DESIGN AND SYNTHESIS OF NOVEL HYDROGELS FOR BIOLOGICAL APPLICATIONS.

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Doctor of Philosophy

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August 1994

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Submitted For The Degree
Of Doctor Of Philosophy

Mark Edward Brennan Smith
August 1994

SUMMARY

The aims of this project were:-

1) the synthesis of a range of new polyether-based vinylic monomers and their incorporation into poly(2-hydroxyethyl methacrylate) (poly(HEMA)) based hydrogel networks, of interest to the contact lens industry.

2) the synthesis of a range alkyltartronic acids, and their derivatives. These molecules may ultimately be used to produce functionalised poly(α-hydroxy acids) of potential interest in either drug delivery or surgical suture applications.

The novel syntheses of a range of both methoxy poly(ethylene glycol) acrylates (MPEGAs) and poly(ethylene glycol) acrylates (PEGAs) are described. Products were obtained in very good yields. These new polyether-based vinylic monomers were copolymerised with 2-hydroxyethyl methacrylate (HEMA) to produce a range of hydrogels. The equilibrium water contents (EWC) and surface properties of these copolymers containing linear polyethers were examined. It was found that the EWC was enhanced by the presence of the hydrophilic polyether chains. The macroscopic surface properties were investigated by measuring the surface free energies of the gels in their hydrated states. At a molecular level surface properties were probed by using *in vitro* cell adhesion studies. Results suggest that the polyether side chains express themselves at the polymer surface, thus dictating the surface properties of the gels. Consequentially, this lead to an advantageous reduction in the surface adhesion of biological species.

A synthesis of a range of alkyltartronic acids is also described. The acids prepared were obtained in very good yields using a novel four-stage synthesis. These acids were modified to give potassium monoethyl alkyltartronates. Although no polyesterification is described in this thesis, these modified alkyltartronic acid derivatives are considered to be potentially excellent starting materials for poly (alkyltartronic acid) synthesis via anhydrocarboxylate or anhydrosulphite cyclic monomers.

Keywords: **2-hydroxyethyl methacrylate, linear polyether, surface properties, alkyl tartronic acid, polymer.**

Dedicated to Mum, Dad, Dan and Becky. I love you all very much.

Also to Dr Glynn Boobyer and Mr Andrew Gould for their early inspiration.

TEACHERS CAN!

ACKNOWLEDGEMENTS

I would like to express my thanks to the following:

My supervisor, Dr Ann Jarvie, for her constant advice, encouragement and friendship throughout the duration of this work.

Professor Brian Tighe for his associate supervision.

SERC and Vickers Laboratories for their financial support.

Dr Brian Green, VG Biotech, Altrincham, for kindly running, and helping to interpret, my ESMS samples at no cost.

Dr Mike Perry for his NMR expertise. Dr Helen Fitton for completing the cell adhesion studies described in this thesis and for her enthusiasm for science. Dr Chris St. Pourcain and Dr Helen Oxley for sharing their knowledge. Steve Ludlow, Mike Lea and Lynne Store for their practical support.

Andy Hall for the especially close friendship that we have developed over the past three years.

Phil 'Make my funk a P-Funk!' Bouic for being exceedingly funky at all times and also, more importantly, for being the best man to know in a crisis.

'The Coca-Cola Company' and 'Philip Morris Products Inc.' for supplying the necessary blend of psycho-stimulants during the completion of this thesis.

Everybody in Labs 208 and 209 for their friendship and love. I'll miss you.

Finally, special thanks to Michelle for allowing me to be a part of her life and for all the love, affection and support she gave me in our time together at Aston.

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LIST OF ABBREVIATIONS

δ (NMR)	Chemical shift
DSC	Differential scanning calorimetry
DTA	Differential thermal analysis
d (NMR)	Doublet
'Ether'	Diethyl ether
ESMS	Electrospray mass spectrometry
EWC	Equilibrium water content
EGDM	Ethylene glycol dimethacrylate
γ_s^d	Dispersive component of surface free energy
γ_s^p	Polar component of surface free energy
γ_s^t	Total surface free energy
GPC	Gel permeation chromatography
HEMA	2-Hydroxyethyl methacrylate
HPLC	High performance liquid chromatography
IR	Infra-red
m (IR)	Medium
m (NMR)	Multiplet
MMA	Methyl methacrylate
m.p.	Melting point
MPEGA	Methoxy poly(ethylene glycol) acrylate
MPEGMA	Methoxy poly(ethylene glycol) methacrylate
-ve (NMR)	Negative peak
NMR	Nuclear magnetic resonance
PEG	Poly(ethylene glycol)
PEGA	Poly(ethylene glycol) acrylate
PEGMA	Poly(ethylene glycol) methacrylate
+ve (NMR)	Positive peak

q (NMR)	Quartet
s (IR)	Strong
s (NMR)	Singlet
t (NMR)	Triplet
TAAC	Tartronic acid anhydrocarboxylate
TAAS	Tartronic acid anhydrosulphite
TBDMS	*tert* - butyldimethylsilyl
TBDPS	*tert* - butyldiphenylsilyl
TEA	Triethylamine
T_g	Glass transition temperature
THF	Tetrahydrofuran
TIPS	Triisopropylsilyl
w (IR)	Weak

CHAPTER 1

INTRODUCTION.

1.1 BIOMATERIALS : A GENERAL OVERVIEW.

A biomaterial is a material designed to fulfil a purpose and to exist, without rejection, at a physiological interface. By the very nature of this definition, biomaterials research can be seen to be a vast area.

In recent years a great amount of progress has been made in the development of biomaterials for biomedical devices. One of the major growth areas has been the design and development of materials, particularly polymers, to fulfil the growing number of biomedical requirements. Some well-established examples of biomedical functions, together with the types of polymer used and the particular problems associated with each function, are summarised in Table 1.1.

Table 1.1 illustrates a number of polymers that provide a range of properties ranging from hard and glassy plastics, through hydrophobic rubbery materials, to soft water-containing hydrogel matrices. The range of materials available allows the particular requirements of prosthesis, for a variety of body sites, to be addressed.

It is apparent that the universal problem, when applying biomaterials as biomedical devices, is that of compatability. It is absolutely essential that an implanted device avoids physiological rejection at the biological interface at which it resides. Rejection can be manifested in many ways depending on the biological environment of the implanted material. For example, a material rejected at a blood interface may cause extremely serious thrombosis in a patient, whilst an inadequate contact lens material may promote tear protein and lipid deposition that will impair the quality of the lens and also give discomfort to the wearer. Development in biomaterial research, therefore, has to take account of both the bulk properties of materials required for a particular purpose and the requirement that the material must be compatible with the

environment into which it is introduced. Combining these requirements can be extremely difficult but has proved to be both necessary and challenging.

