discussed in chapter 2, section 2.3.1.1.

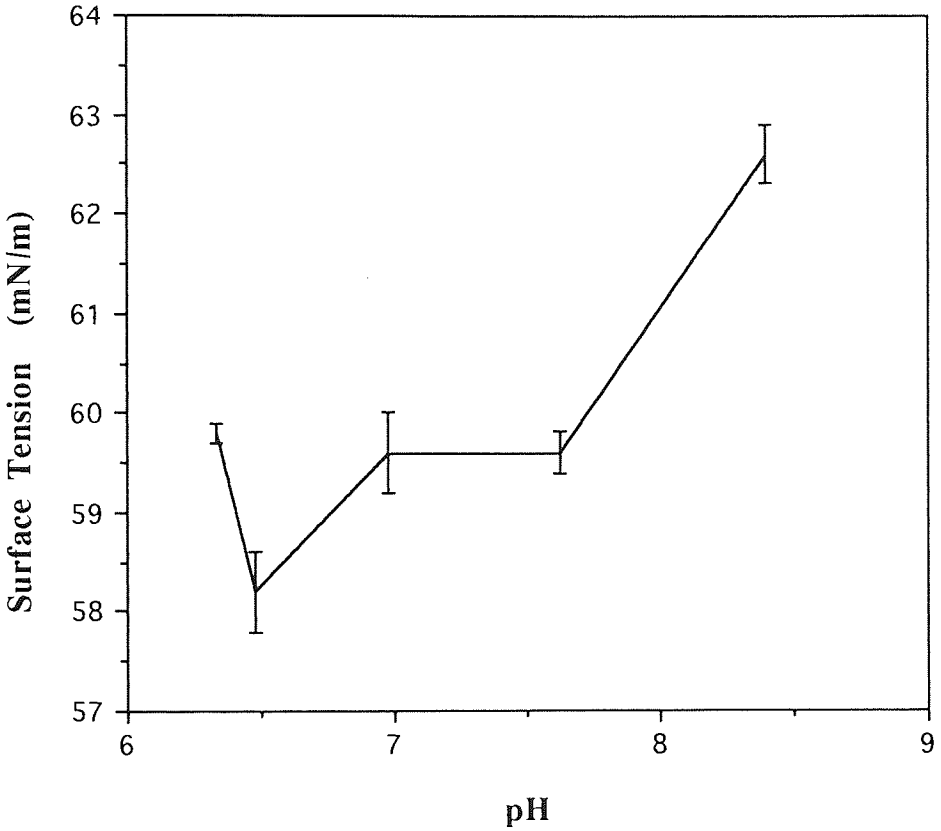
Figure 6.11. Surface tension vs. solution pH - poly(isophthalamides). Low pH range.



The surface tension/pH profile was also investigated for a 0.1% solution of the terpolymer of lysine ethyl ester, diaminosuccinic acid and isophthaloyl chloride and the results are graphically expressed in Figure 6.12. This polymer may have been expected to show greater surface activity than any of the simple copolymers because, of the greater proportion of hydrophobic groups but, in fact, exhibited an intermediate level of

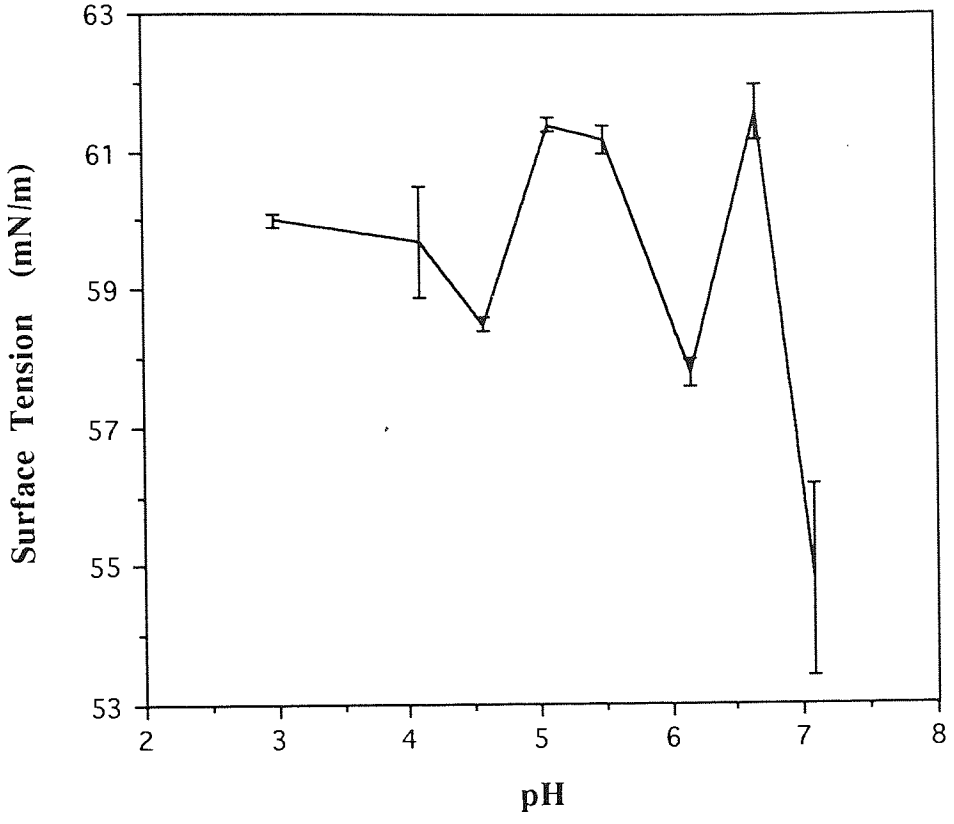
surface activity between that of the lysine and diaminopropionic acid copolymers, at least over the higher pH range (pH 6.3-8.4) where activity was studied. This result supports the contention that the two amino acids were not incorporated into the polymer in discrete blocks, as intended, but are randomly arranged, and thereby, fail to form a particularly amphipathic or surface active product.

Figure 6.12. Surface tension vs. solution pH - 0.1% LETES/ diaminosuccinic acid isophthalamide oligomer.



The surface tension/pH profile of the product of interfacial condensation of tyrosine and isophthaloyl chloride was also investigated and the results are shown in Figure 6.13. In contrast, to the results obtained with the other isophthalamide products, a different pH dependency can be observed, where the surface tension rises on acidification. These observations provide further evidence that the material synthesized was not, in fact, polymeric in nature and confirm the results obtained by GPC.

Figure 6.13. Surface tension vs. solution pH - 0.1% tyrosine isophthalamide oligomer.

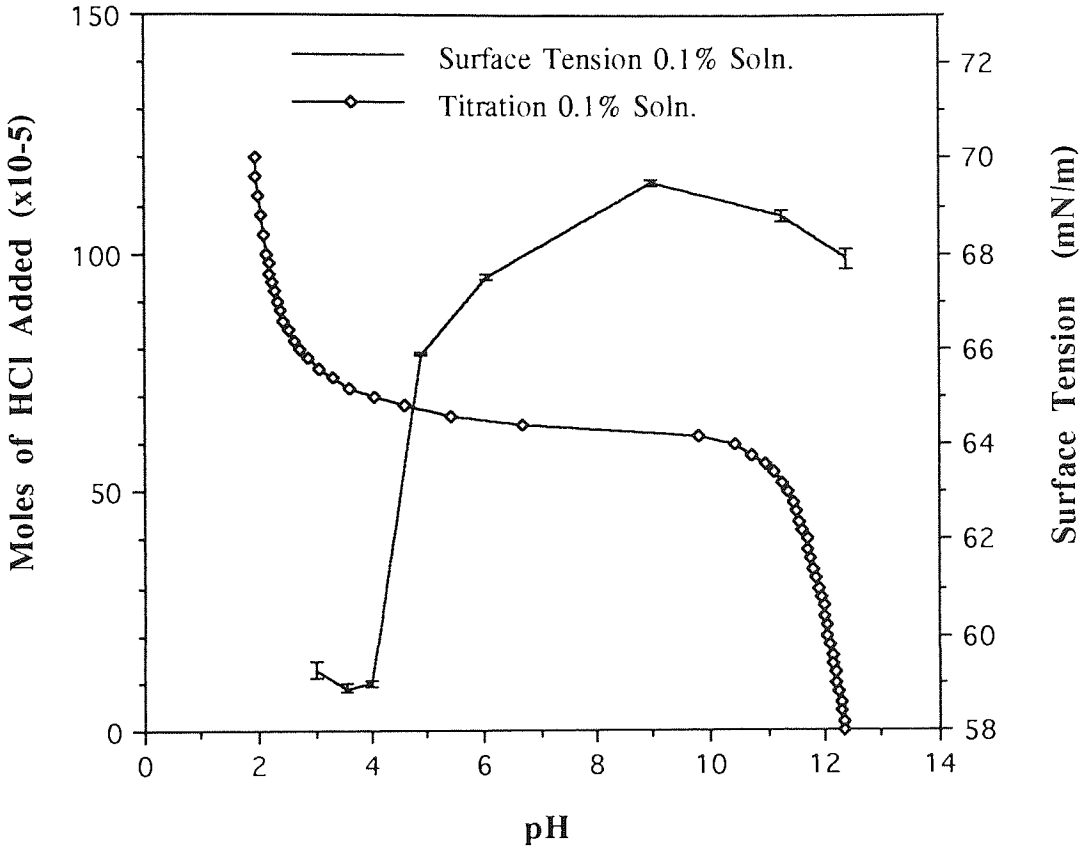


6.2.4.2. Poly(adipamides).

The surface activity of a 0.1% aqueous solution of Poly(lysine ethyl ester adipamide) (PLETESA), synthesized at room temperature and saponified in 0.25% ethanolic sodium hydroxide, was monitored over a broad pH range. The results are graphically represented in Figure 6.14 and reveal an extremely interesting trend, previously alluded to in the poly(isophthalamides), where an initial rise in surface tension can be observed as the solution is acidified, i.e., over a range from pH 12.4 to 4.9, with a peak value at pH 8.96. As the pH is lowered below pH 4.9 the surface tension falls sharply, and this fall is concomitant with the equivalence point in the titration curve, shown as an overlay plot in Figure 6.14. These observations can be explained by assuming that the polymer initially forms intramolecular micelles as charge is lost, in which the hydrophobic groups become associated within the core of the micelle, and thus, are removed from the aqueous environment. The removal of the latter, results in the rise of surface tension observed. With further loss of charge the number of carboxylate groups is no longer sufficient to encompass the hydrophobic core, and hence, the hydrophobic groups become exposed to the aqueous environment, and in so doing, form an amphipathic polymer that causes a fall in surface tension (or

rise in surface activity). When the pH is lowered below the pK_a of the pendant carboxylate groups (approximately pH 4), the remaining charge on the polymer is insufficient to retain the polymer in solution and precipitation ensues.

Figure 6.14. Surface tension vs. solution pH & titration curve of PLETESA synthesized at room temperature - saponified.



Further studies were conducted on a 0.5% aqueous solution of PLETESA, synthesized at low temperatures using the high concentration potassium carbonate technique and subsequently saponified in 0.25% ethanolic sodium hydroxide. The results are graphically presented in Figure 6.15 and show that, as expected, higher concentrations of polymer result in a lower starting surface tension. A comparison of the surface tension/pH profiles of the two solutions is shown in Figure 6.16. The 0.5% polymer solution also showed some evidence of a rise in surface tension upon acidification around the equivalence point as observed with the polymer synthesized at room temperature, i.e., pH 9.8 to 5.4, which is shown by the titration curve plotted as an overlay in Figure 6.15. The similarity between the surface tension/pH profiles of the two polymers synthesized at different temperatures indicates that the same molecular changes are occurring in both polymer solutions, and therefore, the temperature at which the polymer is synthesized does not appear to affect its conformational behaviour in solution.

Figure 6.15. Surface tension vs. solution pH & titration curve of PLETESA synthesized at low temperature - saponified.

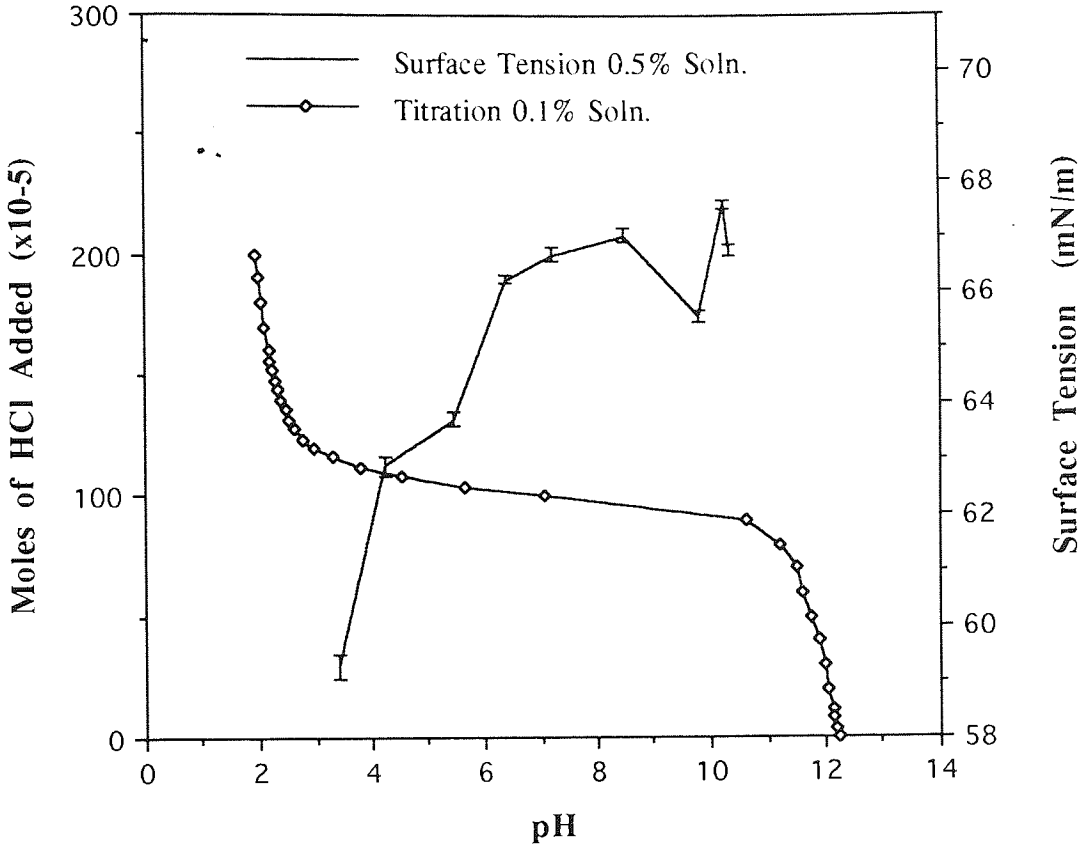
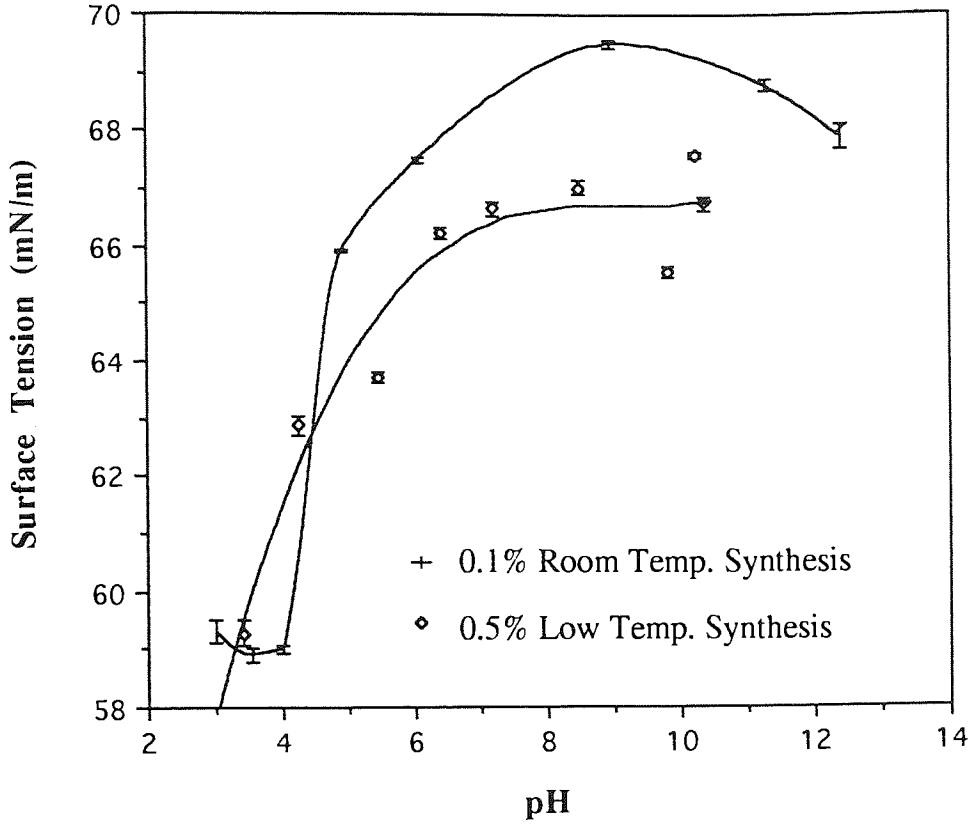


Figure 6.16. Surface tension vs. solution pH of PLETESA - saponified.



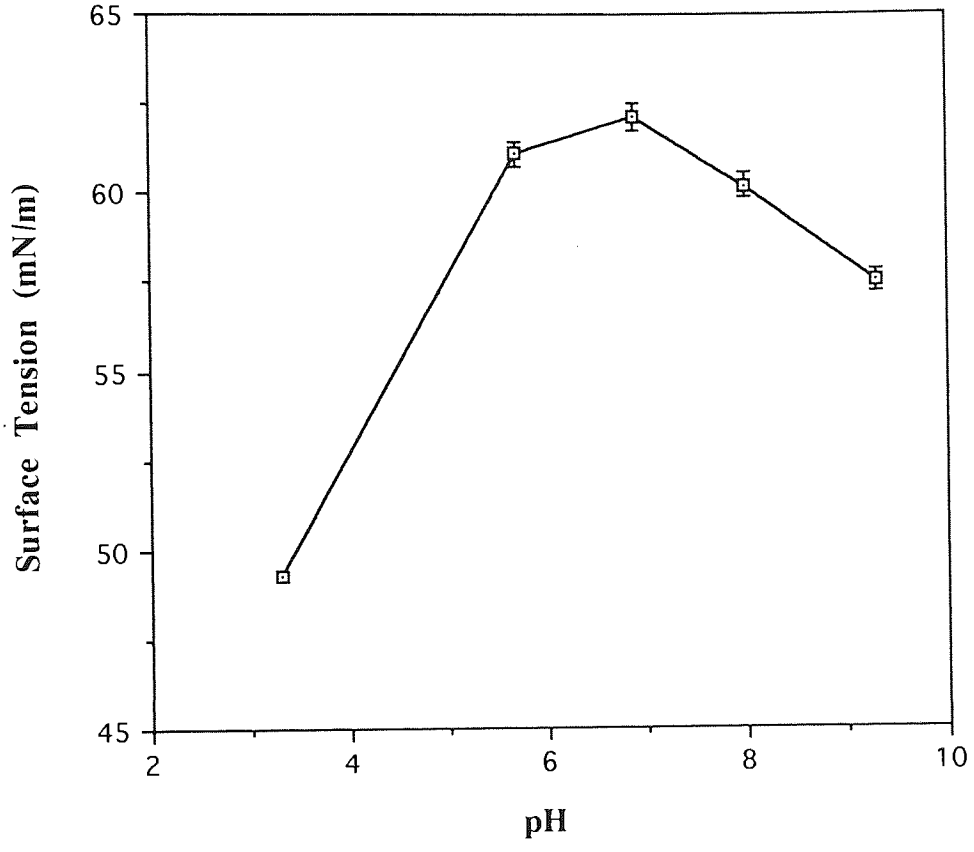
6.2.4.3. Denatured Silk Polypeptide.