This thesis is dedicated to the synthesis of novel molecules of prospective interest to the biomaterials industry. Research has been completed in two areas:-

1) The development of new polyether-based vinylic monomers and their incorporation into poly(2-hydroxyethyl methacrylate) (poly(HEMA)) based hydrogel networks, of interest to the contact lens industry.

2) The development of new α-hydroxy acids, and their derivatives. These molecules may ultimately be used to produce functionalised poly(α-hydroxy acids) of potential interest in either drug delivery or surgical suture applications.

These disparate strands of the thesis may at first sight appear unrelated but are in fact closely related in that the properties of the polymeric products in both cases will be dominated by the polar, pendant side chains. The hydrophilicity of the pendant groups will allow the polymers, in both cases, to interact with and structure water which is a highly necessary property of a potential biomaterial.

The following sections of this thesis will introduce both of these areas of work in more detail. It is hoped that the reader will not only enjoy an historical insight into the previous work and invention completed in these areas, but will also appreciate the small, but never-the-less significant, contribution that this thesis makes to biomaterials research.

FUNCTION	POLYMER	PROBLEMS
Contact Lenses	Hydrogels	1) Biocompatability 2) Permeability 3) Mechanical properties
Surgical Sutures	Poly(α-esters) Poly(glycolic acid) Poly(lactic acid)	1) Biocompatability 2) Biodegradability 3) Mechanical properties
Drug Delivery	Poly(α-esters)	1) Biocompatability 2) Biodegradability 3) Bioerodability 4) Functionality for drug attachment
Hip and Knee Joint	High density polyethylene/ Stainless steel	1) Biocompatability 2) Wear 3) Fatigue
Breast Prothesis	Silicone rubber	1) Tissue compatability 2) Mechanical properties
Urethral prosthesis	Polytetrafluoroethylene Polyethylene terephthalate	1) Biocompatability 2) Attachment 3) Mechanical properties
Vascular Grafts	Woven or knitted polyethylene terephthalate	1) Blood compatability 2) Dynamic mechanical properties
Tendon Prosthesis Ligament Prosthesis	Nylon cord Silicone rubber cord	1) Biocompatability 2) Stress/strain behaviour 3) Ease of anchorage

Table 1.1 : Examples of the Biomedical Application of Polymers.

1.2 HYDROGELS - AN INTRODUCTION.

A hydrogel is, by definition, a cross-linked polymeric matrix which swells, but does not dissolve in water.

Wichterle and Lim[1] reported the synthesis of poly 2-hydroxyethyl methacrylate (poly(HEMA)) in 1960 and highlighted its possible biomedical applications. Subsequently, research has explored possible hydrogel applications in areas such as soft contact lenses[2], wound dressings[3] and synthetic cartilage[4].

However, it has been found that poly(HEMA) has limitations. Even a highly cross-linked poly(HEMA) matrix has relatively poor mechanical properties. Its uses are further restricted by limited biocompatability[5]. This is illustrated in its use in soft contact lenses where, even though it is found to be mechanically adequate, ocular incompatability is observed with the formation of 'white spot deposits'[6] on the lens surface. Some success has been found in improving biocompatability by copolymerising HEMA with a range of vinyl monomers. Graham[7] and Merril[8] have synthesised hydrogels containing predominantly poly(ethylene oxide) for proposed drug- release usage. These showed enhanced biocompatability.

The water maintained within hydrogels will influence the surface, mechanical and transport properties of the subsequent biomaterials. The structuring of water within hydrogels will heavily influence the biocompatability of the materials. The structuring of water in hydroxyalkyl acrylates and methacrylates[5] is discussed in the literature.

In this thesis the author intends to introduce the idea of using vinylic based polyether acrylates, copolymerised with HEMA, as potential biomaterials. Of

particular interest is the prospective use of polyether-modified hydrogels as novel contact lens materials.

The introduction will contain a description of the nature and properties of hydrogels in a more detailed fashion, and also of the role of polyethers in the biomaterials area to date.

1.3 HYDROGELS.

As a hydrogel is a cross-linked polymer matrix which swells, but does not dissolve, in water, then hydrogels can be naturally occuring eg. gelatin, semi-synthetic eg. cellulose, or entirely synthetic. Most synthetic hydrogels are based on polyhydroxymethacrylates and polyhydroxyacrylates. This particular area of work is devoted to the synthesis and copolymerisation of novel polyether acrylate derivatives, with HEMA.

Many properties of hydrogels are influenced by water maintained within the polymer matrix. The water, for example, will facilitate oxygen diffusion through the hydrogel. This is an extremely important factor when considering the development of new soft contact lens materials. Thus one of the most important properties of a hydrogel is its equilibrium water content (EWC). This is defined as:-

$$EWC = \frac{\text{Weight of water present in the hydrated gel} \times 100}{\text{Total weight of hydrated gel}}$$

Copolymerisation of HEMA with more hydrophilic monomers, such as N-vinyl pyrrolidone, will increase the EWC. Copolymerisation with hydrophobic monomers, such as styrene, will have the converse effect. Temperature, pH and the environment of the gel can also affect the EWC.

Therefore, variation in the EWC will have pronounced affects on bulk, surface, mechanical and transport properties[9] of the gel. As EWC increases, there is generally a fall in the tensile strength of the hydrogel, but simultaneously an increase in the oxygen permeability of the gel.

1.4 THE NATURE OF WATER IN HYDROGELS.

It is important to gain knowledge of the interactions between the polymer and imbibed water, to understand how the the interactions affect the surface, interfacial and transport properties of the hydrogel material.

Thus a variety of techniques have been used to study water in hydrogels[10-32] i.e. specific conductivity[10], differential scanning calorimetry (DSC)[19-24], differential thermal analysis (DTA)[25,26], dilatometry[27] and nuclear magnetic resonance spectroscopy (NMR)[28-32] under thermal conditions.

There is a large amount of evidence to suggest that water in hydrogel membranes can exist in more than one state[10-34]. Initial studies focused on cellulose acetate membranes that were of use in reverse osmosis applications. Workers generally agreed that at least two different states of water were present in such membranes. The first of these states involved 'non-freezing' or 'bound' water which was thought to interact strongly with or bind to the polymer. The second involved freezing water which had only weak interactions with the polymer.