Denatured silk polypeptide was used as a model polyelectrolyte to investigate the effects of pH on surface tension. The denatured silk used was prepared according to the procedure of Ambrose,[170] by dissolving raw silk in an aqueous solution containing 60% lithium bromide at 70°C. The resulting solution of silk fibroin was then dialysed to remove the lithium bromide and finally extracted into excess water. An analysis of silk fibroin polypeptide published in the literature indicates that it contains 74% alanine and glycine residues along with smaller amounts non-polar, anionic and cationic residues.[171]

Investigations of the surface tension/pH profile of a saturated aqueous solution of silk fibroin, extracted by the procedure outlined, is shown graphically in Figure 6.16 and demonstrates a similar profile to those obtained with PLETESA. However, in this case, a rise in surface tension was noted upon acidification from pH 9.3 to 6.9, followed by a sharp fall as the pH was lowered to 3.3, presumably as the isoelectric point was reached. The surface tension of 0.01-0.1% gelatin solution has also been reported to show a similar response to pH change, exhibiting a minimal surface tension

at the isoelectric point, where maximal charge shielding, and hence, chain folding occurs.[172]

Figure 6.17. Surface tension vs. solution pH of denatured silk fibroin - saturated aqueous solution.



Similar surface tension/pH profiles, showing a rise in surface tension upon neutralization from pH 9 to pH 7, have frequently been observed in these laboratories in hypercoiling, vinyl-based, anionic copolymers and are similar to those reported by Pop[87] and Boiko[88] and reviewed in chapter 2, section 2.3 of this thesis.

6.3. Interfacial Tension Measurements.

Interfacial tension was measured in order to assess the surfactant behaviour of poly(isophthalamides) at an aqueous interface where the non-aqueous phase presented a more hydrophobic environment than that presented by air. Data obtained using this technique gave a greater insight into the dispersive forces defining the polymer conformation.

6.3.1. Spinning Drop Tensiometry.

Interfacial tension was measured using the spinning drop technique. This technique enables the interfacial tension between two immiscible phases to be defined, by measuring the dimensions of a droplet of test solution in aqueous phase suspended within a rotating organic phase. The diameter of the droplet is directly proportional to the interfacial tension between the two phases. Droplets of high interfacial tension are able to resist the elongating force induced by rotation and this results in a shorter drop length. Based on this relationship, the interfacial tension can be calculated directly by applying equation 6.1:

$$I = e(vd)^3 n^2 \Delta\rho \qquad (6.1)$$

Where:
- e = A constant (3.427×10^{-7})
- v = Magnification factor (0.245)
- d = Droplet diameter
- n = Capillary rotation speed (rpm.)
- $\Delta\rho$ = Phase density difference (2.26 g/cm^3 at 20^{o}C)

The advantage of this technique is the absence of any solid at the interface, an inherent feature of the du Noüy ring or Wilhelmy plate techniques, and hence, there are no contact angle or end effects to contend with. In addition, since surface tension is directly proportional to the third power of the droplet diameter, it follows that interfacial tension can be measured over a wide range,[97] including 'superlow' values as low as 5×10^{-4} dynes/cm (mN/m).[173] However, this technique is most suited to measuring interfacial tensions in the order of 3 mN/m.

6.3.2. Experimental Procedure.

Interfacial tension measurements were made using a Kruss Site 04 Spinning Drop Tensiometer. Diiodomethane was used as the organic phase, into which was injected 2μl samples of either test monomers or poly(isophthalamides) in aqueous solution. All solutions were prefiltered through Whatman N^o. 542 paper. The rotational speed was initially adjusted to approximately 2,000 rpm. and then varied to obtain a droplet size where the length was four times the diameter. The drop diameter and corresponding rotational speeds were then recorded at several points along the length of each droplet until consistent readings were obtained. In some cases measurements were also made on a second droplet of the sample under investigation to check for consistency. The

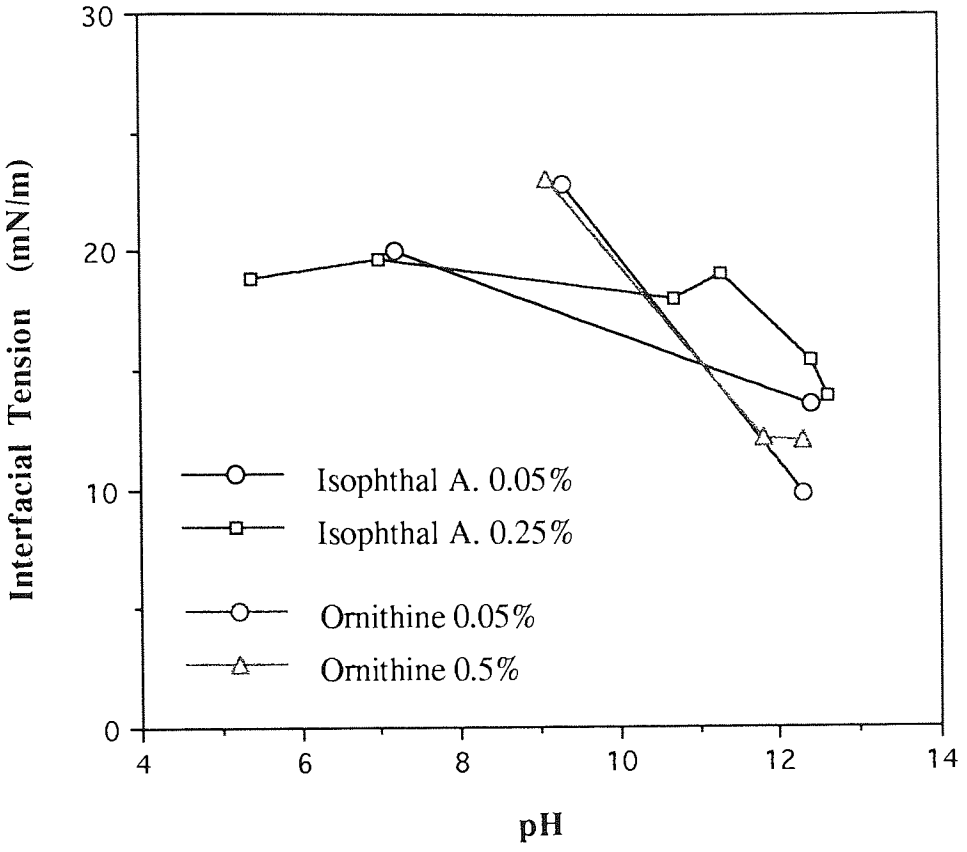
apparatus was maintained at 20-21°C throughout the experiments by a thermostated oil bath.

6.3.3. Interfacial Tension of Starting Monomers and Hydrolytic Degradation Products.

To assess the possible surfactant effects of the presence of residual starting materials or hydrolysed acid chlorides upon the interfacial behaviour of the poly(isophthalamide) solutions, the interfacial tensions of ornithine hydrochloride and isophthalic acid were assessed over a range of pH values. The isophthalic acid used in this study had been extracted from the aqueous phase following an interfacial reaction between isophthaloyl chloride and ornithine and had previously been characterized by FT-IR spectroscopy. FT-IR analysis had suggested that this material represented the major source of contamination in the poly(isophthalamides) after their extraction from aqueous solution.

The interfacial tension/pH profile of 0.05% and 0.5% ornithine in aqueous solution is shown in Figure 6.18 and can be seen to exhibit an increase in interfacial tension with falling pH, rising from 10-12 mN/m at pH 12 to 23 mN/m at pH 9, a level similar to that recorded for HPLC grade water of 24.7 mN/m. This observation may be explained by assuming that the loss of charged carboxylic acid groups upon acidification is counteracted by increased quaternization of the amine groups, and therefore, the overall tendency is to increase the polarity of the molecule and cause desorption from the interface

Figure 6.18. Interfacial tension vs. solution pH: Monomer starting materials.



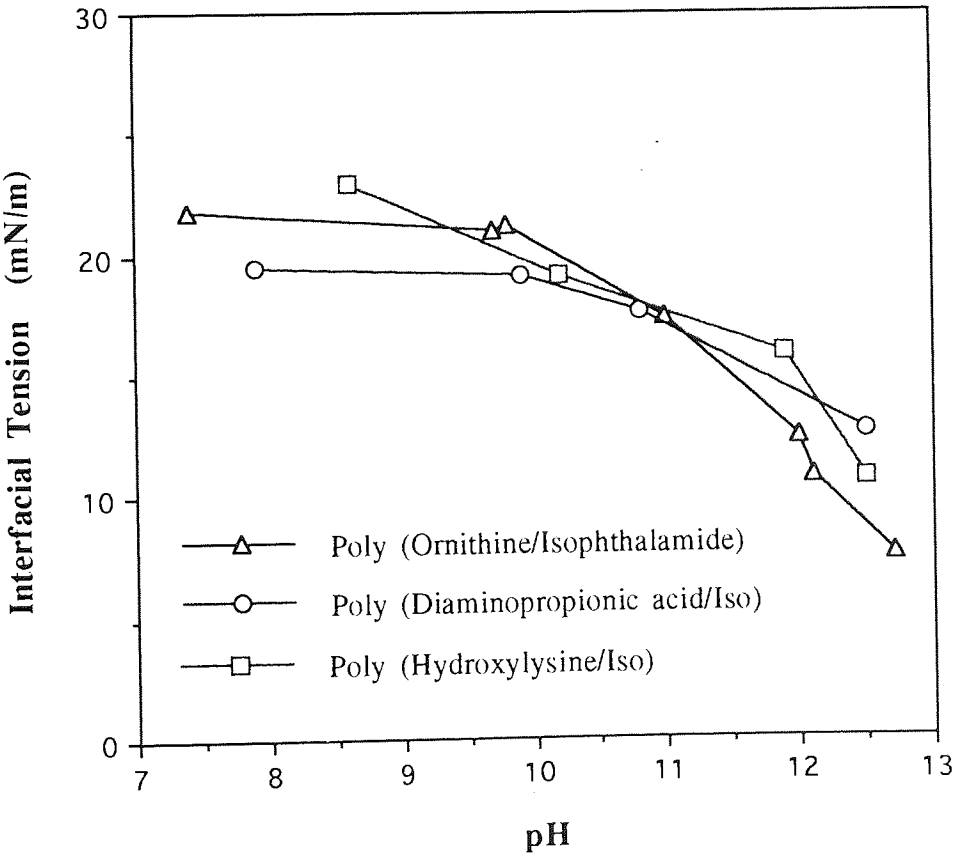
Isophthalic acid solutions at both 0.025% and 0.5% exhibited an increase in interfacial tension from approximately 14 mN/m to 20 mN/m following the reduction in pH from 12.4 to 7 as can be seen in Figure 6.18. This effect may result from the loss of charge on the molecule, desorption from the interface, and perhaps, partition into the organic phase.

Hence, in both isophthalic acid and ornithine solutions, changes in interfacial tension in response to changes in pH were either rather moderate, as in the case of isophthalic acid, or only occurred in highly alkaline conditions, as in the case of ornithine. We can assume that any contamination of the polymers under investigation by these materials is likely to have a noticeable but limited influence on the interfacial properties under investigation.

6.3.4. Interfacial Tension of Poly(isophthalamides).

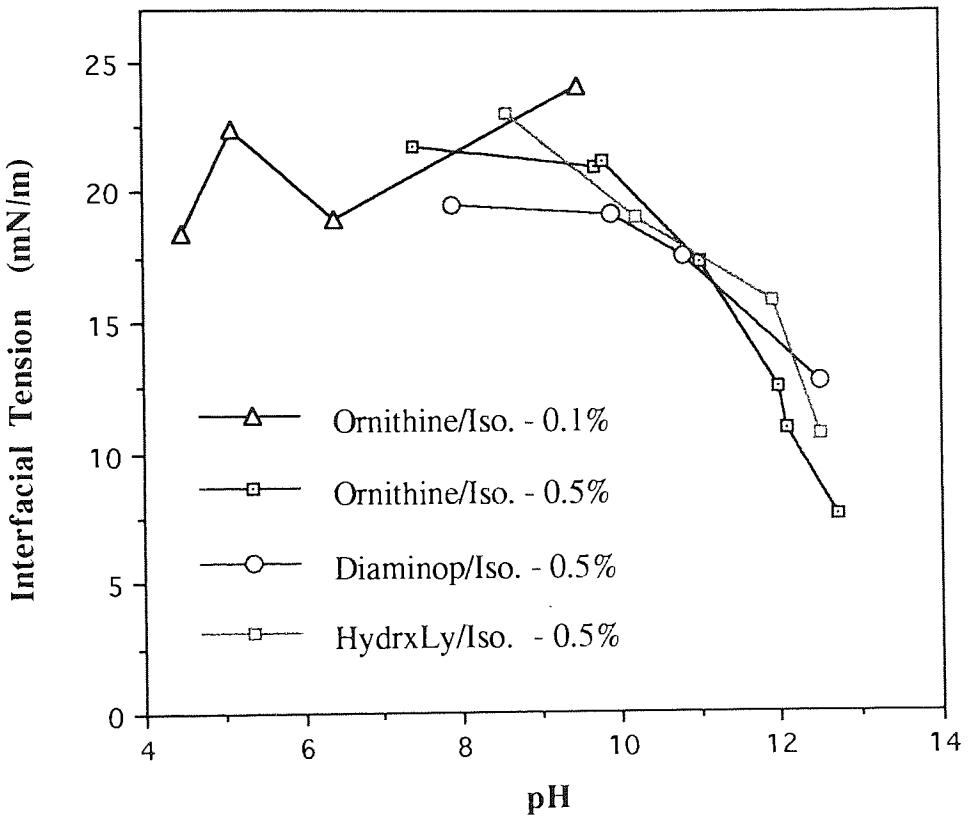
The interfacial tension/pH profiles of 0.5% aqueous solutions of the poly(isophthalamides) based on ornithine, diaminopropionic acid and hydroxylysine were all observed to be similar and are shown graphically in Figure 6.19. In each case the interfacial tension rose from 7.6-12.7 to 19.1-21.8 mN/m as the pH was lowered from 12.5-12.7 to 9.8-10.2, and then remained fairly constant (range 19.5-23.0 mN/m) as the pH was lowered still further to 7.4-8.6. One explanation of these results, may be, that the loss of charge on the carboxylate pendant groups results in chain collapse, forming intramolecular micelles which are not themselves surface active, and therefore, desorb from the interface and partition back into the aqueous phase. An alternative explanation involves a similar mechanism to that proposed for isophthalic acid, where the loss of charge and resultant increase in hydrophobic/hydrophilic balance favours polymer transfer into the organic phase, again leading to desorption from the interface and a rise in interfacial tension.

Figure 6.19. Interfacial tension vs. solution pH - poly(isophthalamides) - 0.5%.



The interfacial behaviour of a 0.1% solution of poly(ornithine isophthalamide) when measured over the pH range from 9.5 to 4.5, exhibited values between 24.1 and 18.4 mN/m, similar to the value of 24.7 mN/m obtained with HPLC grade water. This suggests that the polymer possesses only minimal interfacial activity. The results are shown graphically in Figure 6.20 in comparison to those obtained with the 0.5% poly(isophthalamide) solutions.

Figure 6.20. Interfacial tension vs. solution pH - poly(isophthalamides) - 0.1% & 0.5%.



As revealed by Figure 6.20, a slight fall in interfacial tension of 5 mN/m occurred in the 0.1% solution of poly(ornithine isophthalamide) as the pH was lowered from 9.5 to 6.4, which indicates a rise in interfacial activity, perhaps in response to a further loss of charge and formation of a dumb-bell shaped amphipathic molecule with distinct hydrophobic domains. Lowering of the pH to 4.5 does not indicate a continuation of this trend probably due to a precipitation of the polymer from aqueous solution and its partition into the organic phase.

When considering the results of the poly(isophthalamides) it should be borne in mind that contamination with either 0.05% isophthalic acid, ornithine or possibly other amino acids would result in similar observations to those reported, as described in

section 6.3.3. This would, however, presuppose that the poly(isophthalamide) samples contained some 10% by weight of either unreacted starting material or hydrolysed acid chloride. The latter supposition is an unlikely one, since the extraction procedure involving excess acidic water would be expected to remove all of the unreacted ornithine, whilst the extraction into excess methanol would be expected to remove virtually all of the isophthalic acid.

In conclusion, these interfacial results indicate that the poly(isophthalamides) appear to show some activity, but are less active upon neutralization. This probably arises as a result of the high (1:1) proportion of hydrophobic isophthaloyl residues, to charged carboxylate groups. The polymers, therefore, have a propensity to collapse and form non-surfactant intramolecular micelles. As a result of these interfacial studies the synthetic emphasis, as described in chapter 5, was re-directed towards producing poly(adipamides). In the poly(adipamides) the hydrophilic/hydrophobic balance could, potentially, be more readily controlled so as to favour the formation of an amphipathic polymer conformation with surfactant properties in place of the intramolecular structures exhibited by the poly(isophthalamides).

CHAPTER 7.

ANALYSIS OF PRIMARY AND SECONDARY STRUCTURE IN ALIPHATIC POLYAMIDES BY NMR SPECTROSCOPY

By this art you may contemplate
the variation of the 23 letters...*

* The Anatomy of Melancholy,
Part 2, Sect. II, Mem. IV.[174]

7.1. Introduction and Rationale.

Proton (^{1}H), ^{13}carbon (^{13}C) and proton-^{13}carbon (^{1}H-^{13}C) correlation NMR analyses were used to define the primary structure of poly(lysine ethyl ester adipamide) [PLETESA] base polymer synthesized under low temperature conditions. These studies enabled the resonance assignments to be defined. The assignments could then be used to identify the through bond and through space associations revealed by COSY and NOESY analyses. The latter were then used to define the secondary structure of both the base polymer and the saponified polymer in a polar solvent. The conformation of the base polymer in a non-polar solvent was also investigated.

The ^{1}H and ^{13}C spectra of the PLETESA base, and saponified polymer, synthesized at room temperature were also acquired and compared with those of the polymer synthesized at low temperature, with particular reference, to the identification of any differences in structure which may have resulted from variations in sequence distribution. A quantitative analysis based on the ^{13}C-carbonyl integral values was then conducted to define the efficacy of the saponification process used.

7.2. Primary and Secondary Structure Determination of PLETESA Synthesized at Low Temperature.

7.2.1 ^{1}H-NMR Analysis of PLETESA :

7.2.1.1. Experimental Conditions.

NMR analysis of all samples was conducted in deuterated methanol, unless otherwise stated, using a Bruker AC 300, 300 MHz Spectrometer. All chemical shifts were measured relative to tetramethylsilane which was set as the zero value. Initial experiments defined the proton-NMR spectrum of polymer material synthesized at low temperature (designated PLETESA-HTHT). Both the base polymer and material saponified in 100% ethanol were investigated and the spectral data acquired shown in Table 7.1, examples of the spectra obtained are shown in Figures 7.1 and 7.2, respectively.

Table 7.1. ^{1}H-NMR chemical shifts of low temperature synthesized PLETESA: Base and saponified polymer.

Base Polymer PLETESA - HTHT δH ppm. Split*	Integral value	Saponified Polym. PLETESA - HTHT δH ppm. Split*.	Integral value	Aliphatic CH Assign-ments **
		1.24238		ethanol CH_3
1.16060 trip.	118.2	1.27254	9.48 (comb)	ethyl CH_3
1.31183	91.2	1.39888	21.0	αCCH_2 - γ (1.45)
1.42153 qut. 1.39740	68.0	1.50645	22.2	αCCH_2 - δ (1.70)
1.53064	183.1	1.62017	47.8	..CH_2CH_2..CO
1.67292 qut. 1.69297	45.1	1.82286	11.5	αCCH_2 - β (1.80)
2.09816	80.1	2.19004	22.8	$\alpha CNHCOCH_2$
2.17356	55.0	2.26833	17.8	CH_2CONH
3.04448 trip	72.8	3.15223	17.6	ε CH_2NH (3.02)
3.21278	-	3.2998	-	methanol
		3.65	00.95	ethanol CH_2
4.05221 qut. 4.07884	77.8	4.15 minor qut.	-	ethyl CH_2-O
4.22537 trip.	24.1	4.3	1.38	αCH (4.36)

* Splitting pattern: qut. = quartet and trip. = triplet. In the case of a triplet only the central value is listed and in the case of a quartet the two central values are listed.

** Published δH values for lysine residues within polypeptides are shown in parenthesis.[175]

7.2.1.2. Analysis of ^{1}H-NMR Spectral Data.

The effect of saponification is clearly visible when comparing the spectra acquired from the base polymer and the saponified polymer samples. The δH at 1.16 and 4.05 ppm. correspond to the methyl and methylene groups in the ethyl ester side chains, respectively, and can be seen to be greatly reduced in intensity in the saponified sample, resulting in a reduction of the corresponding integral values at δH 1.27 and 4.15 ppm. However, in the saponified sample, two weak signals are also apparent with δH values of 1.24 and 3.65 ppm. indicating the presence of ethanol. It is likely that ethanol is liberated from the sample as the partially saponified PLETESA continues to saponify in the mildly acid conditions that persist after hydrochloride salt formation.* In both samples, a low intensity δH is present at 3.2-3.3 ppm. indicating the presence of a methyl group, due to methanol contamination of the deuterated solvent. The intensity of the chemical shift noted in this region is far less in the base PLETESA, due to the use of high purity (99.96%) methanol solvent. A strong δH is also present in both samples at 4.8-4.9 ppm. as a result of the hydroxyl groups within residual water, again, present as a contaminant in the deuterated methanol solvent.

The spectral shifts acquired were assigned to their respective chemical groups after conducting a ^{1}H-^{13}C correlation experiment and the assigned ^{1}H-NMR values are shown in Table 7.1.

* Confirmation of the presence of small amounts of ethanol in the saponified sample is also provided in the ^{13}C APT spectrum where two δC emanating from CH_2-O- groupings were noted at 93.5 and 93.7 ppm. indicating the presence of methylene groups within an ethyl chain in two environments, i.e., within an ethyl ester and within ethanol.

Figure 7.1. ^{1}H-NMR of PLETESA - base polymer synthesized at low temperature.

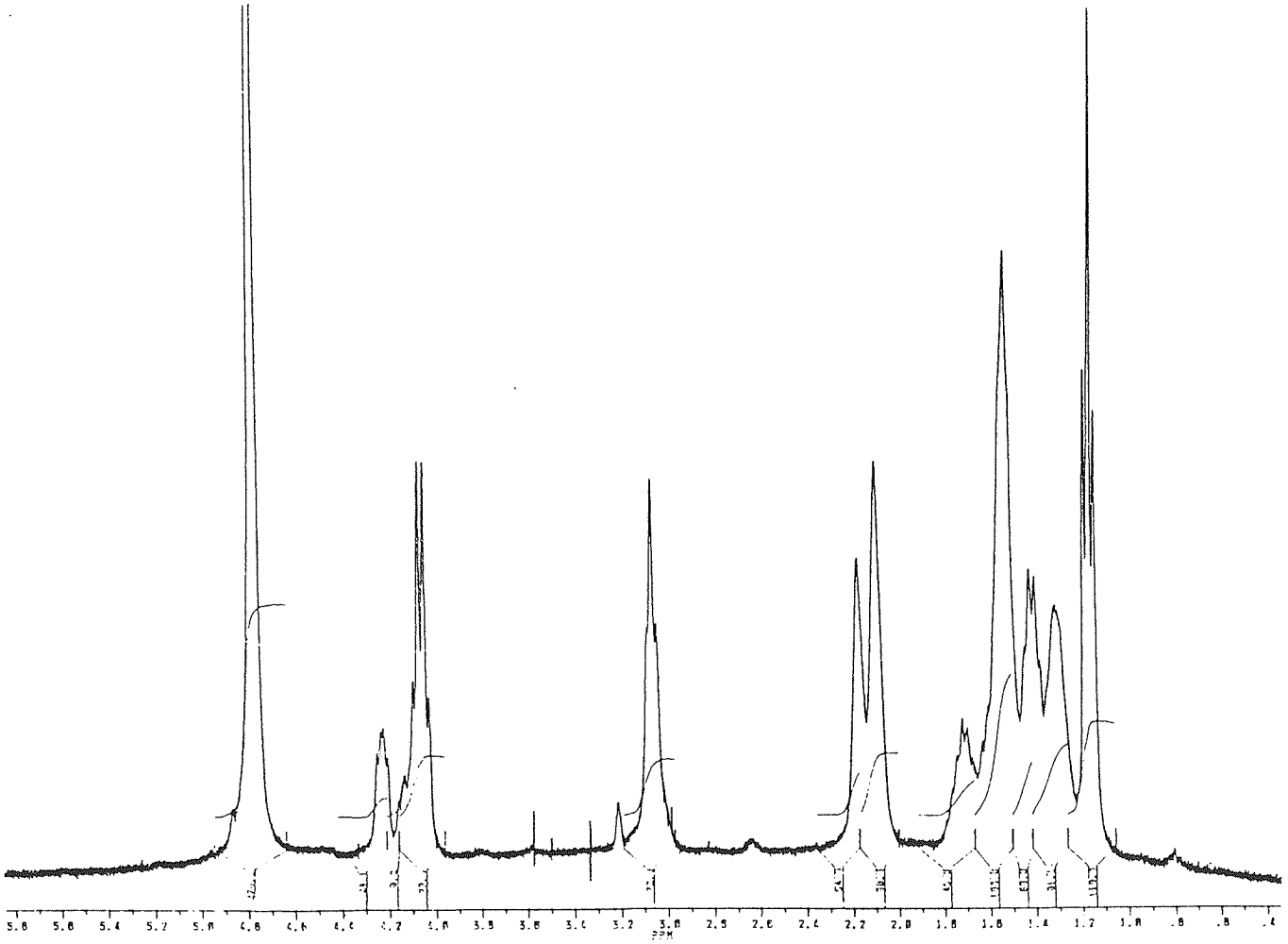
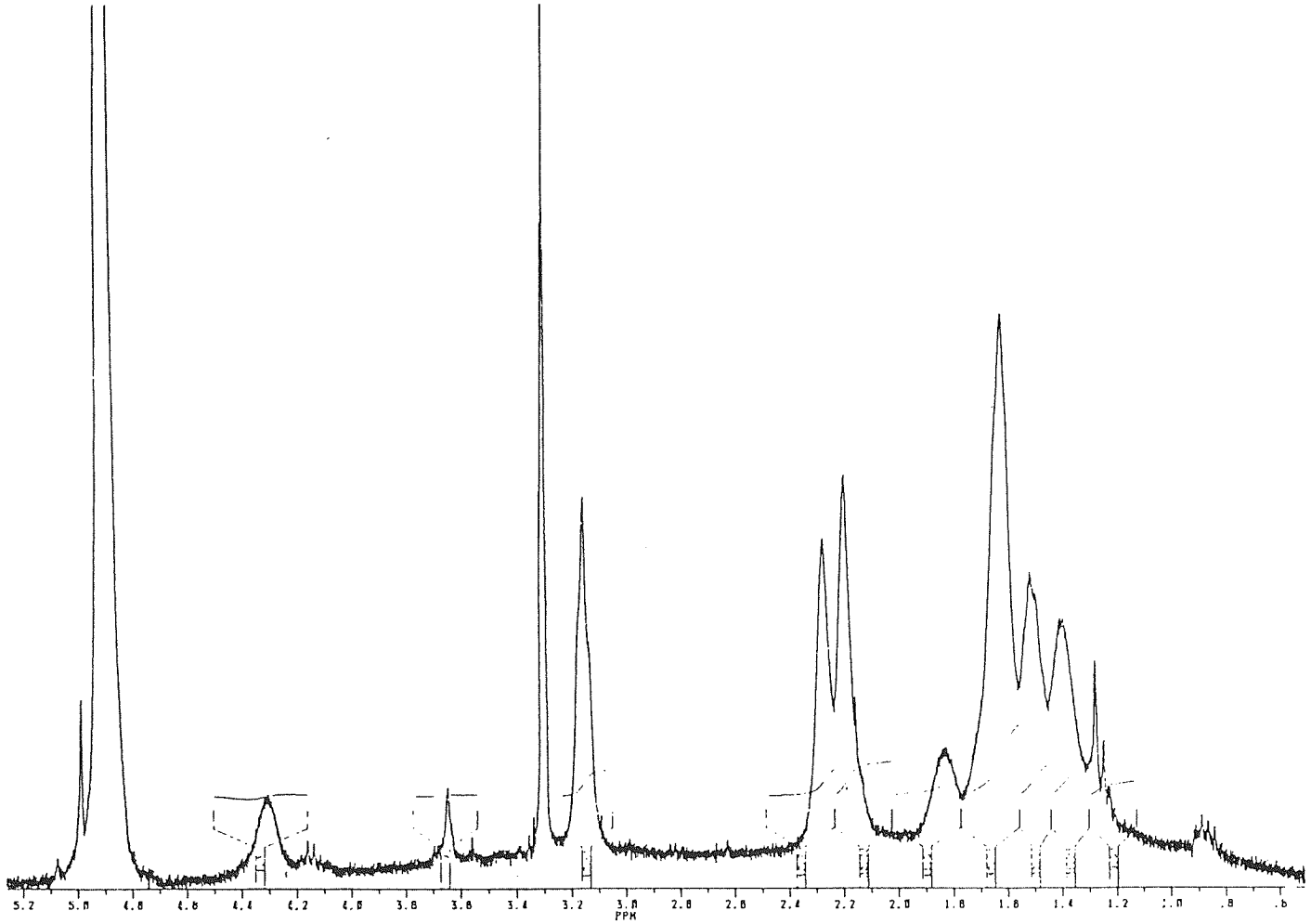


Figure 7.2. ^{1}H-NMR of PLETESA - saponified in 100% ethanol. Synthesized at low temperature.



7.2.2. ^{13}C-NMR Spectral Analysis.

^{13}C-NMR spectra were acquired for saponified PLETESA synthesized, both at low temperature (HTHT), and at room temperature. The chemical shifts recorded are reported in Table 7.2. The attached proton test (APT) and Polarization Enhancement Nurtured During Attached Nucleus Test (PENDANT)[176] were both used to enable the C-H couplings to be resolved into methine and methyl, or methylene and quaternary groups. Examples of the APT and PENDANT spectra are shown in Figures 7.3 and 7.4, respectively.

Table 7.2. ^{13}C-NMR chemical shifts of saponified PLETESA. Using the APT and PENDANT tests.

HTHT Scan N^{o}. 12*	HTHT Scan N^{o}. 10	Room Temp. Scan N^{o}.13*	Room Temp. Scan N^{o}. 11	Room Temp Scan N^{o}. 3 ***
18.393 (-)**		14.571 (-)	14.567 (-) 18.369 (-)	14.582 (-)
24.353	24.352	24.285	24.373	24.287
26.508 26.633	26.453 26.617	26.396 26.557	26.478 26.628	26.359 26.524
30.194	30.001	29.905	30.066	29.792
32.206 33.623	 32.724	31.991 32.139	 32.950	 32.078
 36.789	36.525 36.779	36.315 36.754	36.625 36.785	36.248 36.521
40.004 40.297	 40.163	40.029	 40.202	 40.220
56.146 (-)	65.661 (-) 67.822 (-)	53.518 (-) 53.753 (-)		53.537 (-) 53.780 (-)
58.288		62.240	58.311	62.264

* PENDANT technique used to acquire signals.
** The detection is phased so that methylene and quaternary carbons generate a positive signal while methine and methyl groups generate a negative signal, indicated in the above table by (-)
*** Conducted in acidic conditions.

Figure 7.3. ^{13}C-NMR APT spectrum of PLETESA saponified in 100% ethanol - synthesized at room temperature. Acid conditions (scan 3).

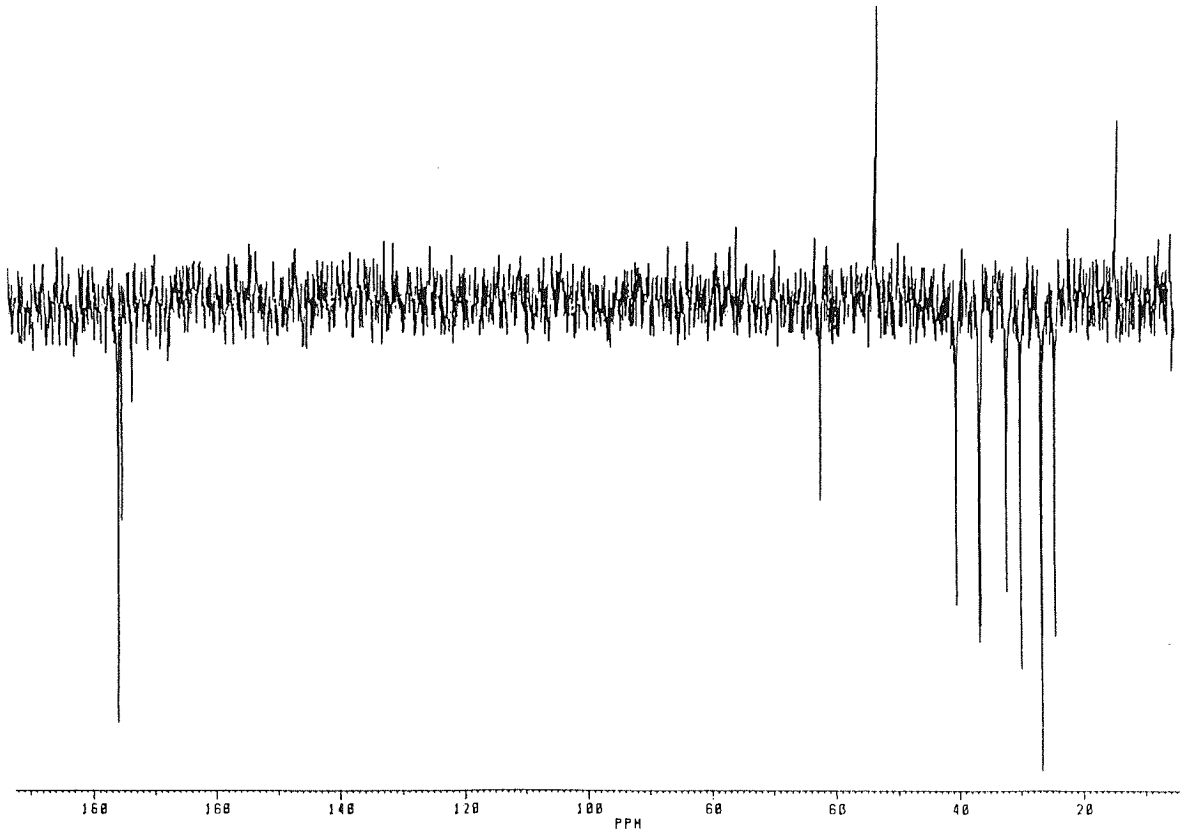
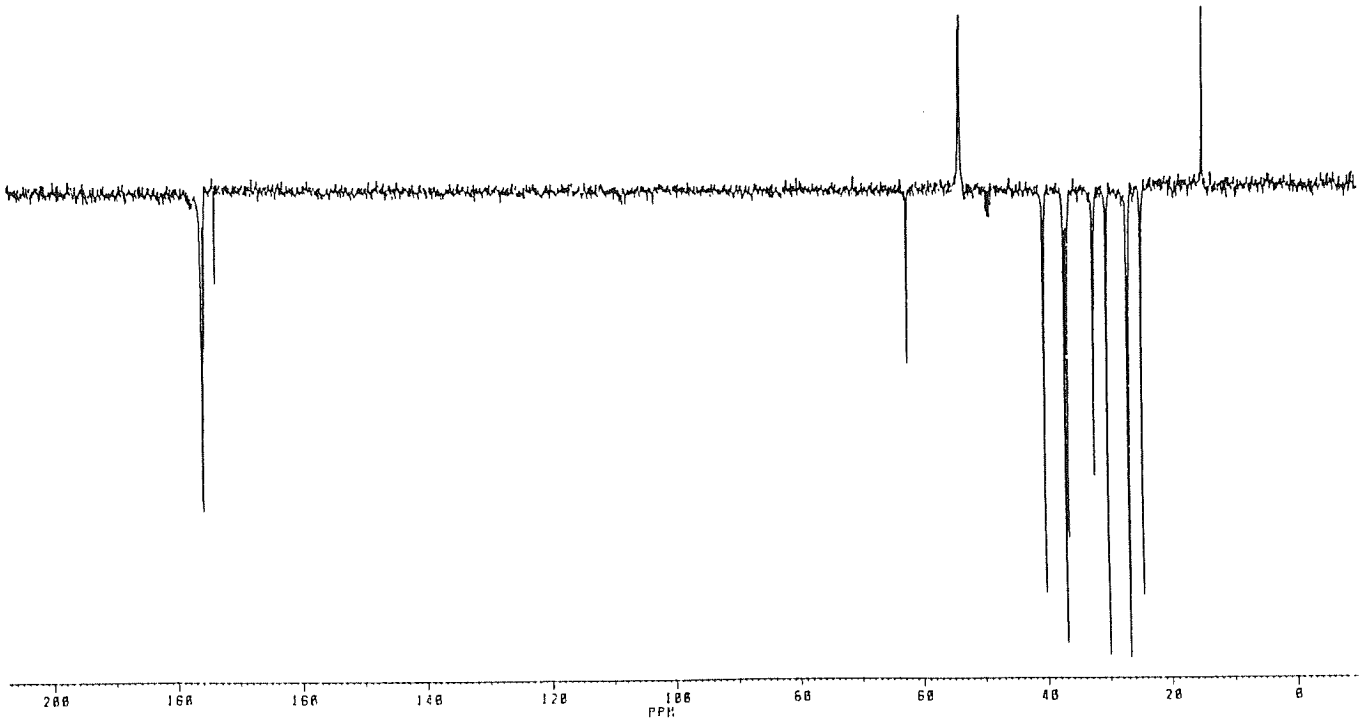


Figure 7.4. ^{13}C-NMR PENDANT spectrum of PLETESA saponified in 100% ethanol - synthesized at room temperature (scan 13).



The δC (chemical shift) values used were calculated using the following estimation of ^{13}C chemical shift within an aliphatic chain, according to equation 7.1.[177]

$$\delta C = -2.3 + \Sigma z \qquad (7.1)$$

where z = substituent constant, values used are those quoted by Macomber[178] *

The negative δC values noted at 14.5 and 18.4 ppm. correspond to the methyl group within the ethyl side chain of PLETESA and also possibly from the methyl group present within ethanol released during degradation of the polymer. These negative δC values correlate to the δH values recorded at 1.24 and 1.27 ppm., respectively, and confirm the presence of ethanol. The negative chemical shift values noted around 53.5-65.6 and 53.8-67.8 ppm. represent the α-carbon methine group, the presence of two methine chemical shifts suggests that the α-methine carbon exists in two environments, as could be expected for methine groups adjacent to saponified and non-saponified groups.

It is evident from Table 7.2 that ten chemical shifts appear to exist in the saponified PLETESA, from material synthesized under both temperature conditions. These are shown in Table 7.3, along with their corresponding calculated values. It can be seen that the observed values agree well with the calculated values and enable the chemical shifts to be assigned with some degree of confidence.

* It should be noted that the -NHCO grouping appears to cause a substituent shift of 29, 11 and -5 ppm. in the α, β, and γ positions, respectively, of the lysine ethyl ester residue, equivalent to those caused by an aliphatic amine group. This is clearly apparent from the negative δC values noted at 53-68 ppm., these almost certainly arise from the methine grouping of the α-carbon atom within the lysine ethyl ester residue, since the only other negative chemical shift would arise from the methyl grouping within the ethyl ester side chain, but in the latter case, the δC value is known from published information[177] to occur in the range of 14-20 ppm. Therefore, the methine δC values noted at 53-68 ppm. can only be accounted for, using the above method of calculation, if the -NHCO substituent has the effect of shifting resonances to a far greater degree than that reported to occur within monomeric amides.[179] Such downfield shifts, to higher frequencies, would indicate a lower level of charge shielding which is, indeed, likely to occur within a polyamide chain upon saponification. Interestingly, the δC values are shifted to higher values in the low temperature synthesized polymer, suggesting a greater degree of deshielding, perhaps as a result of chain coiling. The δC values calculated using these substituent shift values are shown in Table 7.3.

Table 7.3. Aliphatic group assignment of ^{13}C-NMR chemical shift values: Calculated vs. observed values

Assignment of Aliphatic Grouping (Group underlined)	δC Calculated value ppm.	δC Observed value ppm.
-$CH_2CONH\underline{CH_2}$ - ε carbon	41.9	40.0 - 40.2
-$CH_2CONHCH_2\underline{CH_2}$ - δ carbon	33.8	29.8 - 30.2
-$CH_2CONHCH_2CH_2\underline{CH_2}$ - γ carbon	27.7	24.3 - 24.4
-$CH_2CH_2CH_2\underline{CH_2}C\alpha$ -β carbon	35.8	32.0 - 33.6
-$CH_2\underline{CH}$(COOR)NHCO - α carbon	59.9	53.7 - 67.8
-$CH_2\underline{CH}$(COOH)NHCO - α carbon	58.9	53.5 - 65.7
-CH(COOR)$NHCO\underline{CH_2}$-	34.4*	36.2 - 36.6
-CH(COOR)$NHCOCH_2\underline{CH_2}$	24.7*	26.4 - 26.5
-CH(COOR)$NHCOCH_2CH_2\underline{CH_2}$	24.7	26.5 - 26.6
-CH(COOR)$NHCOCH_2CH_2CH_2\underline{CH_2}$	34.4	36.5 - 36.8

* Lower of two similar values, i.e., C_1 and γ positions, see Figure 7.6, due to upfield shift caused by proximity to ester group.