The most widely accepted model of a hydrogel system was developed by Andrade, Jhon et al.[10,14-16,34]. This favours three distinct phases of water where X represents water bound within the polymer matrix, Y represents interfacial water and Z represents bulk water. The model has been confirmed by using a variety of the above techniques and is illustrated in Figure 1.1.

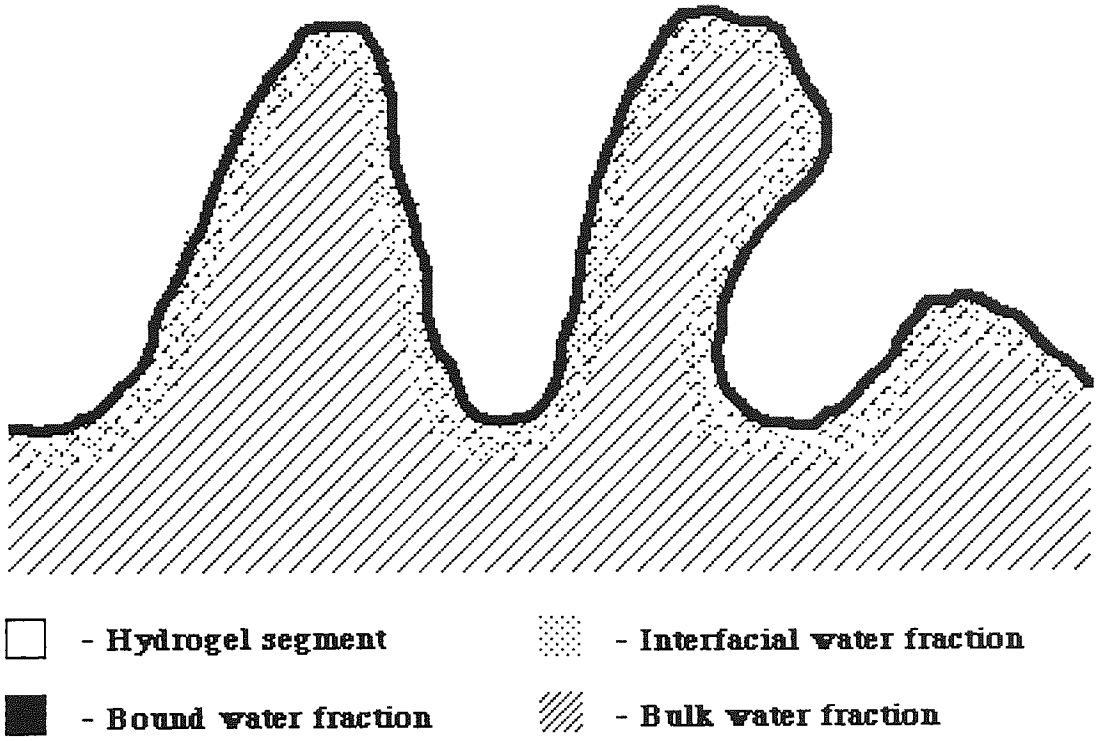


Figure 1.1 A Diagramatic Representation of the Three Phase Model of Imbibed Water

More recently it has been suggested that water bound within the polymer matrix, type X in the previous model, is not a thermodynamic phenonenon caused by 'bonding' between polymer and water[25,26,32]. Instead the authors suggest that, as the polymers studied are well below their T_g at the temperatures used for DSC studies, the 'type X' water molecules are physically prevented from diffusing to the ice crystals forming within the gel, and hence remain unfrozen[26]. Thus the observed phenomenon of 'non-freezing' and 'freezing' water is thought to be kinetic in nature as opposed to thermodynamic. It should be noted, however, that these workers have concentrated on poly(HEMA) and thus neglected the effects of variations in chemical structure on both the water binding and the T_g of the polymer.

1.5 BIOCOMPATABILITY OF HYDROGELS.

Although there is no completely reliable model to predict the biocompatability or biotolerance of a material, it is generally regarded that the surface energy may be an important consideration.

Baier *et al.* [35] suggested that blood compatability may be dependant upon a material having a moderate surface energy. They found that materials exhibiting reasonable biotolerance all had a critical surface tension in the range of 20-30 mN/m. Exceptions to this generalisation included hydrogels.

Andrade[36] proposed the hypothesis of minimal interfacial energy, which indicated that a low interfacial tension between implant material and host environment would improve biotolerance or biocompatability. However, the results from more recent studies by Andrade *et al.* [37] using HEMA:MMA (methyl methacrylate) copolymer surfaces cannot be accounted for on the basis of minimal interfacial energy. Instead, to rationalise their results, they claim an optimum balance between polar and non-polar sites is important to attain biocompatability.

This consideration of surface energy and biocompatability is discussed further in Chapters 3 and 4 of this thesis.

1.6 THE DEVELOPMENT OF POLYETHERS IN BIOMATERIALS.

It should firstly be noted that poly(ethylene glycol)s (PEGs) are defined as those poly(ethylene oxide)s having hydroxyl end groups, and a molecular weight of 20,000 or less[38] .

Poly(ethylene oxide) plays a significant role in reducing the absorption of biological species to substrates[39]. Hydrogels have been synthesised using poly(ethylene oxide) and polyurethane copolymers[7,8,40-43]. More recently poly(ethylene oxide)s have been grafted onto polyurethanes[44], poly(vinyl chloride) [45,46], HEMA : styrene copolymers[47] and poly(ethylene)[48].

Nagaoaka *et al.*, whilst studying the interaction of hydrogel copolymers based on methoxy poly(ethylene glycol) monomethacrylates and poly(vinyl chloride)[49], with blood components, have suggested that the presence of long (>100 repeating CH_2CH_2O units), flexible poly(ethylene glycol) chains at the surface restrict protein absorption and hence thrombogenic reactions. One theory is that the poly(ethylene glycol) chains provide an excluded volume which prevents protein absorption at the polymer surface. This can be illustrated using Figure 1.2 .