A clear ^{13}C resonance pattern emerges from these studies and is illustrated, diagrammatically, in Figure 7.5, where the intensities of the signals arising from the methyl and methylene groups within the ester pendant groups vary according to the degree of saponification. The δC derived from these spectra agree fairly well with the published aliphatic δC values for adipic acid[180] of 23.97 and 33.31 ppm. However, the observed values are somewhat shifted downfield, compared to those reported in monomeric adipic acid, probably as a result of charge deshielding.

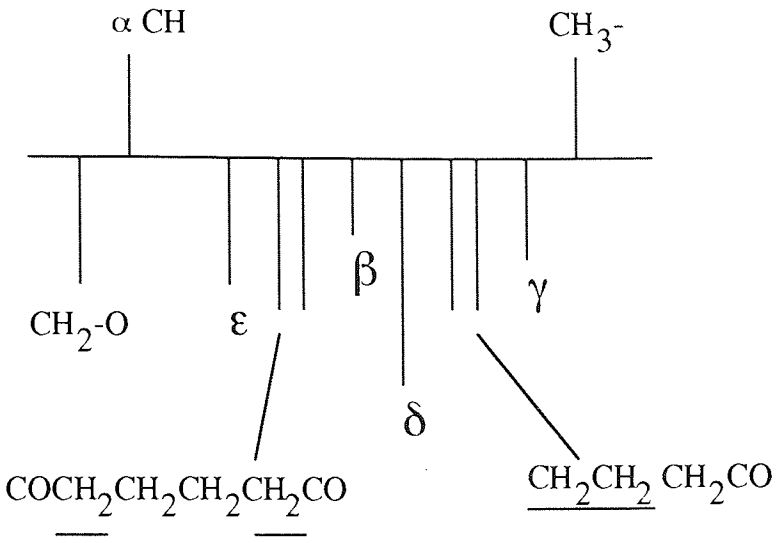


Figure 7.5. Diagrammatic representation of ^{13}C APT or PENDANT spectrum of PLETESA - base polymer.

A more valid comparison is with the δC values reported by Hatfield[181] for Nylon 6 in dilute trifluoroethanol solution. In Nylon 6 each methylene chain is bonded to a carbonyl group and an amide group according to the repeat unit structure: -[-$COCH_2CH_2CH_2CH_2CH_2NH$-]$_n$-. If the carbonyl carbon is designated C_1, then C_2 and C_3 correspond to the adipamide methylene groups of PLETESA, as shown in Figure 7.6.

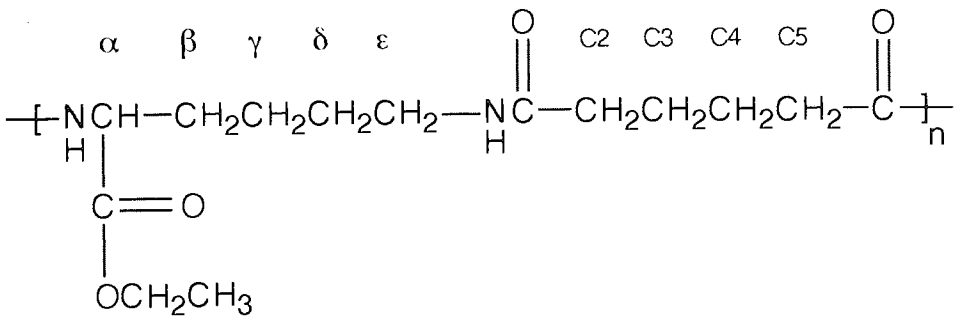


Figure 7.6. PLETESA: Showing relative positions of nominated groups in both the lysine ethyl ester residue and adipamide segment.

Hatfield reports δC values of 37.2 and 26.4 ppm. for C_2 and C_3 respectively. The C_4, C_5 and C_6 methylene groups within Nylon 6 correspond to the γ, δ and ε-carbon atoms of the lysine ethyl ester residue within PLETESA, δC values of 27.1, 29.5 and

40.7 ppm. are reported, respectively, for for these atoms in Nylon 6. Both sets of data are in excellent agreement with the corresponding δC values observed in the saponified sample of PLETESA, as reported in Table 7.2, and confirm that the -NHCO group within a polyamide results in the same substituent shift as that produced by an aliphatic amine group.

7.2.3. ^{1}H-^{13}C-NMR Correlation.

A ^{1}H-^{13}C correlation experiment was then conducted on a sample of saponified PLETESA, synthesized under low temperature conditions. The correlations observed could then be matched with the assignments previously made to define the precise location of the protons. This was crucial for subsequent interpretation of proton-based COSY and NOESY spectra. Table 7.4. shows the results of the ^{1}H-^{13}C correlation experiment carried out.

Table 7.4. ^{1}H-^{13}C-NMR correlation of saponified PLETESA - synthesized at low temperature.

^{1}H Chemical shift ppm.	^{13}C Chemical shift ppm.	Correlated Aliphatic Assignments (Groups underlined)
1.42 (1.45)	25 [24.30]	-CH_2CONHCH_2CH_2<u>CH_2</u> - γ carbon
1.65 *	27 *	-CH(COOR)NHCOCH_2<u>CH_2CH_2</u>
1.55 (1.70)	31 [29.14]	-CH_2CONHCH_2<u>CH_2</u> - δ carbon
2.15	38	-CH(COOR)NHCOCH_2CH_2CH_2<u>CH_2</u>
2.3	37	-CH(COOR)NHCO<u>CH_2</u>-
3.15 (3.02)	41 [41.95]	-CH_2CONH<u>CH_2</u> - ε carbon
1.8 (1.80)**	33 [32.14]*	-NHCH_2CH_2CH_2<u>CH_2</u>Cα -β carbon

* Two similar values the lower of which is assigned to the CH_2 proximate to the ester group as a result of an upfield shift in this position.

** The ^{1}H shift at 1.8 ppm. and the ^{13}C at 33 ppm. do not appear to be correlated with any other groups, but both are weak intensity signals, and therefore, any correlated resonance may be too weak to be detected. Since correlations can be made for all other methylene groups it is probably valid to assume that these two signals correlate with each other.

( ) Published δH for lysine residue in polypeptide chain.[175]

[ ] Published δC for lysine.[180]

The δH values observed in saponified PLETESA are in close agreement with the relative shifts reported for lysine residues within polypeptide chains,[175] the values of which are shown in parenthesis in Table 7.4. The value for the α-methine group within the lysine residue of a polypeptide chain is 4.36 ppm. and this agrees well with the value reported in Table 7.1. However, in the case of a lysine residue within a polypeptide chain, the ε-methylene group is adjacent to an amine, as distinct from the amide positioning found in PLETESA. This provides further evidence that a polymeric amide exerts a similar substituent shift as that exerted by an aliphatic amine.

The δC values observed also correspond to those reported for L-lysine[180] and are shown in Table 7.4 in parenthesis, again, these values confirm that the polymeric -NHCO grouping results in a chemical shift of similar magnitude to that of a monomeric aliphatic amine group.

7.2.4. COSY Analysis.

7.2.4.1. PLETESA - Base Polymer.

The J-J through-bond spin couplings, between covalently linked protons, were investigated by conducting a Correlated Spectroscopy examination or COSY on a sample of saponified PLETESA synthesized at low temperature. The results of the COSY experiment are reported in Table 7.5 and the COSY spectrum is shown in Fig 7.5, where the 1-dimensional spectrum is related to the diagonal elements of the 2-dimensional spectrum by direct projection, and the off-diagonal cross peaks represent couplings between two different protons.[177]

Table 7.5. ^{1}H-NMR Correlation Spectroscopy (COSY) of saponified PLETESA - synthesized at low temperature.

^{1}H Chemical shift ppm.	^{1}H Chemical shift ppm.	J-J Bond Correlations
1.65	2.2 and 2.3	$NHCOCH_2CH_2CH_2$ *
1.5	3.15	δ with ε
1.8	4.35	β with α
1.25	4.15	CH_3-CH_2 ethyl ester

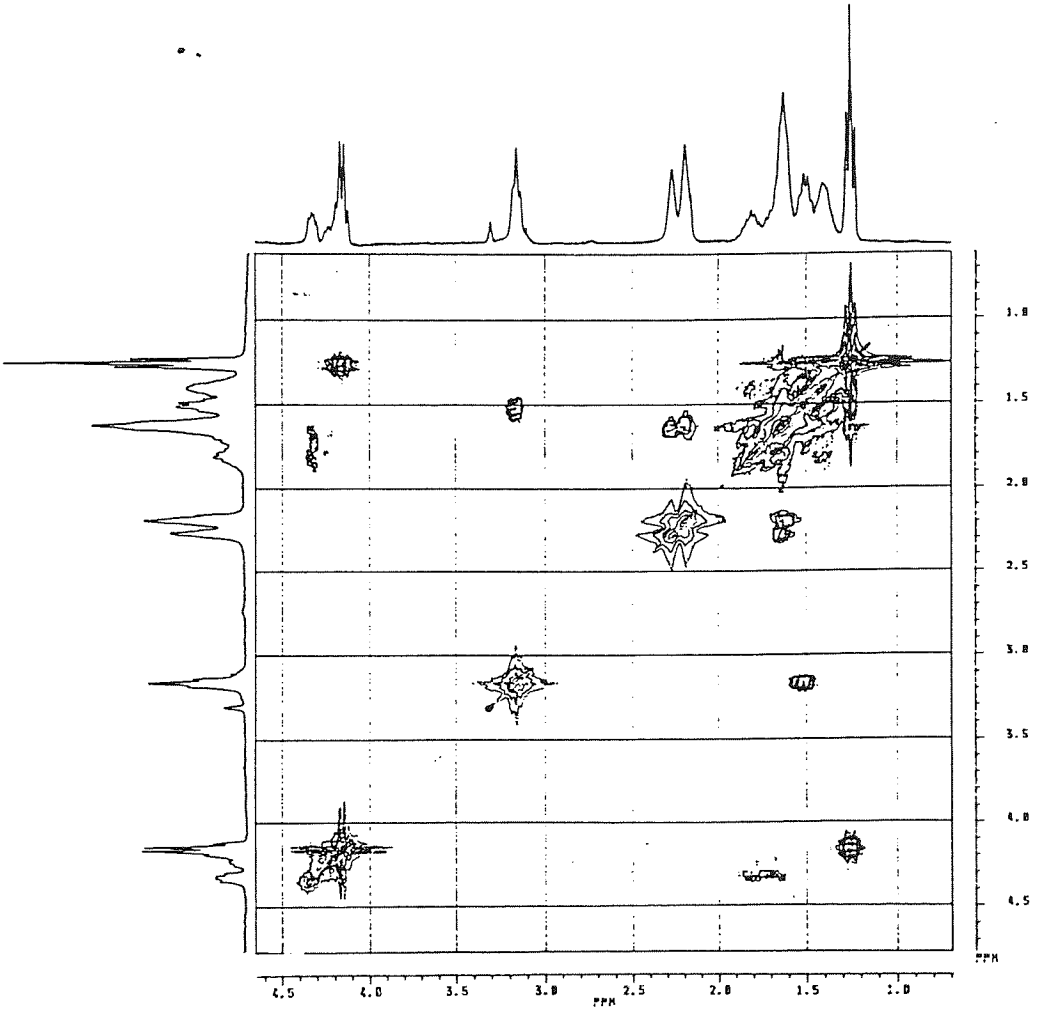
* The two CH_2 groups adjacent to carbonyl groups with δC at 2.2 and 2.3 ppm. both show bonding to the centrally placed CH_2 groups and the latter both exhibit virtually identical δC at 1.65 ppm.

By using the correlations reported in Table 7.5 for J-J through bond coupling, as revealed by COSY, the spatial orientation of various groups covalently bonded to one another could be defined. These correlations confirm the structure shown in Figure 7.6, although, through bond correlations between aliphatic groups in the β and γ position and in the γ and δ positions could also be expected to occur they were not observed. However, such correlations are likely to be obscured by the strong signals apparent along the diagonal axis of the COSY spectra, about which these resonances would be expected to fall as can be from Figure 7.7.

7.2.4.2. PLETESA - Saponified Polymer.

A COSY spectrum was also acquired for the saponified polymer, although, in this case a double quantum filtered, phase sensitive, technique was used to remove any singlet peaks emanating from solvent impurities, and thereby, allowed sample resonances to be optimised.

Figure 7.7. COSY spectrum of PLETESA - base polymer in methanolic solution. Low temperature synthesized.



The COSY spectrum obtained for the saponified polymer was similar to that of the base polymer, with the exception of an additional strong correlation between δH 1.15 and 3.65 ppm., arising from through bond couplings between methyl and ethyl groups within the liberated ethanol. Thus, confirming the presence of the latter, which was also demonstrated in the proton and ^{13}C spectra of the saponified polymer. A correlation between the α and β-carbon atoms within the polymer chain appears to be lacking in the saponified material, possibly because, the experiment was conducted in analar grade, rather than high purity deuterated solvent, this resulted in a reduction of signal intensity and appeared to adversely affect the resonances of these two groups in particular. It would, therefore, be surprising if any correlation were observed.

7.2.5. NOESY Analysis.

Through space resonant effects were investigated by conducting a Nuclear Overhauser Enhanced Spectroscopy examination or NOESY on samples of PLETESA. This technique observes the influence that a particular irradiated nucleus has upon the relaxation of neighbouring nuclei within 2-4 angstroms. NOESY spectra are expressed as 2-dimensional spectra in a similar manner to that of COSY spectra, but in the case of the NOESY spectra, the off-diagonal cross peaks represent couplings between two different protons through space.[177]

7.2.5.1 NOESY Analysis of PLETESA - Base Polymer Conducted in a Polar Solvent.

A sample of unsaponified, PLETESA base polymer, synthesized at low temperature was investigated by NOESY. The experiment was conducted in 99.96% deuterated methanol to minimise any bonding effects between the molecule and contaminant water molecules within the solvent. A mixing time of 200 milliseconds was adopted, as used by Basso *et al.*[144] for investigating the secondary structure of the polypeptide melittin in methanolic solution. A 5 second relaxation delay was also found to give optimal signal correlations. The resonances arising from the solvent were selectively gated off to expose the NOE signals generated by the polymer. The results of the NOESY experiment are shown in Table 7.6 and the spectrum acquired is shown in Figure 7.8.

Figure 7.8. NOESY spectrum of PLETESA - base polymer in methanolic solution. Low temperature synthesized.

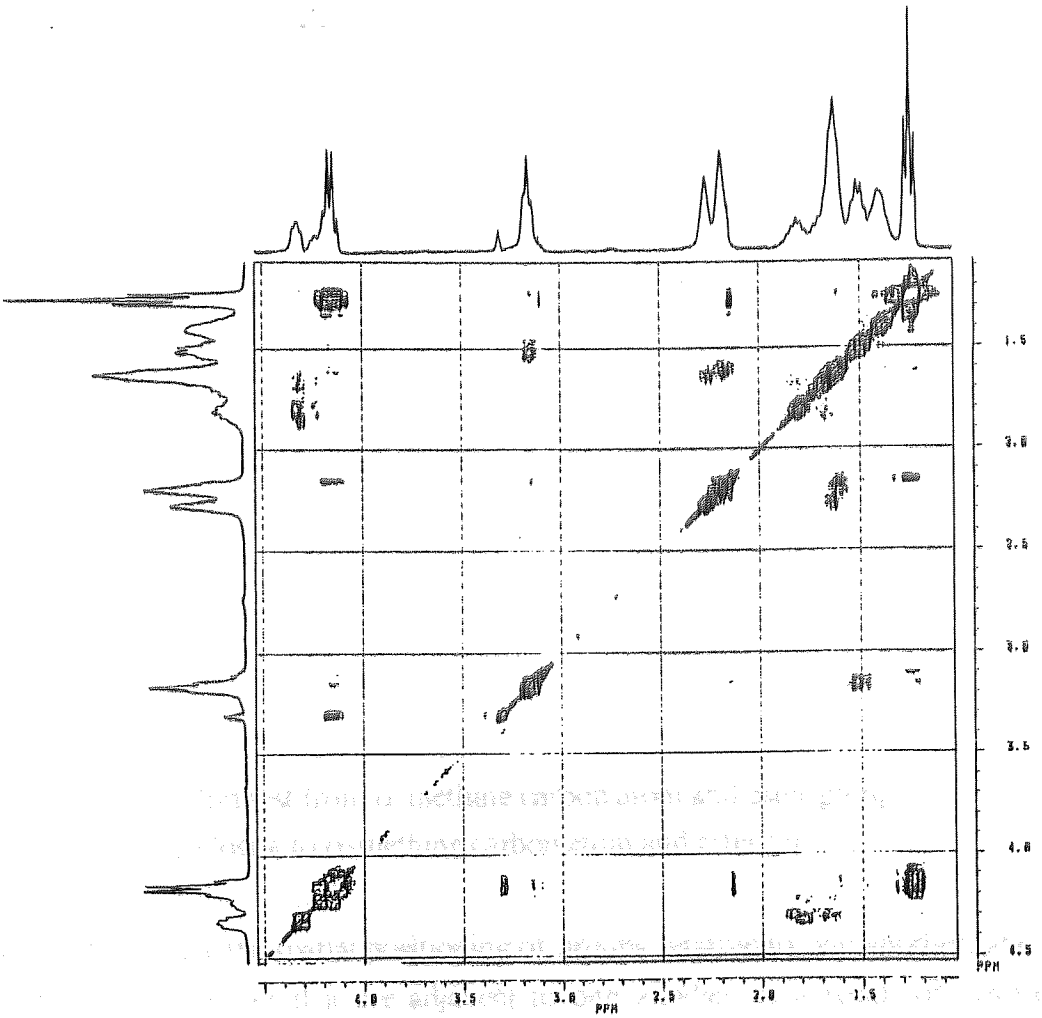


Table 7.6. Nuclear Overhauser enhanced spectroscopy (NOESY) of PLETESA - base polymer in a polar solvent. Low temperature synthesized.

δH ppm.	δH ppm.	Proximate Groups	Signal strength
1.25	2.15	Ethyl chain CH_3 & $\underline{CH_2}CO$	
1.25	3.15	Ethyl chain CH_3 & $\underline{CH_2}NH$	weak
1.25	4.15	Identified in COSY spectrum	
1.5	3.15	Identified in COSY spectrum	
1.65	2.2 & 2.3	Identified in COSY spectrum	
1.8	4.35	Identified in COSY spectrum	
2.15	4.15	$\underline{CH_2}CO$ & CH_2 ethyl chain	
3.15	4.15	$\underline{CH_2}NH$ & CH_2 ethyl chain	weak
3.3	4.15	$\underline{CH_3}OH$ & CH_2 ethyl chain	
1.65	1.25	$COCH_2\underline{CH_2}\underline{CH_2}$* & CH_3 ethyl	very weak
2.15	3.15	$\underline{CH_2}CO$ & $\underline{CH_2}NH$	very weak
4.15	1.62	CH_2 ethyl & $COCH_2\underline{CH_2}\underline{CH_2}$ **	very weak

* Methylene group furthest from α-methine carbon atom and ester group.

** Methylene group closest to α-methine carbon atom and ester group.

The results indicate the spatial positioning of groups relative to one another, and as such, include those groups that are adjacent to one another as a result of covalent bonding, as indicated in the COSY spectrum, and also those groups adjacent to one another through space, as a result of the secondary structure adopted by the molecule in methanolic solution.

In the NOESY analysis presented, in Table 7.6 the strength of the bonding has been estimated from the intensity of the spectral correlation, however, this could be more precisely defined by using a technique known as Differential NOE, where the proton spectrum is quantified and the inter-group association distances defined.

7.2.5.2. Interpretation of NOESY Analysis of PLETESA - Base Polymer Conducted in a Polar Solvent.

The through space associations or dipolar couplings identified in the NOESY spectrum can all be accounted for by the proposed structure illustrated in Figures 7.9 and 7.10. In this structure a single copolymer unit of poly(lysine ethyl ester adipamide) forms a single turn of a helical coil, so as to contain the ethyl ester pendant chain entirely within the coil, the amide carbonyl and hydrogen groups are then exposed to the methanolic solvent phase. The through space association noted between the methyl groups of the methanol contaminant and the methylene groups of the pendant ethyl chains may be arise because, the methanolic hydroxyl groups are hydrogen bonded to carbonyl groups of the polyamide backbone and the latter are in close proximity to the ethyl ester side chains.

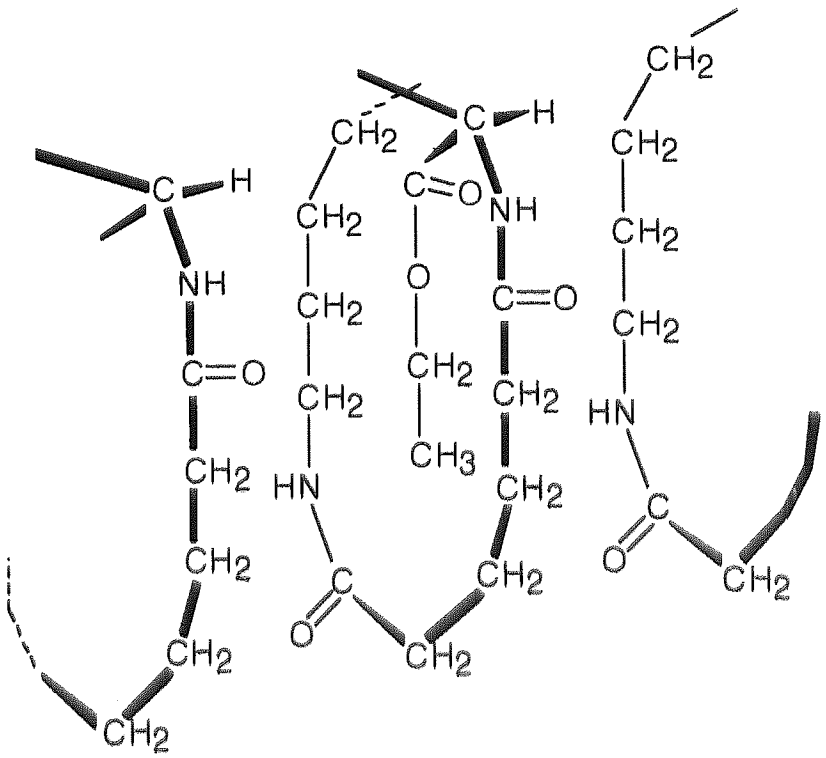


Figure 7.9. Proposed helical structure of PLETESA - base polymer. Dissolved in CD_3OD from NOESY data. Ethyl ester groups are shown within the hydrophobic core

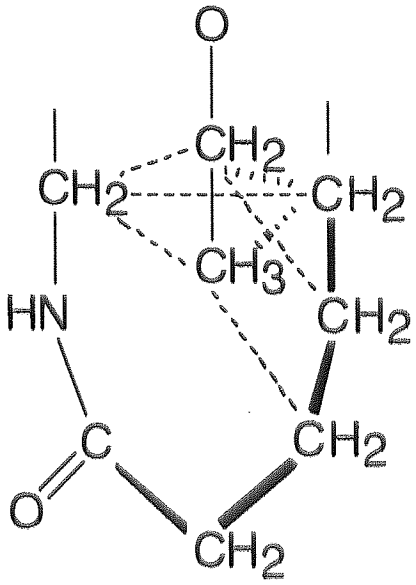


Figure 7.10. Segment of PLETESA - base polymer, showing through space associations revealed by NOESY analysis and the likely conformation adopted. Dashed lines indicate a strong association and dotted lines indicate weak or very weak associations.

This amphipathic coiled structure in which the hydrophobic groups are internalised may account for the curious solvation behaviour of PLETESA; a hydrophobically substituted polyamide that dissolves readily in polar solvents such as water and methanol and can also be slowly solvated in non-polar solvents such as chloroform and methylene chloride. The polymer may be soluble in solvents over a wide polarity range because, it inverts its coiled structure to either expose or internalise the hydrophobic ethyl ester pendant groups, depending upon the polarity of the surrounding environment. Such solvation behaviour is particularly curious when it is considered that related polypeptides, such as the homopolymers of leucine, valine, isoleucine and alanine, that also possess a polyamide structure with pendant aliphatic groups, are completely insoluble in water.[182]

It should be noted, however, that the 'coiled' PLETESA produced directly upon precipitation from the reaction medium is readily soluble in polar solvents such as water and methanol but only dissolves very slowly in non-polar solvents such as chloroform, dichloromethane and benzene. This behaviour is most likely to result from the adoption of a secondary structure such as that shown in Figure 7.9, in which the polar outer 'shell' of the coil effectively acts to prevent interactions between the non-polar solvent molecules and ethyl ester side chains buried within the centre of the coil. Ultimately

random molecular movements would be expected expose the ethyl ester side chains to the non-polar solvent and result in solvation of the polymer.

It is noteworthy, that Katsarva *et al.*[40] have proposed that lysine ethyl ester-based polyurethanes may be used as biodegradable carriers to immobilise labile enzymes and on this basis it can be assumed that the authors' material is not readily solvated in aqueous conditions. In the case of PLETESA, Katsarava[39] clearly states that the polymer does not dissolve in water or organic solvents except in the presence of a small fraction of either methanol or DMF.

The disparity in physical properties between the PLETESA described herein and that described by Katsarava may arise because, the latter polymeric material was synthesized by solution polymerization in hexamethylphosphoric triamide, acetonitrile or dichloroethane and forms mainly low molecular weight material. When an alternative synthetic procedure involving transesterification was adopted,[39] and higher molecular weight PLETESA formed, this material was reported to be soluble in cold but not hot water. The author also reported that this material was optically active. The studies of PLETESA secondary structure presented herein and illustrated in Figure 7.9 would suggest that such solvation behaviour may arise because the polymer adopts a coiled structure at low temperatures and a random structure as the temperature is raised to around 40°C, so exposing the hydrophobic groups to the solvent, and rendering the polymer insoluble. A converse argument can be applied to explain the solvating effect of added polar solvents, as noted by Katsarava, where the polar solvents induce coil formation and render the polymer soluble in water and certain organic solvents.

7.2.5.3. NOESY Analysis of PLETESA - Saponified Polymer, Conducted in a Polar Solvent.

A NOESY experiment was also conducted on a sample of saponified PLETESA, synthesized at low temperature. The results are shown in Table 7.7.

Table 7.7. Nuclear Overhauser enhanced spectroscopy of saponified PLETESA - in a Polar Solvent. Low temperature synthesized.

δH ppm.	δH ppm.	Proximate Groups	Signal strength
1.15	3.6	CH_3 & CH_2O of ethanol	strong
1.2	2.2 & 2.3	CH_3 ethanol & $CO\underline{CH_2}$	weak
1.35	2.2 & 2.3	$NHCH_2CH_2\underline{CH_2}$ & $CO\underline{CH_2}$	weak
1.5	3.15	Identified in COSY spectrum	weak
1.65	3.15	$COCH_2CH_2\underline{CH_2}$ & $\underline{CH_2}NH$	strong
2.2	3.15	$CO\underline{CH_2}$ & $\underline{CH_2}NH$	very weak
2.2	3.3	$CO\underline{CH_2}$ & $\underline{CH_3}OH$	weak

The results shown in Table 7.7 indicate that there are no associations between the ethyl ester aliphatic groups of the pendant chain and other parts of the polymer chain and only a weak or very weak association between the γ CH_2 group and the $\underline{CH_2}CO$ group, and also between the $\underline{CH_2}NH$ and both the mid and end point aliphatic adipamide groups, i.e., C_3/C_4 and C_2 (see Figure 7.6 for explanation of assignments). Ethanol also appears to be present along with a methanol contaminant, as previously identified in the ^1H and ^{13}C spectra, and both are proximate to the $\underline{CH_2}CO$ group, presumably as a result of hydrogen bonding between the alcoholic hydroxyl groups and the polymeric carbonyl groups. The level of methanol impurity was somewhat higher due to the use of analar grade deuterated solvent in this experiment.

These results suggest that the chain is not strongly held in a precise conformation, as would appear to be the case for the unsaponified base polymer. It may, however, adopt a loosely coiled or folded structure in which the aliphatic segments are folded back on one another so as to position the amide groups at the apices of the folds and expose them to the polar solvent. This would also minimise the intrusion of the hydrophobic aliphatic groups into the polar environment, and as such, would be energetically favoured. A representation of the proposed structure is shown in Figure 7.11.

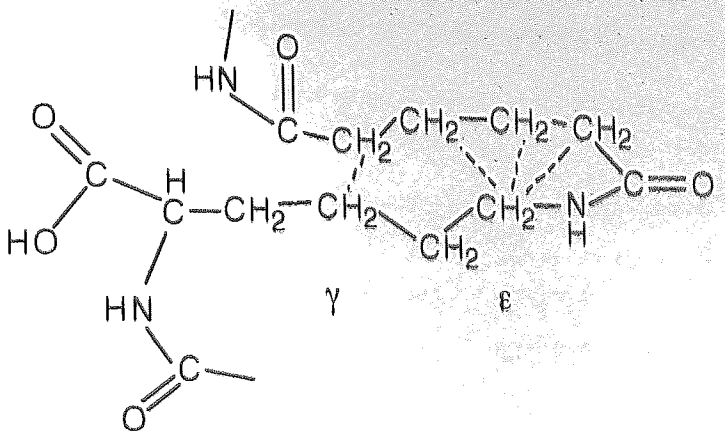


Figure 7.11. A possible secondary structure of PLETESA synthesized at low temperature and saponified in 100% ethanol. Illustrating the loosely folded chain segments with hydrophilic groups exposed to the polar solvent. Dotted lines show weak or very weak associations revealed by NOESY analysis.

7.2.5.4. NOESY Analysis of PLETESA - Base Polymer Conducted in a Non-polar Solvent. Low Temperature Synthesized.

To test the proposed hypothesis that PLETESA base polymer forms a coiled intramolecular micelle in polar solvents, its secondary structure was further investigated using a non-polar solvent system. If the proposed molecular model was correct then in a non-polar solvent PLETESA could be expected to invert its structure to expose the hydrophobic ethyl ester side chains, and thereby, lose its coiled structure and the related NOESY through space associations.

A sample of PLETESA base polymer was dissolved in 0.5mls of deuterated methanol (99.96%) and then added to 1.5mls of deuterated dichloromethane. The presence of excess dichloromethane markedly lowered the polarity of the solvent medium. A proton, NOESY and ^{13}C PENDANT spectra were then acquired and the results of the NOESY experiment are shown in Table 7.8.

Table 7.8. Nuclear Overhauser enhanced spectroscopy of PLETESA - base polymer in a non-polar solvent. Low temperature synthesized.

δH ppm.	δH ppm.	Proximate Groups	Signal strength
1.15	3.6	CH_3 & CH_2 of ethanol	
1.25	4.15	Identified in COSY spectrum	
1.5	3.15	Identified in COSY spectrum	
1.60	2.2 & 2.3	Identified in COSY spectrum	
1.8	4.35	Identified in COSY spectrum	
1.25	4.4	Ethyl chain CH_3 & CH_3OH	very weak
3.6	4.15	CH_2 of ethanol & CH_2 ethyl chain	very weak
4.15	4.4	CH_2 ethyl chain & CH_3OH	very weak
4.15	5.4	CH_2 ethyl chain & CH_2Cl_2	very weak

* Furthest from α-methine carbon atom and ester group.

** Closest to α-methine carbon atom and ester group.

The proton spectrum was found to be identical to that obtained for the polymer when dissolved in methanol, with the exception of a very small quantity of ethanol released as a result of slow saponification. Indicating that the primary structure of the polymer was virtually identical in both solvent systems, with the exception that 0.37% of the ethyl ester groups had undergone saponification in the non-polar solvent. Saponified polymer was also identified in the ^{13}C PENDANT spectrum by the presence of a small additional peak at 174.105 ppm. indicative of a carboxylic acid carbonyl chemical shift, although the amount could not be quantified.

The NOESY spectrum demonstrates that all of the through space bonds resulting from the coiled structure, in the polar medium, were lost as the polarity of the solvent was reduced by the addition of dichloromethane. The remaining associations were either those identified in the COSY spectrum, where atoms are adjacent to one another by virtue of covalent bonding, or by very weak associations of the ethyl ester side chains with components of the solvent. An association was also noted between the methyl and methylene groups of the liberated ethanol identified in the proton spectrum.

These observations support the hypothesis that in non-polar conditions the coiled structure 'springs' open to expose the hydrophobic ester side chains to the solvent. The exposed side chains may be more prone to hydrolysis, by reacting with small amounts of residual water present in the solvent, thus accounting for the small percentage of saponification noted in the non-polar solvent. This compares to the situation in methanol, where no saponification of PLETESA-base polymer occurred, perhaps because, the ester groups were enclosed within the hydrophobic centre of the polymer coil, and were therefore, resistant to hydrolysis by being effectively partitioned from the solvent medium.

These comparative studies of secondary structure reported on PLETESA free base in polar and non-polar solvent, and on partially saponified PLETESA in a polar solvent, suggest that a non-polar medium has a much greater influence on polymer conformation than the degree of ionization. Although, the precise level of ionization of the saponified polymer cannot be defined in methanolic conditions, it does not appear to form such a compact structure as that observed in the base polymer. This observation tends to confirm that hydrophobic forces are the primary determinant in defining polymer structure in polar solutions.

7.3. Primary Structure Determination of PLETESA Synthesized at Room Temperature.

7.3.1. ^{1}H-NMR Analysis of PLETESA - Saponified, Synthesized at Room Temperature.

To investigate the structure of the saponified PLETESA synthesized at room temperature, a ^{1}H-NMR spectrum of a sample of the polymer dissolved in deuterated methanol was acquired. The results are shown in Table 7.9. The solution was acidified by the addition of hydrochloric acid and the experiment repeated to observe the effect of deionizing the pendant carboxylate groups.

The δH values of the saponified PLETESA synthesized at room temperature are similar to those recorded with the saponified PLETESA synthesized at low temperatures and reported in Table 7.1. Analysis of the integral values enables the stoichiometric ratios to be roughly defined, although not as precisely as is possible for a monomeric compound because, of the overlap of spectral resonances that occurs in the spectra of polymer molecules. The relative proportions of methylene groups in the C_2 and C_5 position of the adipamide grouping are roughly equivalent to those in the C_3 and C_4 positions, which is expected for the proposed structure shown in Figure 7.6. The proportions of α-carbon methine to ε-methylene groups within the lysine ethyl ester residue of the polymer are also approximately equivalent to one another, and their combined values are equivalent to the combined totals for the adipamide methylene groups, confirming a 1:1 ratio for the lysine ethyl ester residue to the adipamide sequence. There is also a 1:1 ratio of methyl to methylene groups in the ester side chain as expected for an ethyl grouping. The ratio of ester methylene groups to α-carbon methine groups is approximately 2:3 indicating that the polymer is 66% esterified. Overall these data provide confirmatory evidence of the structure proposed for partially saponified PLETESA.

Acidification resulted in a change of the NH doublet at 7.96-8.19 ppm. to a singlet peak at 8.23 ppm. This suggests that the environment of the amide bond changes as the polymer is neutralised and supports the concept that a change in molecular shape occurs as the polymer chain is extended by charge repulsion.

Table 7.9. ^{1}H-NMR chemical shifts of PLETESA - saponified material. Synthesized at room temperature.

Saponified/Basic PLETESA/Rm. Tp. δH ppm. Split*	Integral	Saponified/Acidic PLETESA/Rm. Tp. δH ppm. Split*,	Aliphatic CH Assignment
1.23090 trip.	30.0	1.22954 trip.	ethyl CH_3
1.4572	76.6	1.39322	αCCH_2 - γ
1.49519 1.51292	87.5	1.49141 1.51116	$aCCH_2$ - δ
1.62102	226.2	1.61358	..CH_2CH_2..CO
1.82206 1.83628	52.7	1.82495 1.84835	αCCH_2 - β
2.19136 2.27034	183.2	2.21460 2.26759	αCNHCOCH_2 CH_2CONH
3.16233	87.1	3.16575	ε CH_2NH
3.30440 sept.	16.6	3.28781	methanol
4.13914 qut. 4.16189	25.7	4.12282 qut. 4.14620	ethyl CH_2-O
4.33448 trip.	45.6	4.31800 trip.	αCH
7.96302 8.19247	9.4 11.8	8.22631	NH

* Splitting pattern: qut. = quartet, trip. = triplet and sept. = septet, in the case of a triplet or septet only the central value is listed and in the case of a quartet the two central values are listed.

7.3.2. ^{13}C-NMR Analysis of Aliphatic Groups.

^{13}Carbon-NMR analysis was conducted on PLETESA-base polymer synthesized at room temperature and on polymer samples saponified in 100, 95, 90 and 75% ethanol (i.e., in 0, 5, 10 & 25% methanol). The aliphatic shifts are shown in Table 7.10. and the carbonyl shifs and their associated integral values are shown in Tables 7.11 a & b and 7.12, respectively.

Table 7.10. ^{13}C-NMR aliphatic chemical shifts of PLETESA - base polymer and polymer saponified in 100-75% ethanol.

Base Polymer δC ppm.	Saponified in Ethanol/Methanol Solution δC ppm.			
	100%	95/5%	90/10%	75/25% *
14.585	14.561	14.579	14.430	
24.318	24.275	24.318	24.016	24.076
26.455 trip.	26.384 26.545	26.419 26.587	26.142 26.328	26.180 26.363
29.961	29.888	29.923	29.551	29.591
32.041	32.115	32.153	31.665 31.865	31.695 31.892
36.284 36.784	36.293 36.736	36.330 36.771	36.066 36.567	36.160 36.597
40.013	40.010	40.049	40.024	40.094
53.777	53.501 53.737	53.541 53.776	53.671	53.628 53.710
62.242	62.226	62.266	62.430	

* NOE gated off to remove hydrogen bonding effects.

7.3.3. ^{13}C-NMR Analysis of PLETESA - Saponified, Synthesized at Room Temperature and Low Temperature.

^{13}C-NMR cannot be used as a quantitative technique unless the NOE is gated off and the nuclei under investigation are allowed to fully relax, to enable all of the emitted signal to be acquired. In order to achieve this, the relaxation times must firstly be defined. Hence, to assess the relative proportions of the carbonyl groups present in the PLETESA polymers under investigation, a series of experiments were undertaken to define these values and a typical spectrum is shown in Figure 7.12. The corresponding δC values and their associated integral values are shown in Table 7.11 a & b.

Figure 7.12. ^{13}C-NMR of PLETESA - saponified in 100% ethanol. Room temperature synthesized.