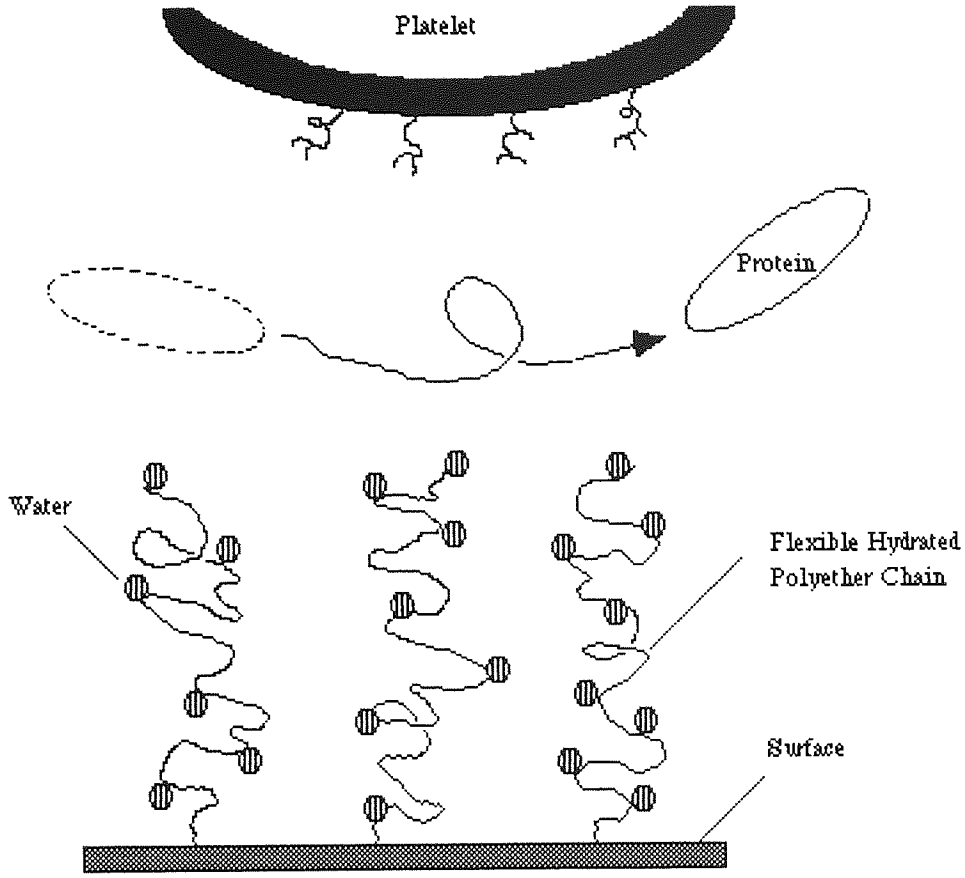


Figure 1.2. The Interaction of Blood Components with Hydrated Poly(ethylene oxide) Chains at the Polymer Surface

Andrade *et al.* have also synthesised copolymers of methoxy poly(ethylene glycol) monomethacrylates and alkyl methacrylates for use as protein resistant coatings and polymeric surfactants for the removal of proteins[50].

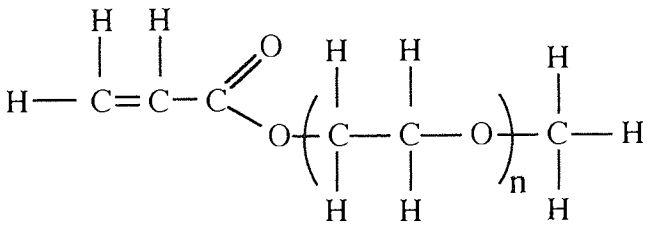
The interaction of poly(ethylene glycol)s with water have been studied by many workers using many techniques. One of the most recent studies by Schreiner *et al.* [51] showed, using proton NMR, that two water molecules are associated with each ether group. The work was carried out using poly(ethylene glycol) hydrates of molecular weight 400. Further to this Graham *et al.* [52] whilst studying poly(ethylene glycol) based hydrogels, have identified both mono and trihydrates present in the gel. They further tenuously propose that the stable trihydrate may have a helical structure. Their work, however, was carried out using predominantly PEG-6000; thus there is no

evidence to suggest that smaller chains eg. MW<1000, need necessarily adopt a similar helix. Such a factor may be significant if indeed, as illustrated in Figure 1.2, poly(ethylene oxide)s prevent absorption of protein molecules, at a polymer surface, by providing an excluded volume.

1.7 AIMS OF PROJECT WORK.

Following on from the aforementioned knowledge of polyethers in biomedicine to date, the aims of this piece of work are to:-

1) synthesise methoxy poly(ethylene glycol) monoacrylates (MPEGA)s **1** of various molecular weights.



1

MPEG350-A n= 7-8

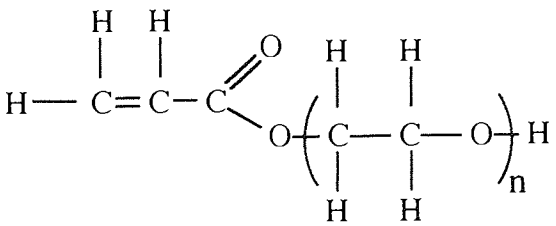
MPEG550-A n= 11-12

MPEG750-A n= 16-17

MPEG2000-A n= 44-45

MPEG5000-A n= 112-113

2) synthesise poly(ethylene glycol) monoacrylates (PEGA)s **2** of various molecular weights.



2

PEG200-A n= 4-5

PEG400-A n= 8-9

PEG600-A n= 13-14

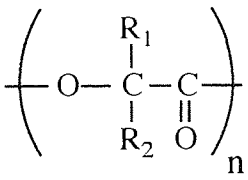
PEG1000-A n= 22-23

3) incorporate the various adducts into poly(HEMA) based hydrogels.

4) study some of the bulk and surface properties of the hydrogels produced. These studies should give some indication as to how the materials may be expected to behave at the ocular interface.

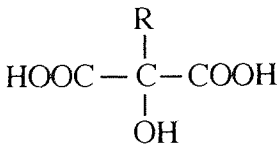
1.8 ALKYLTARTRONIC ACIDS AS INTERESTING PRECURSORS OF NOVEL POLY(α-HYDROXYACIDS).

Until fairly recently, little attention has been paid to poly(α-hydroxyacids) (poly(α-esters)). Poly(α-hydroxyacids) have the following general structure:-



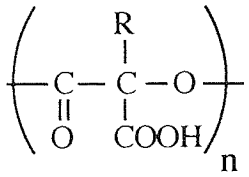
This lack of attention has been in part due to the lack of practical applications, but mainly due to the very limited number of synthetic routes available. In recent years, however, poly(glycolic acid) (R_1=R_2=H) and poly(lactic acid) (R_1=H, R_2=CH_3) have received attention as biodegradable polymers for surgical sutures, drug delivery systems and other biomedical devices. These developments have progressed hand in hand with novel polymerisation techniques designed specifically for the polymerisation of α-hydroxyacids.