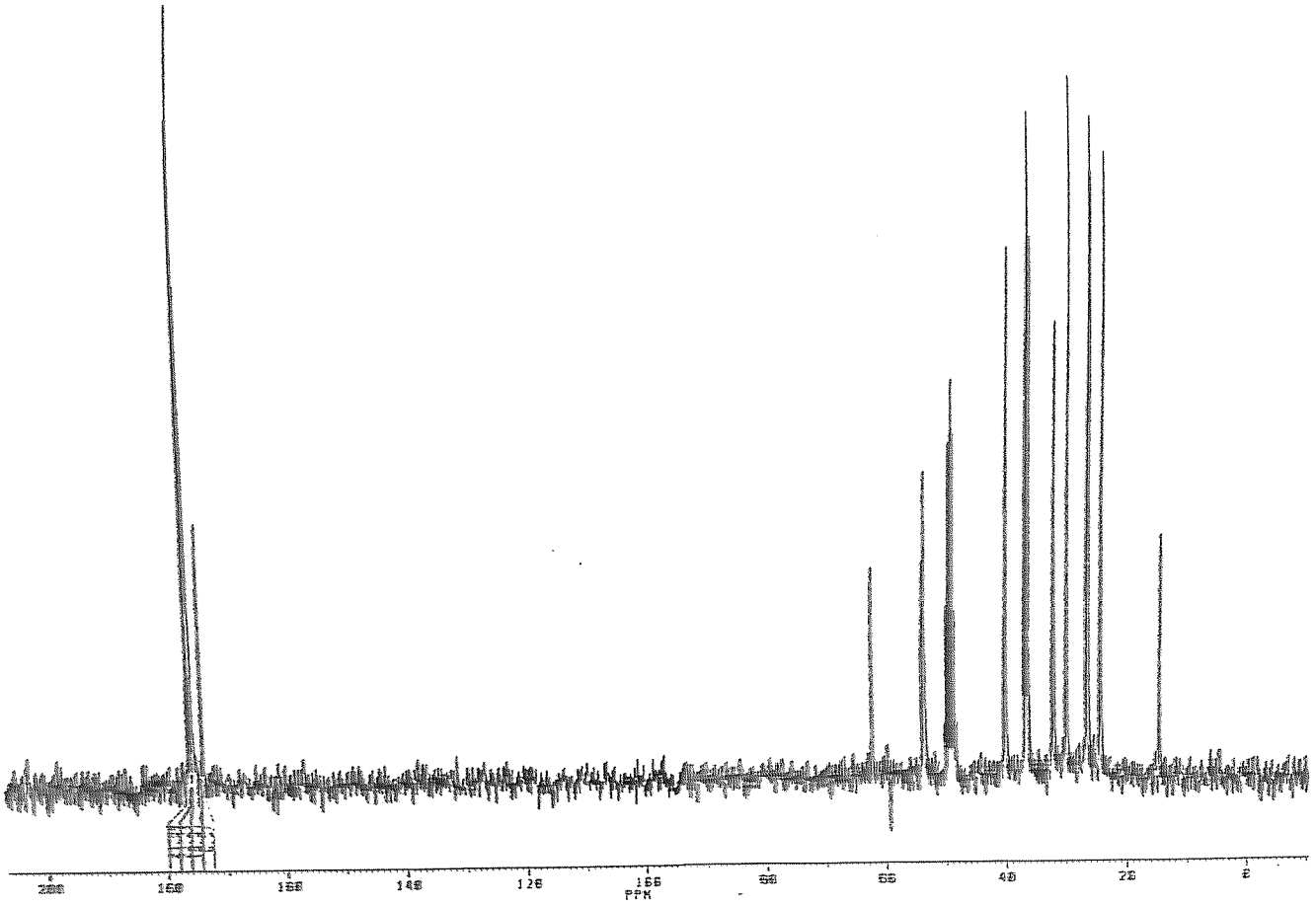


Table 7.11 (a). ^{13}C-NMR carbonyl chemical shifts * of PLETESA - base polymer and polymer saponified in 100% ethanol synthesized at room temperature. Compared to PLETESA saponified in 100% ethanol and synthesized at low temperature.

Room Temperature Synthesized PLETESA				Low Temp. PLETESA	
Base Polymer		Saponified in 100% Ethanol [a]		Saponified in 100% Ethanol [b]	
δC ppm.	Integral	δC ppm.	Integral	δC ppm.	Integral
173.770	1027.395	173.734	6.57	174.349	17.41
		175.471	19.94	174.986	79.03
175.697	1003.266	175.723	22.23	175.633	100.48
175.948	960.503	175.892	24.08	176.707	44.83
				179.363	62.90
δC ppm.	T1** secs +\- S.D.	δC ppm.	T1** secs +\- S.D.	δC ppm.	T1** secs +\- S.D.
173.757	3.902+\- 0.021	173.735	3.391+\- 0.02	174.948	2.600+\- 0.037
		c		c	
175.681	3.523+\- 0.023	175.453	3.715+\- 0.056	175.613	3.219+\- 0.024
		c		c	
175.932	5.274+\- 0.017	175.713	2.506+\- 0.015	175.988	5.393+\- 0.058
		c		c	
				179.324	3.307+\- 0.017

* Inverse gated. ** Relaxation Time. a - saponified in 0.5% NaOH alcoholic solution. b - saponified in 0.25% NaOH alcoholic solution. c - data taken from ^{13}C PENDANT spectrum.

Table 7.11 (b) ^{13}C-NMR carbonyl chemical shifts* of PLETESA - saponified in 95-75% ethanol. Synthesized at room temperature.

Room Temperature Synthesized PLETESA					
Saponified in 95/5% Ethanol/Methanol a		Saponified in 90/10% Ethanol/Methanol a		Saponified in 75/25% Ethanol/Methanol a	
δC ppm.	Integral	δC ppm.	Integral	δC ppm.	Integral
173.785	100.000	174.047	28.194	174.090	0.767
		174.536	100.000	174.576	6.252
175.527	463.964	175.845	57.423	175.897	9.745
175.783	490.774	176.091	161.940	176.176	16.708
175.949	590.799	176.288	60.649	176.268	8.328
		–	114.473	176.361	6.196
δC ppm.	T1** secs +\- S.D.	δC ppm.	T1** secs +\- S.D.	δC ppm.	T1** secs +\- S.D.
173.770	2.877+\- 0.096	174.021	3.253+\- 0.052		
		174.523	3.024+\- 0.031		
		175.820	1.933+\- 0.014	175.862	1.685+\- 0.028
175.764	2.561+\- 0.008	176.099	1.853+\- 0.012		
		176.266	1.617+\- 0.031	176.238	2.305+\- 0.012

* Inverse gated. ** Relaxation Time.
a = saponified in 0.5% NaOH alcoholic solution.

7.3.4. Comparison of ^{13}C-NMR Carbonyl Spectra in Saponified PLETESA Synthesized at Room Temperature and at Low Temperature.

The integral values reported in Tables 7.11 a & b were used to calculate the relative ratios of the groups assigned to particular carbonyl shifts. This data was then used as a method of assessing the efficacy of saponification and to obtain some impression of the hydrophilic/hydrophobic balance within the saponified polymers. The relative ratios obtained are shown in Table 7.12. The integral values for the carbonyl groups proximate to the α-carbon methine group were taken as one because, the chemical shifts noted for these groupings did not appear to split upon saponification, and hence, any changes in the relative proportions of the other groups that occurred as a result of saponification could be monitored by reference to these groups.

Table 7.12. ^{13}C carbonyl assignments and relative ratios of assigned groups.

^{13}C Carbonyl Assignment	Relative Ratios of Carbonyl Groups					
	Room Temperature Synthesized PLETESA					Low Temp PLETESA Saponifd.*
	Base Polymer	Saponified in Ethanol/Methanol 0.5% NaOH				
		100% EtOH	95% EtOH	90% EtOH	75% EtOH	100% EtOH
Methyl ester				0.62	0.37	
Ethyl Ester	1.02	0.30	0.20	0.17	0.05	0.17
Carboxylate		0.90	0.95	0.35	0.58	0.79
α CHNHCO	1.00	1.00	1.00	1.00	1.00	1.00
$CH_2NHCOCH_2$	0.96	1.08	1.20	0.37	0.49	0.45
$CH_2NHCOCH_2$				0.71	0.37	0.63
% Saponification	00.0	75.0	82.6	30.7	58.0	82.3

* Saponified in 0.25% NaOH alcoholic solution.

7.3.4.1. Interpretation of ^{13}C Aliphatic Chemical Shifts.

The aliphatic δC values noted in Table 7.10 for both the base PLETESA and the polymer saponified in 100-75% ethanol are remarkably similar for all five materials and characteristic of the δC values previously defined. The exception to this is the absence of an ethyl ester shift in the sample saponified in 75% ethanol/ 25% methanol, probably as a result of transesterification and the formation of a methyl ester.

7.3.4.2. Interpretation of ^{13}C Carbonyl Chemical Shifts.

The most unusual feature of the ^{13}C-NMR spectra acquired from all samples of PLETESA tested, as shown in Figure 7.12 and Table 7.11 (a) & (b), were the prominent carbonyl peaks which were markedly shifted upfield to lower frequencies, indicating high levels of shielding, possibly as a result of intense intrachain hydrogen bonding. Carbonyl resonances are usually extremely weak signals[177] and can only be observed in the presence of a powerful magnetic influence, such as a paramagnetic salt, which acts as a relaxant.

The three chemical shifts observed for unsaponified PLETESA (base polymer) shown in Table 7.11 (a) represent carbonyl groups within the ester side chain and an amide carbonyl group in two different environments, i.e., adjacent to the α-carbon methine group, where the δC value is shifted upfield by the proximity of a second carbonyl group, and an amide group sandwiched between two methylene chains. The existence of two different amide environments is also indicated by the observation of two different relaxation times. The amide group closest to the α-carbon atom relaxes much faster, probably because, this group experiences increased hydrogen bonding with the proximate ester group. The latter group also exhibits a short relaxation time. The relative ratios of the three carbonyls can be seen from Table 7.12 to be close to unity.

The δC values observed demonstrate that saponification in 100% ethanol, of polymer synthesized at room temperature, results in the formation of carboxylate groups, as revealed by the appearance of an additional resonance at 175.471 ppm. This effect is accompanied by a slight downfield shift in the resonance of the amide carbonyl group adjacent to the α-carbon methine group, as a result of the replacement of the ester substituent with a carboxylate group. Published data indicates that carboxylate groups, do indeed, have this effect upon substituents in the β position.[178] The carbonyl-carboxylate chemical shift observed corresponds well with the 175.7 ppm. reported by

Henry *et al.*[183] for lysine residues within protein chains, which are also said to be shifted downfield.

Alternatively, the additional peaks noted at 175.0-175.5 ppm. may represent the release of lysine by degradation of the polymer, since the δC reported for the carboxylate group of L-lysine is 174.89 ppm.[180] The ratio of ester to carboxylate-carbonyl integral values shown in Table 7.12. suggest that some 75% of this polymer is saponified. This compares to an estimate of 66% esterification obtained by assessment of the aliphatic δC integral values. The disparity between the two estimates may arise from the relative weakness of the carbonyl signals and their high degree of overlap as compared to those of the aliphatic signals.

The effect of raising the proportion of methanol in the saponifying solvent can be observed in Tables 7.11(a) & (b), where the chemical shifts indicate that the proportion of ester groups fall progressively with an increase in the percentage of methanol. A high proportion of ethyl ester groups would appear to be converted to the methyl ester in the 90 and 75% ethanol solutions, i.e., 10 and 25% methanol, by a process of transesterification. This latter process occurs because the ethyl group is a better leaving group than the methyl group and once the methyl ester is formed it is less prone to saponification. The proportion of methylated pendant groups, as a percentage of all pendant groups, is 54.4% and 37.0% in the polymers saponified in 10% and 25% methanol, respectively. Although the relative percentage of methylated to ethylated ester groups is 78% and 88%, respectively. Methylation would, therefore, appear to render the polymer less prone to saponification, since the 54.4% methylated polymer is only 30.7% saponified, whereas, the 37.0% methylated polymer is 58% saponified, this probably occurs because the methyl substituent is a poorer leaving group.

However, the situation is somewhat more complex than this simplistic explanation would suppose, since the highest degree of methylation does not occur in the presence of the greatest concentration of methanol (25%). Presumably solvation factors are also important in maintaining the polymer in solution. In the 25% methanol solution the saponified polymer remains solvated in the more polar solvent for a longer period, and therefore, is subject to a greater degree of saponification, i.e., 58%, leading to a reduction in proportion of both ethyl and methyl esters. Whereas, in the 10% methanol solution, methylation renders the polymer less soluble in the largely (90%) ethanolic (less polar) solvent, and hence, at low degrees of saponification the polymer becomes insoluble. The proportion of saponified material verses the solvation mixture is shown graphically in Figure 7.13.

Figure 7.13. Effect of solvent polarity on the percentage of PLETESA side chains saponified.

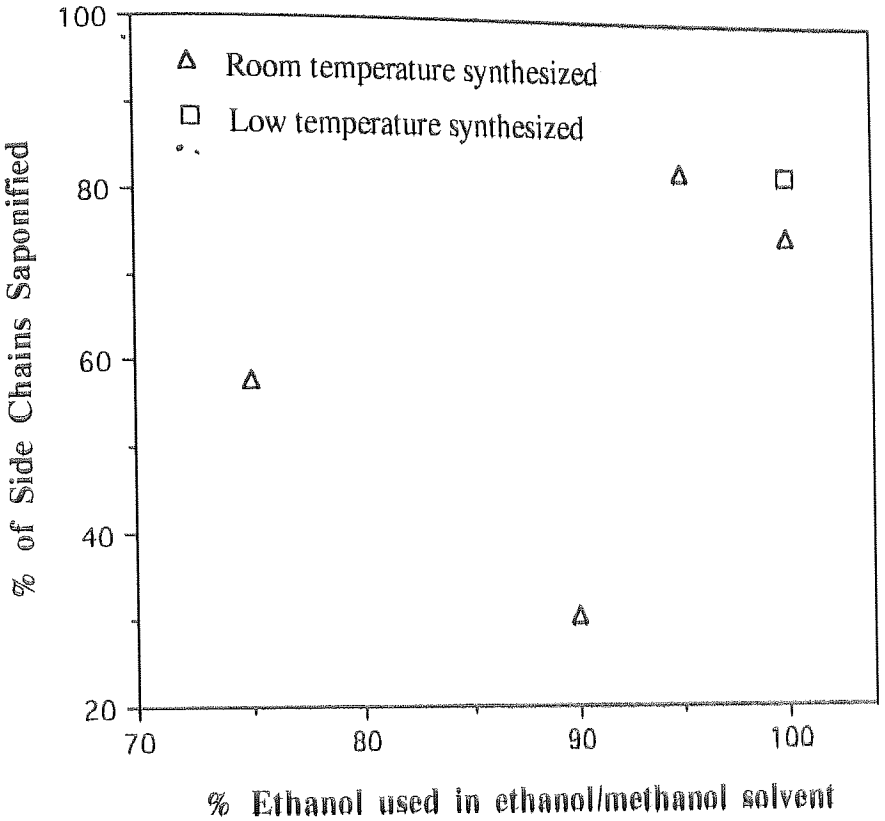


Figure 7.13 shows that as a result of the methylating and solvating effects of methanol, as described above, a monotonic reduction in saponification, with increased solvent polarity, is not observed with PLETESA, largely because, the solubility of the polymer and its saponified derivative is finely balanced between the solvent polarities of ethanol and methanol. Hence, controlled saponification by selective solvation does not appear to offer a method for synthesizing derivatives where the degree of saponification can be precisely controlled.

It is also apparent from the ^{13}C carbonyl spectrum of the polymer synthesized at room temperature, that the amide carbon groups sandwiched between the methylene bridges show a split chemical shift as the methanol concentration is raised to 10% and 25%. This may arise as a result of partial charge repulsion and limited chain extension, exposing some of the formerly shielded aliphatic groups to the solvent, and thereby, creating two environments. In polymers where a high degree of saponification was achieved, charge repulsion will increase and the chain is likely to become fully extended. This is confirmed by the observation of only one amide environment under these conditions. Alternatively, the splitting of the carbonyl resonances may simply

have resulted from the formation of a more hydrophilic environment as ethyl ester groups are replaced with methyl ester groups.

Two environments are also observed for the amide groups adjacent to the C_2 methylene groups in the low temperature synthesized polymer, saponified in 100% ethanol. In the latter sample, the carbonyl δC from the C_2 amide group is markedly shifted downfield to 179.4 ppm., possibly due to inclusion of the amide group within a coiled microdomain, despite the 82.3% saponification and high degree of charge repulsion. As result of the regular backbone sequence, i.e., HTHT, and the presence of a secondary structure, the polymer may be able to resist the effects of charge repulsion. It should be noted, that although, this polymer was saponified in half the concentration of alkali used to saponify the corresponding polymer synthesized at room temperature, it appears to be saponified to a greater extent. A downfield shift is also observed in proteins, where δC values from carbonyl groups within α-helical coils appear above 176 ppm.[184] However, there is no such splitting observed among the ester carbonyls within the saponified PLETESA synthesized at low temperature, which would tend to argue against the presence of any secondary structure.

In conclusion, the spectral data acquired does not provide firm evidence to suggest the presence of four distinct amide environments which might be expected from the four sequence triads which could exist in PLETESA, as illustrated in Figure 5.2. This suggests that only one sequence triad is formed in material synthesized by the interfacial technique, possibly due to the high rates of reaction that characterize this method of synthesis.

CHAPTER 8

DISCUSSION

'And certainly the glass was beginning to melt away,
just like a bright silvery mist.' [185]

8.1. Biodegradable Hypercoiling Polymers: A Synthetic Metaphor for Apoprotein.

The living process is essentially one of association, most basically to form membranes that compartmentalize reactions, and thereby, order chemical processes. Just as the chemist contains and controls reactions in the reaction vessel, membranes enable flexible and adaptive environmental containment. This may be viewed as an example of 'primary organisation'. Association of cells into multicellular structures enable groups of cells to specialise in particular functions, in order to respond more readily to their environment, e.g. by developing nerve networks, muscle cells, etc. This can be considered as 'secondary organisation'. The latter process leads to a 'tertiary organisation' where the organism is aware of is own existence, and this is perhaps the most responsive state of all. The development of each organisational stage offers the organism an increased chance of survival, so favouring its continued development. We can reason that each stage must have been preceded by the evolution of new polymeric structural forms which conveyed new functional capabilities. Hence, evolution in its most basic form, can be considered as the development of polymeric structures with increasingly sophisticated and spatially refined architectures. By acting in a pluralistic unison such polymers associate to achieve the functions characteristic of advanced organisms.

Within most multicellular organisms the vast majority of the cells cannot absorb agents directly from the outside environment, because they are positioned beyond the distances over which diffusional processes operate efficiently. As a result, the development of multicellular capability presents living organisms with several fundamental problems, principally related to the transportation of nutrients and gaseous exchange. This has necessitated the development of a transport system, but poses the organism with a paradoxical problem: how can aqueous insoluble lipoidal material, from which the cells are assembled, be transported through the aqueous milieu of any interstitial fluids or circulatory system.

To absorb oxygen a multicellular organism requires a large, usually internalised, surface area for adequate gaseous exchange. To achieve this, in turn, requires the formation of a multi-chambered structure, e.g. the alveoli of the lung. In terrestrial, air-breathing, animals gaseous exchange has to occur across a fluid boundary, and the omniscient fluid in living systems is water. However, the high surface tension of water would act to collapse such small chambers by polar interactions and high cohesive forces.

For the lung to operate effectively the surface tension must be reduced by the introduction of a natural surfactant. This is achieved by surface adsorption of a hydrophobic lipoprotein layer. In the systemic circulation an analogous problem is presented by the transportation of lipoidal material and this has been solved by the development of proteins possessing an amphipathic helical structure. A similar structural motif is found in lung surfactant protein (SP-B) and appears to be the key development in resolving the problems associated with multicellular transport. It seem unlikely that the fundamental problem of lipid solubilization in aqueous systems would have been resolved by two entirely unique surfactant systems and more likely that lung surfactant protein (SP-B) is a adaptation of structural motifs found in plasma lipoprotein and shares phylogeny with the latter.

On a functional basis, lipoprotein and lung surfactant protein SP-B are related to one another, in that, the solution they represent to the evolution of multicellular capability offers a holistic answer that must have enabled quantum advances in bio-organisation to occur, i.e., multicellular life in its multifarious forms, to progress or possibly to have evolved initially. Even if these events did not predate the formation of organised living structures, then one suspects, that they occurred at a primordial stage. They are not the events of the more subtle, adaptive or later stages, of evolutionary change and must be considered in the formative category.

The contention of this thesis is that biodegradable hypercoiling polymers may act as protein correlates and mimic the functions of the apoproteins found in the lung and in the blood plasma, and therefore, may be used in the treatment of neonatal RDS or as an aqueous soluble vehicle for the systemic delivery of lipid soluble drugs.

8.2. Discussion of Experimental Work.

The synthetic studies reported in this thesis have shown that simple diamino monocarboxylic acids can be used to synthesize anionic polyamides by a process of stirred interfacial polycondensation with acid chlorides. The basic synthetic method identified in these studies involved reacting a 0.2M aqueous solution of a diamine containing 0.6-0.75M sodium carbonate (pH 10-11) with 0.22-0.2M aromatic acid chloride dissolved in an organic phase, usually a chlorinated solvent. Polymeric material formed when isophthaloyl chloride was reacted with L-lysine, L-ornithine and DL-diaminopropionic acid, resulted in the formation of water soluble, and methanol insoluble, resins which exhibited a $\bar{M}_W$ in the range of 12,000-26,000.