Alkyltartronic acids have the following general structure:-



3

They therefore contain two carboxyl functions. If the acid could be polymerised linearly, such that only one carboxyl function per molecule formed an ester linkage, then a functionalised poly(α-hydroxy acid) could be synthesised. Such a polymer would have the following repeat unit:-



4

It is anticipated that a range of poly(alkyltartronic acid)s **4** could have the following interesting properties:-

1) the polymers obtained would have pendant carboxyl groups that would induce functionality in the polymer backbone. This induced functionality could make polymers and copolymers of alkyltartronic acids potentially valuable as say drug-carrying matrices.

2) the presence of pendant carboxyl chains would make these polymers hydrophilic. Hydrophilicity is an important consideration in biomaterial or biomedical application, as the ability of materials to interact favourably in an aqueous environment, *in vivo*, tends to induce a greater biotolerance at a biological interface. The hydrophilicity of the polymers could be controlled by the nature of the alkyl group, also pendant to the polymer chain.

3) the juxtaposed nature of the pendant alkyl and carboxyl groups means that there will be an inductive effect on the acid functions of the polymers by the alkyl groups. The presence of the carboxyl groups themselves will make the solubility of the polymer pH-dependent. The small changes in inductive

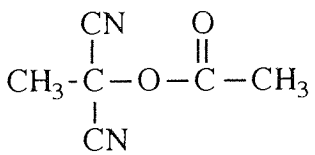
effects observed by altering the nature of the pendant alkyl groups should create a series of polymers with slightly different, but very precise, pH-dependent solution properties.

1.9 THE SYNTHESIS OF TARTRONIC AND ALKYLTARTRONIC ACIDS.

The synthesis of tartronic acid itself, (**3**; R=H), is very well documented. The first recorded synthesis was made by Dessaignes[53] in 1852 by treating tartaric acid with fuming nitric acid and phosphorus pentoxide. This method was modified by Osten[54] in 1905, and is the most quoted in the related literature.

Other syntheses quoted by Gruber[55] and Kekule[56] pass through a dioxytartaric acid intermediate. Dobinson[57] offered a route to tartronic acid via the ozonisation of malonic acid in aqueous solution, although this led to a mixture of products and purification involved tedious elutions from an ion exchange column.

Although the synthesis of tartronic acid is well documented, the synthesis of alkyltartronic acids is not. Bardroff[58] describes the synthesis of methyltartronic acid (**3**; R=CH_3) using 1,1- dicyanoethyl acetate **5** as a starting material.



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By conversion of the cyano groups to amide functions and by subsequent hydrolysis of both the amide functions and the acetate ester, the desired product was obtained.

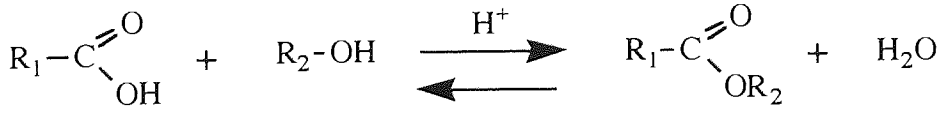
The only general synthetic pathway to alkyltartronic acids **3** was published by Grandjean[59] in 1969. He describes a four stage synthesis from which he was able to obtain **3** for R= ethyl, propyl, butyl, pentyl, hexyl, heptyl, isopentyl, cyclohexyl and benzyl. This publication is not only extremely lacking in experimental detail but also only allows for synthesis on a very small scale, for reasons that will become evident in Chapter 5 of this document.

A major objective of this thesis is to develop a novel general synthesis for alkyltartronic acids **3**, that would allow the creation of a range of products in bulk quantities and in good yields.

1.10 NOVEL DERIVATIVES OF ALKYLTARONIC ACIDS

Alkyltartronic acids **3** provide 'building blocks' from which novel functionalised polyesters could potentially be obtained. In this section, however, the author will demonstrate that these hydroxy acids can be further developed into theoretically more suitable monomers. To illustrate this an understanding of the existing methods available for the synthesis of poly(α-hydroxy acid)s, and their historical application to similar systems, must be obtained.

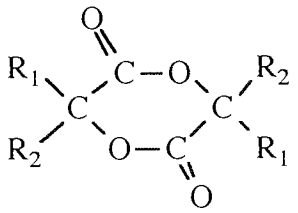
The synthesis of esters by direct combination of a carboxylic acid and an alcohol, in the presence of a mineral acid catalyst, is historically well documented and is perhaps the classic method of esterification:-



It would seem reasonable that this synthetic technique could be applied to the polymerisation of hydroxy acids, as such acids contain both carboxyl and hydroxyl

functions and the desired product is a polyester. However, this synthesis is reversible and this often leads to unfavourable equilibrium reactions between monomer, eliminated water and polymer resulting in a product with a very low molecular weight.

In the prescence of a dehydrating agent, such as H_2SO_4, α-hydroxyacids dimerise to form the relevent six-membered glycolide. Glycolides have the following general structure:-



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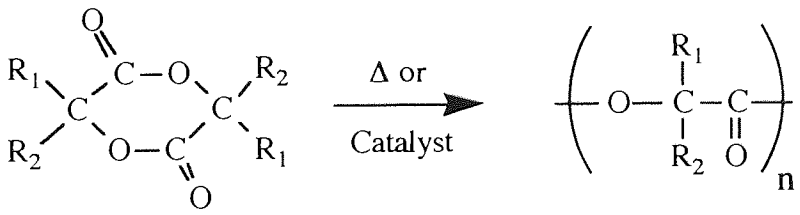
Polymerisation of both glycolide (**6**; R_1=R_2=H) and lactide (**6**; R_1=H, R_2=CH_3) occurs readily using heat and zinc chloride[60-62].



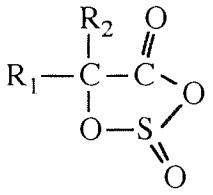
6

For 1,1,4,4-tetramethylglycolide (**6**; R_1=R_2=CH_3), no polymerisation was seen to occur[63] using similar conditions. Delbig[64] reported later that 1,1,4,4-tetramethylglycolide could be polymerised at elevated temperatures using lithium tertiary butoxide as a catalyst although there was substantial doubt as to the authenticity of this claim. Generally, increasing the substitution on the glycolide ring reduces the polymerisability of the ring[65]. This is primarily due to a competitive proton abstraction from the β-carbon atoms of ring substituents that causes

dehydration of the hydroxy acid molecules that constitute the ring. The result is that the ring will fall apart leading to either a complete failure in polymer synthesis, or at best the production of a contaminated polymer of very low molecular weight.

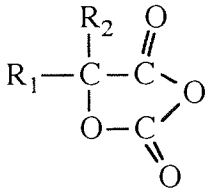
It can be seen, therefore, that the ring-opening of glycolides **6** is a very restrictive technique and that more general synthetic routes, specifically designed for the production of poly(α-hydroxy acid)s, have had to be developed.

In a series of papers[66-70] the use of the decomposition of α-hydroxy acid anhydrosulphites **7**:-



7

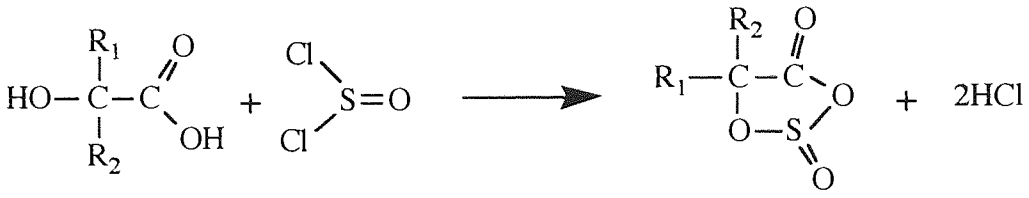
and α-hydroxy acid anhydrocarboxylates **8**:-



8

as routes to poly(α-hydroxy acid)s, has been described.

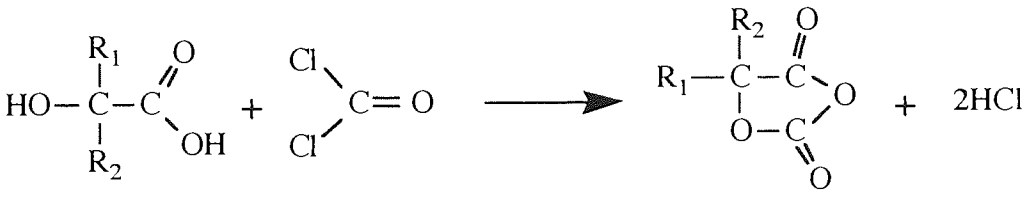
Anhydrosulphites **7** are synthesised by the action of thionyl chloride on an α-hydroxy acid:-



7

Blaise and Montague[71] first reported the synthesis of the anhydrosulphites **7** of both lactic acid (**7**; R_1=H, R_2=CH_3) and α-hydroxy isobutyric acid (**7**; R_1=R_2=CH_3).

Anhydrocarboxylates **8** are synthesised by the action of phosgene on an α-hydroxy acid:-

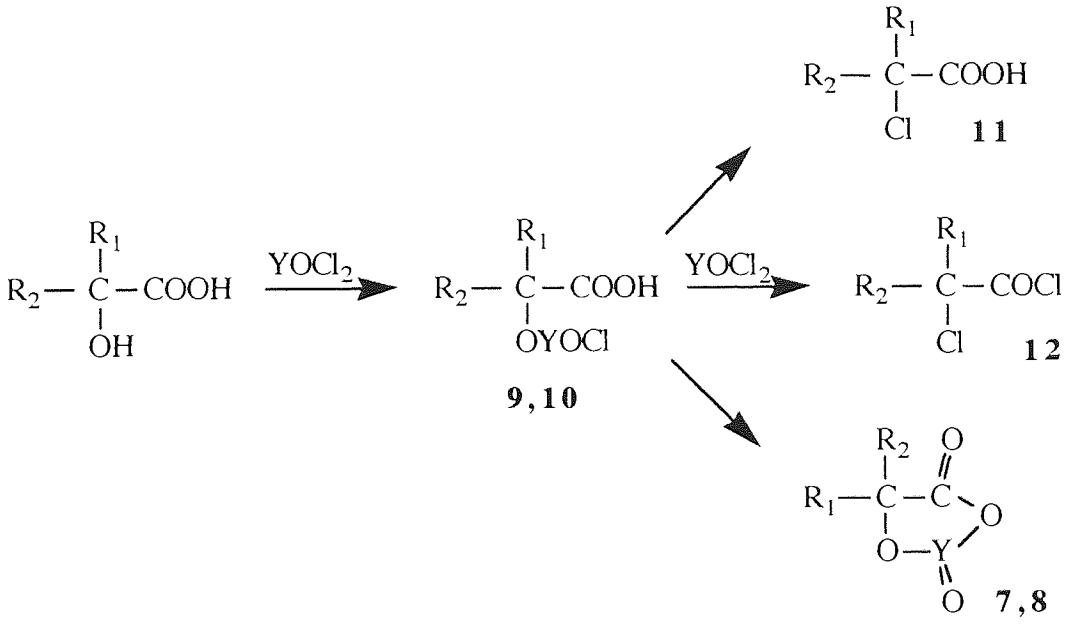


8

Davies[72] first published the syntheses of anhydrocarboxylates **8** of glycolic (**8**; R_1=R_2=H), lactic (**8**; R_1=H, R_2=CH_3) and α-hydroxy phenylacetic acid (**8**; R_1=H, R_2=C_6H_5). He described the purification of these products by recrystallisation from low boiling, anhydrous solvents such as ether. Tighe's[73] attempts to reproduce the work of Davies were fairly successful although it was found that recrystallisation was not an adequate technique when pursuing products of high purity. Modifications were hence made to the synthetic procedure to reduce the amount of impurity present in the crude products.

The synthesis of both anhydrosulphite **7** and anhydrocarboxylate **8** via direct combination of the α-hydroxy acid with either thionyl chloride or phosgene appears to pass through the alkyl chlorosulphinate **9** or alkyl chloroformate **10** respectively.

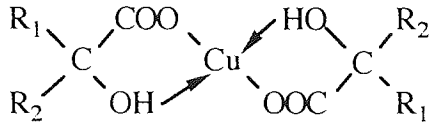
Attack by thionyl chloride or phosgene occurs, therefore, at the α-hydroxyl group of the acid with the evolution of HCl. The desired cyclic product then forms by reaction of the carboxyl group with either the chlorosulphinate or chloroformate function with further evolution of HCl. Unfortunately, in both systems, side-reactions do occur producing both α-chloro acid **11** and α-chloro acid chloride impurities **12**. This can be demonstrated schematically as follows:-