The reaction was essentially found to depend upon the relative solubilities and stabilities of the two components in their adjacent phases, i.e., the solubility of the diamine in the organic phase and that of the acid chloride in the aqueous phase. The reaction can be considered as a product of two competing rates; the rate of ingress of the diamine into the organic phase, that is to the locus of the polymerization (at the organic phase of the interface), and conversely, the rate of loss of acid chloride by dissolution, and subsequent hydrolysis, into the aqueous phase. The balance between these two competing rates is the principal factor in defining the rate of polymerization. The ideal situation for the formation polymer, is one in which the acid chloride is poorly soluble in the aqueous phase, while the diamine is readily soluble in the organic phase.

The aromatic acid chlorides are generally less soluble in the aqueous phase and less reactive compared to their aliphatic equivalents, and as a result, their rate of loss by hydrolysis was found to be reduced during interfacial reactions. The aromatic acid chlorides, were therefore, maintained at the interface for a longer period to react with the diamino acids or their derivatives. The experimental results showed that sodium hydroxide (0.3M) could not be used as an acid acceptor in the reaction, because as a strong base it caused extensive hydrolysis of the acid chloride. Its replacement with sodium carbonate (0.76M) greatly improved the yields of polymer obtained. It was found to be important to use a sufficient quantity of base to maintain alkaline conditions throughout the reaction (i.e. pH 8-10) if high polymer yields were to be obtained. It was also observed, when using isophthaloyl chloride, that approximately half of the organic phase was solubilized in the aqueous phase at the end of each reaction. This effect probably resulted from the formation of isophthalic acid during the reaction, which acted as a solubilizing hydrotope. Increasing the hydrophilicity of the diamine, e.g. by using hydroxylysine in place of lysine, was found to result in the formation of a

polymer of lower molecular weight, probably as a result of the reduced solubility of the diamine in the organic phase and a lower rate of ingress of the latter into the locus of polymerization.

It was not possible to produce poly(ester/amides) based on tyrosine or serine, or poly(anhydrides) based on malonic acid, by interfacial reaction with isophthaloyl chloride. The failure of the reaction involving tyrosine was probably due to the strongly basic (pH>12.5) conditions needed to maintain the tyrosine in aqueous solution. Aliphatic hydroxyl groups also exhibited poor interfacial reactivity towards isophthaloyl chloride, as a result of their high aqueous solubility and effective partition into the aqueous phase. These studies suggest that cross-linking between poly(isophthalamides) bearing pendant hydroxyl or carboxylic acid groups is unlikely to occur. In addition, it was not possible to incorporate polyethylene oxide (mol. wt. 3,350) blocks and produce an amphipathic 'block' copolymer, by interfacial reaction of isophthaloyl chloride with POE bis(amine). The failure of this reaction may have arisen from the low amine end-group density of the POE used.

Interfacial reactions of diamines with phthaloyl chloride did not result in the production of substantial quantities of polymeric material, possibly due to the formation of phthalamides (imides) by reaction of phthaloyl chloride with a single amino acid amine group, thereby end-capping the reaction and preventing polymerization. This effect was partially overcome by use of the short chain amino acid, DL-diaminopropionic acid, where the proximity between the amine group substituted at the ε-carbon atom and the carboxylic acid group substituted at the α-carbon atom probably acts to sterically hinder imide formation. The latter effect may account for the formation of polymer-like material when using this amino acid. Lesser amounts of material were produced with ornithine, where the intrachain distance is increased by one methylene group. Some evidence was obtained to suggest that the presence of the indole ring within L-tryptophan may exert a similar steric effect and enable polymerization to occur between phthaloyl chloride and this amino acid, although the product synthesized by the reaction appeared to be unstable. A similar reaction between tryptophan and isophthaloyl chloride also resulted in the production of an unstable, probably, monomeric product.

Reactions between terephthaloyl chloride and lysine failed to produce any poly(teraphthalamide), possibly because, hexane was used as the organic phase and so limited the ingress of lysine into the locus of polymerization, since lysine possesses a very low solubility in this solvent.

High molecular weight poly(adipamides) were synthesized by interfacial reaction of adipoyl chloride with L-lysine ethyl ester (LETES). The purpose of this synthetic strategy was to synthesize a polyamide without the constraining influence imposed by the presence of aromatic groupings within the polymer backbone. This approach offered the additional synthetic versatility of being able to modify the number of hydrophobic groups in the polymer backbone, by partial saponification of the ester side chains. However, interfacial reactions between adipoyl chloride and lysine, ornithine and 5-hydroxytryptophan did not result in the formation of polyamides. Probably as a result of the low solubilities of these amines within the organic phase, compared to the high solubility of the adipoyl chloride in the aqueous phase, and consequent high rates of acid chloride hydrolysis.

By using LETES the solubility of the diamine in the organic was greatly increased, such that the rate if ingress of the diamine into the locus of polymerization outpaced the rate of loss of the acid chloride by hydrolysis. Substantial yields of poly(lysine ethyl ester adipamide) [PLETESA], e.g. up to 17%, were obtained by interfacially reacting 0.2M-0.4M LETES in an aqueous phase, containing either 0.94-2.0M sodium carbonate or 5M potassium carbonate, with 0.25-0.83M adipoyl chloride in an organic phase. The high concentrations of salts used probably acted to limit the solubility of the acid chloride in the aqueous phase, and thereby, reduced its rate of loss by hydrolysis, so retaining the active species at the interface and favouring the formation of polymer material. PLETESA with a M_W of 126,000 was obtained.

The NMR studies on PLETESA base polymer and saponified polymer synthesized at room and at low temperature supported the structure proposed for this polymer. It is noteworthy, that the substituent shift resulting from the -NHCO grouping was found to be greater than that of monomeric amides and equivalent to the value noted in polyamides such as Nylon 6. Thus, providing additional evidence of the formation of a polymer. These studies also indicated that in the saponified state the degree of saponification was 66% when assessed by δH values and 75% when assessed by quantified δC carbonyl values.

Poly(lysine ethyl ester glutaramide) was also produced by the interfacial method used to synthesize PLETESA and is believed to be a novel product. Studies showed that polyamides could not, however, be formed by interfacial polymerization when succinyl or fumaryl chlorides were used in place of adipoyl chloride. This was probably the result of the short interchain distance within these acid chlorides, which tended to favour the formation of imides and resulted in end-capping of the reaction.

Attempts to selectively modify the hydrophobic character of PLETESA, by saponification in ethanol/methanol mixed solvents of differing polarity, resulted in partial cleavage of the polymer backbone. The strong ^{13}C-NMR carbonyl resonances exhibited by these poly(adipamides) enabled the degree of esterification to be quantified. ^{13}C-NMR studies demonstrated that modification of solvent polarity could not be used as a method of controlling the degree of saponification by selective solvation. The results indicated that the synthetic method adopted led to the formation of the corresponding methyl ester by a process of transesterification. The failure to obtain a direct correlation between the degree of saponification and the solvent polarity was probably the result of selective solvation of the saponified polymers at particular solvent polarities, combined with the formation of the methyl ester that made the outcome of the saponification process rather unpredictable.

Low temperature conditions were also used to facilitate the synthesis of PLETESA with head/tail sequence distribution. Although, the synthetic conditions did not appear to influence the surface tension/pH profiles, and by inference, the conformation of the polymer in solution. This result would tend to suggest that the microstructure of the polymer chains was similar when synthesized under both temperature conditions. This finding was confirmed by ^{1}H and ^{13}C-NMR studies of the primary structure of the polymers, which failed to reveal any differences between the materials produced under the two reaction conditions. It can be concluded from these studies that, as a result of the high rates of synthesis that occur during interfacial synthesis only one sequence triad is formed, i.e., the HTHT triad. Indeed, the NOESY studies of the base polymer suggested that the conformation of the polymer could most accurately be described by an HTHT sequence distribution. However, there was some evidence from potentiometric titration studies on 0.1% solutions of saponified PLETESA that the low temperature synthetic process resulted in the formation of a different product. The latter polymer appeared to behave as a weaker acid, perhaps because, its pendant carboxylic groups were more effectively shielded or sterically hindered. These findings indicate the presence of a secondary structure, resulting from a defined sequence distribution within the polymer backbone. Compared to the random distribution found in polymer synthesized at room temperature. The potentiometric titration may be a more sensitive measure of conformational form than the other two methods used to probe polymer structure.

The synthetic studies demonstrate the feasibility of using interfacial polymerization as a simple, cost effective and rapid method for producing high molecular weight amino acid-based polyamides. Either in the form of anionic poly(isophthalamides) or hydrophobic poly(adipamides). Where the latter can be saponified to form more

hydrophilic derivatives. The ability to use a range of acid chlorides and amino acids or their derivatives, with differing hydrophobic and hydrophilic properties, presents us with a 'molecular paint box' from which to select monomers to meet particular requirements. This synthetic versatility offers great promise for producing biodegradable polymers with a balance of hydrophobic and hydrophilic characteristics, a facility that is normally associated with biopolymers. In addition, the polymers described appear to satisfy the specifications initially outlined for an easily synthesized, biodegradable, hypercoiling or hydrophobically associating polymer.

When considering the analytical studies aimed at elucidating the structural and physical characteristics of the polymer materials, it is clear that a basic theme runs throughout the work, and that theme is one of association. Surface tension was used as a rapid, but sensitive, method of monitoring conformational transitions of polymers in solution. The surface tension/pH profiles of 0.5% solutions of the amino acid-based poly(isophthalamides) showed some indication of a rise in surface activity upon acidification, possibly as a result of intramolecular micellation, this was followed by a marked fall in surface tension when the solutions were acidified further. The polymers became maximally surface active as they approached their pK_a values, presumably as microdomains led to the formation a surface active amphipathic molecular conformation. Complete loss of charge resulted in precipitation of the polymers from aqueous solution. The surface behaviour of the monomeric starting materials, which may be present in low concentrations as contaminants within the polymers, showed only minimal surface activity in response to pH change, and are therefore, unlikely to have influenced the results. However, the principal hydrolytic breakdown product, isophthalic acid, was surface active (56-49 mN/m) although in the concentrations likely to be present in the extracted polymers, its effects are likely to be negligible.

A similar fall of interfacial tension, against diiodomethane, occurred in response to a reduction in pH, when measured over a range from pH 7.5-12.5. This effect may also have resulted from intramolecular micellation. Such effects probably arise from the high ratio of hydrophobic to hydrophilic groups inherent in the structure of the poly(isophthalamides). There is some evidence form the interfacial studies, conducted on 0.1% poly(ornithine isophthalamide), that the interfacial tension falls as the pH of the solution is reduced below pH 7.5. This would suggest that the interfacial tension/pH profile is similar to that of the surface tension/pH profile. Similar effects have previously been reported[87,88] to occur in copolymers of maleic acid and styrene which can be considered to be structurally and functionally equivalent to the poly(isophthalamides).

Further evidence of domain formation is provided by potentiometric titrations of 0.1% solutions of the poly(isophthalamides), which exhibit two distinct pK_a values upon neutralisation. These may reflect the change in polyacid conformation from an extended chain to a collapsed coil. Where the poly(isophthalamides) behave as stronger and weaker acids, respectively. Alternatively, the presence of a second pK_a value may be a consequence of carboxylic acid groups located at the end of the polymer chains.

The surface tension/pH profiles of 0.1% and 0.5% solutions of partially (66-75% in the case of low temp synthesized polymer) saponified PLETESA, synthesized at both room and low temperature, respectively, also indicate that intramolecular micelles were formed upon loss of charge. An initial rise in surface tension was noted, followed by a marked fall as further charge was lost, presumably as a result of chain collapse and formation of amphipathic, dumb-bell shaped molecules with distinct hydrophobic and hydrophilic domains. This response was more apparent in the poly(adipamides) than in the poly(isophthalamides), possibly because, of their greater backbone flexibility, and hence, ability to orientate their chain structure into particular conformations. Further acidification of the saponified PLETESA solution caused a further fall in surface tension, as a result of a shift in the balance of hydrophilic to hydrophobic groups which accompanied the loss of charge and ultimately resulted in precipitation. A similar surface tension/pH profile was obtained with a solution of denatured silk fibroin and suggests that natural polypeptides may be subject to the same type of pH induced conformational changes as those described in poly(isophthalamides) and saponified PLETESA.

The fall in surface tension noted on reducting solution pH with the poly(adipamides) appeared to be concentration dependant, when comparing 0.1 and 0.5% solutions. This suggests a potential to form inter, as distinct from intra, molecular micelles, such as those formed in conventional surfactants above their critical micellar concentration. The formation of intramolecular micelles at low concentrations and intermolecular micelles at higher concentrations has also been reported[164] to occur in other polymeric surfactants, e.g. block copolymers of ethylene oxide and propylene oxide.

Hypercoiling or hydrophobic association of the polyamides in polar solvent was studied by using 2-dimensional NMR. This technique has been frequently used to the study the conformation of proteins in solution,[186] but has not previously been applied to hypercoiling polymers. The data produced by this technique provided the strongest evidence for the ability of PLETESA to form intramolecular micelles. Studies on the unsaponified base polymer, in a polar solvent, indicate that each repeat unit of the

polymer chain appears to form a single turn of a coiled structure, in which the hydrophobic ethyl ester side chains are contained within the centre of the coil, and thereby, excluded from the polar environment. In non-polar conditions (dichloromethane/methanol, 3:1) the coil 'springs' open and the intrachain associations, previously noted in the NOESY spectra, were lost.

2-dimensional NMR studies of the saponified PLETESA, synthesized at low temperature also reveal weak through space associations within the polymer between the backbone methylene groups, as a result of their exclusion from the polar environment. However, since these studies were conducted in methanol the precise degree of charge cannot be defined, and therefore, the polymer may not be in a similar conformation to that adopted in aqueous solution at a defined pH, i.e., the conformational state monitored by the surface studies. Notwithstanding this limitation, the NMR results show that intramolecular associations occur in partially saponified PLETESA and indicate the type of conformation that may result in the formation of intramolecular micelles, such as alluded to by surface tension studies, although they do not identify its precise form.

When in the partially saponified state, the hydrophobic/hydrophilic balance within PLETESA is different from that found in the base polymer, and therefore, this polymer would not be expected to remain as an intramolecular coil upon loss of charge, because of the shift in favour of hydrophobicity, which would tend to favour microdomain formation and creation of a more surface active, amphipathic, structure.

The NMR studies also revealed that saponified PLETESA was prone to hydrolysis, resulting in the release of ethanol. This effect was was found to be greatly reduced in the base polymer in polar solvent conditions as compared to non-polar conditions. This observation again supports the concept of intramolecular micellation, where the ethyl ester groups are effectively removed from the polar environment and partitioned into the hydrophobic core of the polymer and in this environment are not subject to hydrolysis.

The remarkable solubility profile exhibited by unsaponified PLETESA base polymer can be explained by the secondary structure described, i.e., when in polar media such as water and methanol, the molecule is coiled so as to contain its ethyl ester side chains within the centre of the helical chain, but when dissolved in non-polar solvents, such as carbon tetrachloride and chloroform, its molecular shape is changed to expose the ethyl ester side chains as the helix becomes everted. The ethyl ester side chains can be considered to act as the 'trigger' for coiling. The functional groups in this case are, most intriguingly, not those normally defined as such by organic chemists,

e.g. amines or carboxylic acids, but in fact, the 'non-functional' hydrophobic, ethyl ester side chains. The paradox is clear - the greatest functional definer in aqueous systems is no 'function' at all. The reason for this apparent anomaly, is that the largest free energy gain in an aqueous environment results from the hydrophobic effect, where hydrophobic groups are excluded from the aqueous environment. As a result, the hydrophobic effect is the principal molecular driving force in aqueous systems, e.g. living systems.

To use a literary allegory, we have 'stepped through the looking glass', where lack of reactivity is the most powerful force in defining functionality in a chemical system, when the system comprises, partially hydrophobic polymer molecules in a highly polar environment. Since all living systems are aqueous polymeric systems, then all living systems must be circumscribed by these rules, and may perhaps, behave in a manner analogous to that of PLETESA. Although, it must be considered that proteins may be more constrained in their ability to move by the proximity of their amide groups and resultant rigidity in their backbones. PLETESA may perhaps, best be viewed, as a hypermobile correlate of protein structure and functionally more akin to the lipid-bound poly(hydroxybutyrate)[148] found in certain bacterial cell membranes. In the latter polyester, the backbone mobility is sufficiently high to form a helical coil with a diameter large enough to confine its hydrophilic groups within the centre of the coil. This arrangement may be analogous to the hydrophobic core formed within PLETESA, in order to contain the ethyl ester side chains.

The backbone of polypeptides does not enable the formation of a coil of sufficient diameter to contain the 'reactive' amino acid side chain residues. Hence, functionality can only be achieved within a polypeptide coil by spatial separation of the amino acid side chains onto particular facets on the exterior surface of the polypeptide coil, as is supremely exemplified by the plasma apoproteins. Indeed, it has been reported[187] that in a series of uncharged hydroxyalkylated poly(glutamines) the effect of increasing the number of methylene groups in the hydrophobic side chains acts to stabilise the polypeptide chain in an α-helical conformation when in aqueous solution. In this case, the side chain associations were peripheral to the central polypeptide core. A similar phenomenon has also been observed to occur in cationic polypeptides such as poly(lysine), where adoption of a helical conformation maximises hydrophobic bonding and the entropic gain contributed by the loss of associated water molecules overcomes the effects of charge repulsion between the side chain groups.[188] In view of these constraints, inherent in the structure of polypeptides, PLETESA may be seen to achieve functions and structural forms on a molecular level that can only be achieved by

proteins on a supramolecular level. Although, the resulting protein structures would, undeniably, offer a far greater degree of conformational stability.

It is particularly intriguing to speculate about the behaviour of hydrophobically triggered molecules such as PLETESA at interfaces. It is likely that they will change their structure at the interface between two media with widely differing polarities. In this sense, the polymers would have to be considered as dynamic surfactants, i.e., they may be 'surface reactive,' as distinct from surface active, or perhaps, will only exhibit surface active behaviour upon interaction with other hydrophobically associating molecules.

CHAPTER 9

CONCLUSIONS

&

SUGGESTIONS FOR FURTHER WORK

9.1. Conclusions.

A literature review of hypercoiling polymer structures and biological surfactants identified the differences between monomeric and polymeric surfactant behaviour and highlighted the role played by polymeric surfactants in nature, such as those found in lung surfactant and plasma apoproteins. Hypercoiling polymers may be the synthetic counterparts of the lipid-associating biopolymers. Hence, the use of hypercoiling polymers as replacements for apoproteins in deficiency states, such as RDS, is proposed. However, to make such products compatible with biological systems and applicable as human medicines, it is important that these materials possess an ability to degrade *in vivo*. The objective of the work described in this thesis, was therefore, to synthesize a biodegradable hypercoiling or hydrophobically associating polymer which could be used as a replacement for lung surfactant apoprotein, which would potentially hydrolyse into either natural metabolites or harmless, non-toxic products.

The synthetic work undertaken has shown that stirred interfacial polymerization can be used to synthesize a range of poly(isophthalamides) and poly(adipamides) that meet this specification, based on L-lysine, L-lysine ethyl ester and L-ornithine and isophthaloyl chloride or adipoyl chloride. The reaction was shown to be the product of the rate of diamine ingress into the organic phase at the locus of polymerization, compared to the rate of loss of the diacid chloride, by dissolution and hydrolysis into the aqueous phase. Sodium or potassium carbonates when used in high concentrations were found to be effective as acid acceptors and high molecular weight products could be extracted by the selective solvation of low molecular weight material into methanol. Using these techniques poly(isophthalamides) with a M_W ranging from 12,000 to 26,000 were produced. Phthaloyl chloride was not found to produce substantial yields of polymer and FT-IR analysis suggested that this was the result of phthalamide (imide) formation and reaction end-capping. The one exception to this was the reaction with DL-diaminopropionic acid, where steric hindrance, caused by the short intra-amine distance, acted to prevent imide formation, and thereby, favoured polymerization.

The interfacial technique has previously been described in the literature as a method of synthesizing polyamides based on lysine and adipoyl chloride, but as the results reported in this thesis confirm, the use of lysine, with its high aqueous solubility, results in the formation of only low molecular weight material. Interfacial studies using adipoyl and glutaryl chloride, found that these materials produced polyamides when reacted with L-lysine ethyl ester with a Mw of 126,000 and 26,000, respectively. Poly(lysine ethyl ester glutaramide) is not thought to have been previously reported.

The polyamides synthesized were resinous materials and the unsaponified PLETESA base polymer, diplayed a curious solubility profile which had previously been alluded to in the literature but not understood.