Where Y= S or C

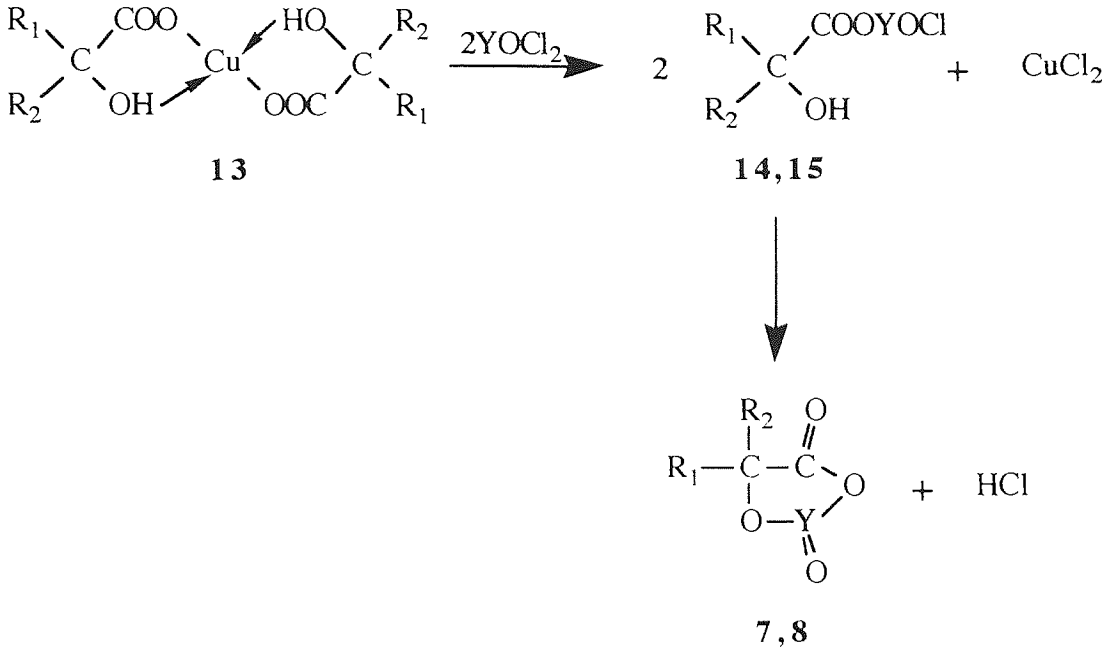
Such impurities interfere with the polymerisation of anhydrosulphite **7** and anhydrocarboxylate **8** monomers and hence must be removed. Thomas[74] concluded that the most rapid and efficient way of removing chlorinated impurities is by stirring the crude products, in anhydrous ether, with baked silver oxide. The resulting silver chloride is then filtered off. Anhydrosulphites **7** thus obtained can then be distilled, under vacuum, to remove the parent acid impurity. Anhydrocarboxylates **8** can be similarly purified using vacuum sublimation.

Although, generally, these cyclic anhydrides can be purified using the above techniques there is a desire to reduce chlorinated impurities to a minimum in the actual synthesis of the cyclic monomers. The use of the metal carboxylate, most commonly the copper (II) salt **13**:-



13

in the synthesis of both anhydrosulphite **7** and anhydrocarboxylate **8** monomers, has advantages over the direct use of α-hydroxy acids. As mentioned earlier, direct combination of the hydroxy acid with either thionyl chloride or phosgene initially forms the alkyl chlorosulphinate **9** or alkyl chloroformate **10** respectively. It was also shown that this intermediate could either proceed to form the desired cyclic anhydride or alternatively unwanted chlorinated impurities. It is generally considered that the mechanism of reaction between thionyl chloride, or phosgene, and the metal carboxylate **13** of the α-hydroxy acid differs from that when using the parent acid. The suggestion is that the thionyl chloride or phosgene attacks at the carboxylate ion rather than the α-hydroxyl group to form the acyl chlorosulphinate **14** or acyl chloroformate **15** respectively, with the elimination of the metal chloride. The anhydrosulphite **7** or anhydrocarboxylate **8** is then formed by elimination of HCl from either the acyl chlorosulphinate **14** or acyl chloroformate **15**. This can be shown schematically as follows:-

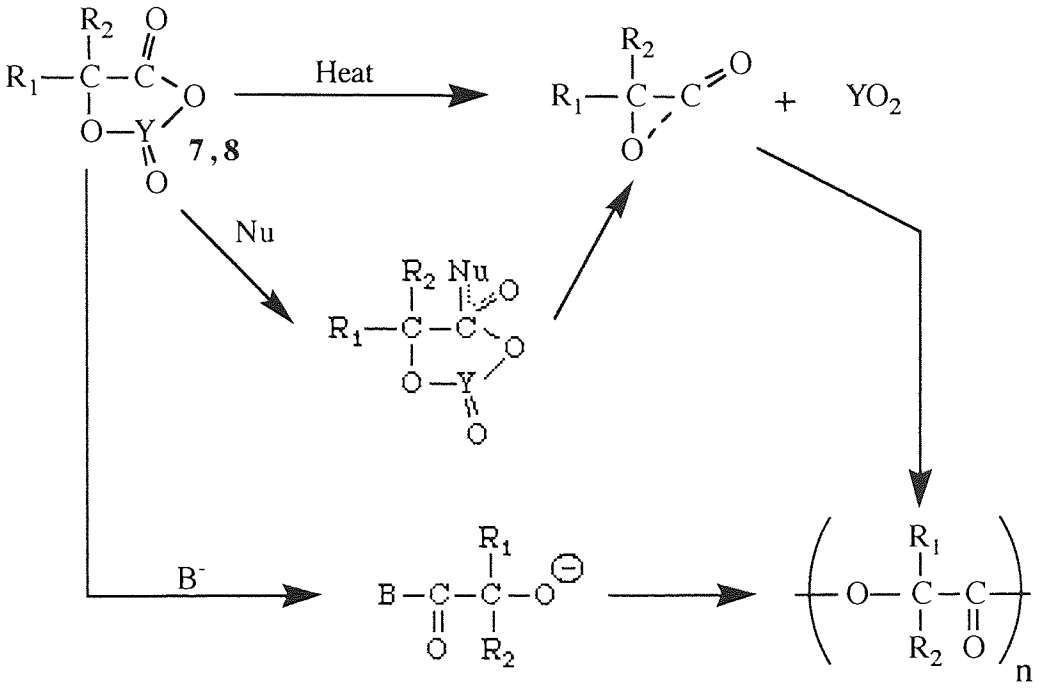


13 14,15 7,8

Where Y= S or C

Since acyl chlorosulphinates **14** and acyl chloroformates **15** are rather more stable than their alkyl analogues **9,10**, the former are less likely to eliminate sulphur dioxide, or carbon dioxide, to form chlorine containing impurities, before the desired ring closure can occur. Cyclic anhydride products obtained using the α-hydroxy carboxylate **13**, as a starting material, will therefore be of a higher purity, and in higher yields, than for analogous systems employing the unadulterated α-hydroxy acid. The crude products can still be purified using the procedures introduced earlier in this section..