2-Dimensional NMR analysis, using the NOESY technique, suggests that these characteristics are the result of the formation of an endohydrophobic-exohydrophilic coil. The ethyl ester side chains appear to act as a trigger mechanism to collapse the polymer into a defined secondary structure, where the ethyl ester groups are contained within the coil and the amide groups of the polymer backbone are exposed to the polar solvent. This arrangement is lost when the polymer is dissolved in a non-polar environment, as the polymer chain 'springs' open to expose the ethyl ester side chains to the solvent. The intramolecular micelles formed within PLETESA in a polar solvent are an example of polymeric hydrophobic association or hypercoiling, not in this case, induced by a loss of charge, as in most vinyl-based hypercoiling polymers, but by changes in solvent polarity. Indicating the importance of non-polar hydrophobic effects in defining secondary structure of polymers in aqueous solution.

Partial hydrolysis of PLETESA base polymer in alcoholic sodium hydroxide solutions of varying polarity resulted in the formation of lower molecular weight (M_W 28,000) saponified polymer. But NMR analysis of the product showed that it was not possible to control the degree of saponification, in any predictable manner, by variation of the solvent polarity. In addition, there was no evidence from either surface or NMR investigations of the formation of different sequence distributions within the backbone of PLETESA, when synthesized at different temperatures. Although, some evidence of the presence of two molecular types was provided by potentiometric titration. 2-Dimensional NMR NOESY analysis of the saponified polymer identified through space associations between the methylene groups which are indicative of an ability to form intramolecular associations in polar solvents, although this tendancy may not be as pronounced as that found in the base polymer, because of the lower degree of hydrophobicity presenct in the saponified material.

Surface and interfacial tension measurements were used as a method of monitoring conformational changes in response to pH. The studies of surface tension demonstrated that amino acid-based poly(isophthalamides) and poly(adipamides) undergo a pH induced conformational transition when deionized in aqueous solution, whereby, the extended polymer chain initially forms an intramolecular micellar structure, which is non-surface active. Further loss of charge results in a fall in surface tension which is probably concomitant with the formation of an amphipathic molecule. A complete loss of charge results in precipitation of the polymer from aqueous solution.

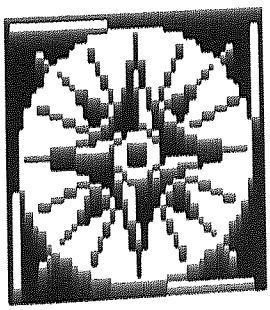
The contribution of the work described in this thesis to our understanding of hypercoiling or hydrophobically associating molecules has been to highlight the distinct molecular events that occur upon collapse of the charged polymers when dissolved in polar solutions, i.e., from extended chain, through intramolecular micellar and amphipathic conformations and finally to insoluble precipitate. Although these events have been described before they do not appear to have been viewed as a continuum and their dynamic surface chemical implications have not been recognized.

The conformational states are accompanied by the expression of distinct surface characteristics and these are particularly relevant to understanding the behaviour of biopolymers and, in particular, the apoproteins. It is hoped that by understanding the secondary properties of simple correlates of protein molecules, such as those exemplified by PLETESA, we will be able to mimic the functions of biopolymers more effectively.

9.2. Suggestions for Further Work.

Further studies should also attempt to define the precise dimensions of the intramolecular coil formed within PLETESA base polymer, by using the technique of differential NOESY analysis. This would enable the size of the hydrophobic core to be defined and its ability to carry drug molecules to be assessed. The secondary structure of the poly(isophthalamides) in polar solution should also be investigated by 2-dimensional NMR analysis, to observe whether or not they, too, form intramolecular micelles, as is suggested by results from surface and interfacial tension studies. This technique should also be extended to study poly(lysine ethyl ester glutaramide), to observe the effect of a shorter intrachain distance on the ability of the polymer to form an intramolecular coil. Additional surface tension studies should also be conducted to define the critical micellar concentrations of the amino acid-based poly(isophthalamides) and poly(adipamides).

The most important work that remains to be done, if the studies reported in this thesis are to be successfully applied to human medicine, and hence to be of benefit to a wider public, is to combine PLETESA base polymer with those phospholipids found in lung surfactant, e.g. DPPC and PG, and to observe if lipopolymer micelles, analogous to the lipoprotein assemblies found in lung surfactant, are formed. The surface behaviour of such lipopolymer conjugates should then be tested under conditions of surface compression, such as those encountered in the lung during breathing. These studies should be conducted with a view to producing an affordable artificial lung surfactant for the treatment of RDS.



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APPENDIX

FT-IR Spectral Data Tables

All Fourier transformed infrared spectra contained in this appendix were obtained from samples in solid form and collected by the attenuated total internal reflection (ATR) technique using a Nicolet 510 FT-Spectrometer.

Sample		Spectrum No.	Amide I	Amide II	H--H Bonding		C=O (CO	O-H OH)	COO- Asym	COO- Sym	C=O Ester	C-O / Amino Esters,	O-H Acids, COOH	C-H	CH_2 Sciss	Other Bonds	
Lysine (0.1M)	wall deposit	30		1524				2470				1218				784	ortho sub ring
																1737	5 memb imide
	aqu ppt.	44		1545	3263					1381				2859		1737	5 memb imide
					3389									2941			
	wall ppt.	155	1627	1538	3282	3067	1709	2518	1589			1229	1305	2859	1431		
								2619						2932			
Diamino propionic acid (0.2M)	free acid	49	1631			3089		2577	1588	1389			1310	2954		1502	N-H amine
	aqu ppt.	43			3269	3061			1583	1387			1311				
					3364												
	CCl_4	34			3275	3061			1583	1387			1318			1154	C-OH (COOH)
					3351												
	'rope'	59	1636	1538	3202	3068	1709	2520	1588	1389			1304	2826		1168	C-O aromatic
								2620						2883		1247	C-O (COOH)
Diamino pimelic acid (0.2M)	free acid	53	1631	1524	3132			2620	1581	1375		1211	1332	2997		1069	C-O (COOH)
								2705								1481	C-O aromatic
																1638	NH_3
	aqu ppt.	56			3474	3418	1688	2520		1403						1061	C-O
					3552	3232		2648								1609	NH_3
	CCl_4	48	1631	1524	3253	3068	1716	2627	1588	1396			1311	2940		1018	C-OH
					3395												
Ornithine (0.18M)	free acid	50			3410	3097									1446	1617	NH_3
					3475										1474		
	aqu ppt.	33	1627	1551	3427	3054	1709		1596	1375			1305	2928	1438	1084	C-O (COOH)
																1154	CO aromatic
NaOH acceptor	aqu - ppt. (acetone)	35		1551						1393				2945	1438	1078	CO (COOH)
																1602	NH_3
																1766	acid Cl-
	CCl_4 after aqu extr.	36			3471									2852	1438	1071	C-O (COOH)
														2922	1457	1122	
																1280	CO aromatic
																1728	5 memb imide
	CCl_4 aqu	54		1545	3431	3061			1574	1382				2954	1481	1076	C-O (COOH)
																1410	

Table A1. Interfacial reactions with phthaloyl chloride I.

Sample		Spectrum No.	Amide I	Amide II	H--H Bonding	C=O (CO OH)		COO- Asym	COO- Sym	C=O Ester	C-O / Amino Esters,	O-H Acids, COOH	C-H	CH_2 Sciss	Other Bonds	
Ornithine (0.2M)	wall ppt.	65	1633	1539	3282 3067	1709	2524 2606	1564			1236	1305	2934	1438		
	interface ppt	66	1627	1545	3319 3073	1707		1589	1406		1241	1311	2871	1438	1128	
Na_2CO_3 acceptor	aqu ppt.	97	1640	1539	3289 3067	1716	2524 2606	1589			1222	1305	2928	1438		
	$CHCl_3$	98	1646	1538	3275 3067	1715			1393			1280	2827 2877 2960	1450	1071 1121 1766	C-O 5 memb imide
Trypto-phan (0.2M)	wall ppt.	69	1646	1526	3405 3054	1709	2511 2612		1393			1248	2982	1431	1097 1343 1842	C-O COOH aliphat.
	rope ppt.	70	1646	1526	3408 3054	1709	2530 2624	1596			1229		2928	1457 1481	1097 1337 1848	C-O (COOH)
	re-reacted	71	1652	1532	3395 3054	1709	2530 2625						2928	1457	1097 1254 1343 1842	C-O (COOH) amide III COOH aliphat.

Table A2. Interfacial reactions with phthaloyl chloride II.

Sample		Spectrum No.	Amide I	Amide II	H--H Bonding	C=O (CO OH)		COO- Asym	COO- Sym	C=O Ester	C-O / Amino Esters,	O-H Acids, COOH	C-H	CH_2 Sciss	Other Bonds	
LETES (0.2M)	aqu ppt.	94	1640	1532	3332	3061	1722	2644 2555			1217	1292	2928 2865	1438	1020	C-C ester
Lysine unstirred (0.76M)	aqu acid	74	1633	1532	3288	3067	1722	2600			1217	1299	2928 2859	1438	1021	C-C
	aqu alk	75	1633	1538		3073			1589	1734	1204	1292			1179 1128	C-O C-O
Lysine stirred (0.2M)	resin - MeOH insoluble	77	1640	1532	3307	3067	1721	2587 2511		1381	1223	1299	2928 2859	1438	1097 926	C-O -OH
	aqu post ppt.	76	1639	1545	3319	3073			1589	1400		1299	2922 2852		1179 1128	C-O C-O
Hydroxy lysine (0.1M)	resin MeOH insoluble	151	1646	1532	3319	3067	1716	2593 2492		1381			2928		1090 1248	C-O Amide III
	MeOH soluble	119	1640	1539	3319	3067	1722	2499 2619			1261	1292	2928	1444	1090	C-O
Ornithine (0.2M)	resin MeOH insoluble	154	1646	1539	3288	3067	1722	2499 2612		1387	1217	1299	2928 2871	1438	913 1097	COOH Dimer C-O
	MeOH soluble	80	1633	1539	3301	3073	1716	2524 2619			1217	1299	2852 2928	1444	920	O-H COOH Dimer
	resin hexane phase	107	1653	1533	3284	3063	1716	2507 2595		1382		1299	2867 2930	1432	1091 1249	C-O (COOH) Amide III
	aqu extr hexane phase	112		1558			1697			1381		1292	2877	1431	1097 1248 1608	C-O (COOH) Amide III Amine
high [CO3]	resin	99	1640	1532	3301	3067	1721	2619			1217	1299	2871 2953	1431		
	resin + Soxhlet	101	1640	1532	3301	3067				1734	1210	1299	2865 2947	1431	1097 1172	C-O Me - ester
	Soxhlet MeOH phase	102	1640	1532	3307	3067	1728 mix ester			1728	1217	1299	2947	1438	1172	Me - ester

Table A3. Interfacial reactions with isophthaloyl chloride I.

Sample		Spectrum No.	Amide I	Amide II	H--H Bonding		C=O (CO OH)	O-H	COO- Asym	COO- Sym	C=O Ester	C-O / Amino Esters,	O-H Acids, COOH	C-H	CH_2 Sciss	Other Bonds	
Diamino propionic acid (0.2M)	resin	95	1643	1532	3281	3061	1722	2505 2505		1381			1299	2928	1431	1097 1248	C-O (COOH) amide III
Trypto-phan (0.2M)	ethanol soln.	110	1646	1526	3345	3054	1716	2499 2600		1387		1223	1337	2928	1438	1008 1097	 C-O (COOH)
Tyrosine (0.2M)	resin	90	1646	1532	3301	3067	1728 mix ester	2511 2600		1381	1728	1216	1299	2808 2928	1438	1059 1097	C-O ester C-O (COOH)
	resin	88	1646	1519	3321	3067	1702	2530 2852		1393		1236	1292	2852 2922	1438	1090 1608	C-O (COOH) NH_3+ def.
	aqu ppt.	178			3357	3079	1684 aryl	2549 2669	1582			1229	1305	2852 2922	1419	940 1513	O-H def. (COOH) H_2O cryst.
Butane 1, 4, diol	aqu	92				3086	1684 aryl	2549 2669	1577	1419			1305	2827 2882		920 1071 1160 1614	O-H def. C-O (COOH) NH_3+ def.

Table A4. Interfacial reactions with isophthaloyl chloride II.

Sample		Spectrum No.	Amide I	Amide II	H--H Bonding	C=O (CO OH)	O-H	COO- Asym	COO- Sym	C=O Ester	C-O / Amino Esters,	O-H Acids, COOH	C-H	CH_2 Seiss	Other Bonds	
Diamino propionic acid (0.125M) LETES (0.125)		96	1640	1532	3301 3067	1716	2505 2612		1381		1229	1299	2928	1431	1103 1791	C-O (COOH) acid Cl-
POE $(NH_2)_2$	free base in MeOH	109				1715			1356			1280	2877	1463	1084 1109 1356	C-O (COOH) C-O ether COO^-
(0.003M)	$CHCl_3$	117	1652		3357		2587 2663	1570	1336 1412	1734		1260	2846 2915	1463	1021 1241 1507 1608	C-O ester amide III amine amine def
	aqu ppt.	114	1633	1539		1709						1299	2852 2922	1463	1103 1241 1349	C-O amide III COO^-
PEG $(NH_2)_2$ (0.003M)	MeOH insol.	108	1640	1532	3307 3067	1722	2505 2600		1381			1292	2871 2923	1438	1097 1242	C-O (COOH) amide III
	MeOH soluble	118	1639	1539	3319 3073	1716	2530 2619					1299	2877 2953	1444	1097 1261	C-O (COOH) amide III
	aqu acid	116			3382 3073			1564	1387				2877		1084 1602 3282	C-O (COO) amine def. amine

Table A5. Interfacial reactions with isophthaloyl chloride - 'block' polymers III.

Sample		Spectrum No.	Amide I	Amide II	H--H Bonding	C=O (CO OH)	O-H	COO- Asym	COO- Sym	C=O Ester	C-O / Amino Esters,	O-H Acids, COOH	C-H	CH_2 Sciss	Other Bonds
Lysine (0.2M)	aqu high CO_3	120	1633	1545	3275 3073	1722	2593		1362			1280	2859 2928		1071 C-N amine 1135 C-O (COOH)
	aqu hexane	122	1646	1551	3300 3092	1721			1380		1242		2859 2928	1438	1091 C-N amine
Lysine (0.33M)	aqu	164	1621	1545	3282	1697			1412				2865 2928	1457	1191 C-O str. 1280 O-H (COOH)
LETES (0.2M)	free base ex Sigma	124			3395		2644		1380	1734	1223 1015	1292	2934		1507 NH_3^+ 1601 amine NH_2
Na_2CO_3	resin	125	1640	1545	3288 3080	1716	2518 2612		1362		1223 1027		2852 2928	1450	1147 C-O-C ester sym str.
	CCl_4/ hexane	126				1728 mix ester			1375	1728		1280	2865 2953	1457	1071 C-N amine 1122 C-O-C ester
	aqu NaOH	140	1627	1545	3275 3080			1589	1400			1292	2859 2934	1444	1084 C-O ester 1141 C-N amine
PLETES/A (0.33M)	resin CCl_4 /hexane	127	1646	1545	3282 3073				1368	1734	1021 1261		2865 2934	1450	1185 C-O-C ester
timed saponif	prehyro-lysis	129	1640	1545	3282 3073				1368	1734	1021 1261		2865 2934	1444	1185 C-O-C ester
EtOH	15 mins	130	1640	1545	3275 3073				1374	1735	1255 1027		2865 2935	1457	1185 C-O-C ester
NaOH	30 mins	131	1627	1545	3282 3073				1375	1734	1261 1027		2865 2934	1457	1185 C-O-C ester
(0.01M)	60 mins	132	1627	1545	3282 3073				1375	1734	1261 1027		2865 2934	1457	1185 C-O-C ester
	overnight	133	1640	1545	3282 3073				1375	1734	1254 1027		2865 2934	1457	1185 C-O-C ester

Table A6. Interfacial reactions with adipoyl chloride and saponification of PLETESA I.

Sample		Spectrum No.	Amide I	Amide II	H--H Bonding	C=O (CO OH)	COO- Asym	COO- Sym	C=O Ester	C-O / Amino Esters,	O-H Acids, COOH	C-H	CH_2 Sciss	Other Bonds	
PLETES/ A (0.2M) timed saponifn. NaOH (0.05M)	30 mins	134	1621	1545	3282 3073			1375	1734	1021	1299	2865 2947	1462	1085 1185	C-O C-O-C
	1 hr.	135	1627	1545	3288 3073			1381	1734	1027	1292	2865 2947	1469	1084 1185	C-O C-O-C
	2 hrs.	136	1621	1545	3282 3080			1380	1734	1027	1305	2865 2945	1475	1084 1185	C-O C-O-C
	4.5 hrs.	137	1620	1551	3288 3080			1380	1728		1305	2859 2928	1465	1116 1160	C-O C-O-C
	overnight	139	1623					1382				2862 2947 2983	1474	1082 1190	C-O C-O-C
PLETES/ A	resin	138	1640	1545	3288 3079		1589	1400	1734	1027	1292	2865 2928	1438	1147 1185	
post sapf.	resin acidf.	142	1633	1545	3301 3092			1349	1734	1021	1261	2865 2928	1450	1098	C-O dimer
PLETES/ A resin	100% EtOH	38			3288	1696		1343 1375 1400	1734			2852 2922	1438	1078 1614	C-O dimer N-H def amine
saponif.	75% EtOH	39			3358	1697	1563	1406	1734	1040		2852 2922	1456	1077 1115	C-O dimer
in % EtOH	90% EtOH	165	1640	1545	3282 3073	1722 2511 mix 2600 ester		1368	1722	1033 1222		2865 2928	1450	1090 1147	C-O dimer
LETES (0.4M) low temp	resin ppt.	161	1640	1545	3282 3067	2605		1375	1734	1027	1255	2865 2934	1450	1181 1255	C-O-C ester as
	CCl_4 (hexane)	162								1071	1279	2865 2928	1457	1128	C-O-C ester sy
Na_2CO_3	Sapf. ppt.	173	1640	1545	3269 3073	1715 2606				1223		2865 2934	1450	1084	

Table A7. Interfacial reactions with adipoyl chloride and saponification of PLETESA II.

Sample		Spectrum No.	Amide I	Amide II	H--H Bonding	C=O (CO OH)	COO- Asym	COO- Sym	C=O Ester	C-O / Amino Esters,	O-H Acids, COOH	C-H	CH_2 Seiss	Other Bonds
LETES (0.286M)	resin ppt.	166	1640	1545	3282 3073			1375	1734	1027 1190	1255	2865 2934	1450	1255 amide III
Low temp	MeOH sol resin	167	1640	1545	3288 3067			1375	1734	1021 1185		2865 2928	1450	1254 amide III
K_2CO_3	MeOH inso β sheet	170	1640	1545	3282 3073			1393	1734	1021 1185	1305	2865 2941	1443	1084 C-O 1245 amide III
PLETES A	sapf. ppt.	168	1640	1551	3282 3073	1709	1596	1406			1318	2865 2928	1438	1084 1760 carbonate
(K_2CO_3) EtOH	sapf. ppt. acidif.	169	1646	1545	3256 3073	1709 2593		1368		1021 1210		2865 2934	1445	
NaOH 0.0625M	sapf. ethanol	171	1646	1545	3275 3079	1702		1406 1356		1027		2865 2934		1084 1147 1760 carbonate
	saponif. ethanol acid	172	1640	1545	3275 3098	1709		1367		1223		2865 2934	1438	1084 C-O 1147
Succinyl chloride	CCl_4/ hexane	145	1640	1558	3282 3073	1728		1400	1728		1280	2865 2953	1450	1071 C-O 1122 C-O-H
LETES (0.33M)	aqu	144	1640	1551	3288 3073			1400	1734	1210 1021	1261	2865 2934		1084 C-O
Glutaryl chloride	resin	149	1646	1545	3282 3073			1368	1734	1191 1027		2865 2934	1445	
LETES (0.17M)	hexane wash	160				1728		1381	1728	1014	1261 1273	2852 2922 2960	1457	1071 C-O 1122 C-O-H
Fumaryl chloride LETES (0.17M)	CCl_4/ hexane	150			3294 3073	1728	1589		1381	1241 1008		2859 2922	1457	1109 (COOH) 1627 C=C alkene

Table A8. Interfacial reactions with aliphatic acid chlorides.