Finally the pure cyclic anhydride monomers are allowed to decompose, under controlled conditions, to form poly(α-hydroxy acid)s. There have been three principle mechanisms postulated for the decomposition of both anhydrosulphites **7** and anhydrocarboxylates **8**. These are thermal decomposition[69], tertiary base initiated polymerisation[75] and hydroxyl initiated polymerisation[76], which can be shown schematically as follows:-



Where Y= S or C

Nu= Tertiary Base e.g. Pyridine, Triethylamine

B^-= Strong Base e.g. Hydroxyl, Alkoxide

Both anhydrosulphites **7** and anhydrocarboxylates **8** are extremely moisture sensitive. Interaction with moisture causes the cyclic anhydride to largely revert back to its parent acid. It is therefore imperative that both the synthesis of the cyclic monomers and the polymerisation is carried out with anhydrous reagents in an ultra-dry environment.

Al-Mesfer and Tighe[77] published a substantial paper describing the formation of polymers of tartronic acid (**3**; R=H). They acknowledge the potential appeal of such functionalised polymers in biomedical applications. The paper describes the initial synthesis of both tartronic acid anhydrosulphite (TAAS) (**7**; R_1=COOH, R_2=H) and tartronic acid anhydrocarboxylate (TAAC) (**8**; R_1=COOH, R_2=H) monomers, and their purification. Attempts to polymerise these monomers are also described. The similarities between alkyltartronic acids and tartronic acid itself are obvious. Important

deductions can therefore be made from this report that can be directed to the synthesis of alkyltartronic acids and their derivatives.

Synthesis of TAAS was carried out by direct combination of tartronic acid with thionyl chloride. TAAC was prepared by reacting the parent acid with phosgene. As discussed earlier in this chapter, the presence of chloride containing impurities, in the synthesis of these cyclic anhydrides, is minimised by using the copper(II) salt of the hydroxy acid **13**. However, the authors observed that this was not applicable in the case of tartronic acid due to the presence of two equivalent carboxyl groups in the parent acid. In other words it was impossible to react one of the carboxyl functions without first protecting the other. The products obtained were purified somewhat by stirring with baked silver oxide. It is normal procedure to further purify anhydrosulphites **7** by vacuum distillation and anhydrocarboxylates **8** by vacuum sublimation. It was found, however, that the high boiling point of TAAS, coupled with its low thermal stability, precluded the use of vacuum distillation as a purification technique. Distillation at the required temperature lead to the complete decomposition of the anhydrosulphite. Similar difficulties arose in the attempted purification of TAAC by vacuum sublimation. Attempts to polymerise these cyclic anhydrides of tartronic acid were therefore made without further sample purification.

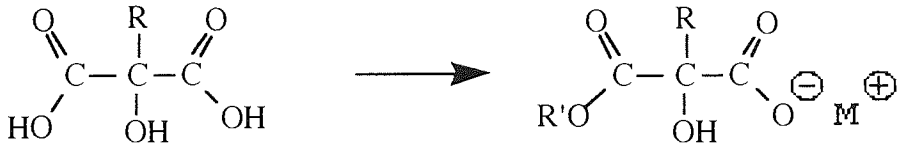
Ring-opening polymerisations of both TAAS and TAAC were carried out thermally and catalytically. Results showed that some polymer was obtained on polymerisation of TAAS. Attempts to polymerise TAAC were more successful. The TAAC ring appeared to be more stable than its related anhydrosulphite thus allowing greater control over ring decomposition. The weight average molecular weights of these polymers were found to be typically in the range of 3000-4000 Daltons.

Many of the polymers formed by Al-Mesfer and Tighe[77] were, however, discoloured and impure. It is clear that this synthetic technique could be used as the

basis for producing polymers of both tartronic acid and alkyltartronic acids. However, the production of polymers of sufficient purity to be of biomedical interest would require improvements to be made to the system.

Therefore, a further objective of this project was to investigate ways of 'tailoring' alkyltartronic acids, so that cyclic anhydrides and polymers, of potentially greater purity, might be prepared. This could overcome, somewhat, the difficulties encountered in monomer purification by Al-Mesfer and Tighe[77].

It was considered that conversion of the alkyltartronic acids **3** to their monoalkyl esters **16** prior to cyclic anhydride synthesis would be beneficial:-



3 **16**

The protection of one carboxyl function, as an alkyl ester, would allow the activation of the other carboxyl group by conversion to the metal carboxylate **16**. This would encourage the formation of acyl intermediates during a prospective cyclic anhydride synthesis and could therefore reduce the concentration of unwanted chlorinated impurities in the final product. In addition to this, the presence of an ester, rather than a carboxyl-function, on the cyclic anhydride ring, should significantly reduce the boiling points of prospective anhydrosulphites and melting points of anhydrocarboxylates. This might allow the purification of cyclic anhydride samples by either vacuum distillation or vacuum sublimation. These techniques were precluded in the work of Al Mesfer and Tighe.

1.12 ENZYMES IN ORGANIC SOLVENTS : A POSSIBLE FUTURE APPROACH TO THE POLYMERISATION OF ALKYLTARTRONIC ACIDS ?.

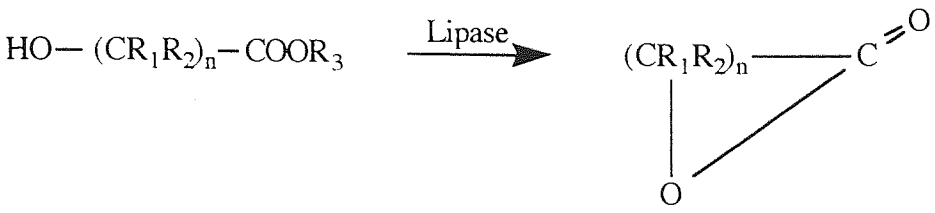
Enzymes offer a natural source of extremely powerful catalysis and can induce high levels of functional, regio- and stereo- selectivity in product formation where purely chemical approaches would induce none.

Research over the last twenty years, most particularly the last decade, has shown that many enzymes retain their activity in practically anhydrous solvents. Comprehensive reviews on this subject have been published by Klibanov[78] and Dordick[79]. The hypothesis proposed, to account for this activity, is that to maintain the enzyme in its catalytic conformation requires only a thin hydration layer of water. It is proposed that this layer effectively buffers the enzyme from the bulk organic solvent, so preventing enzyme denaturisation.

Lipases, esterases, proteases and carbohydrases have been used successfully, in aqueous solutions, to catalyse hydrolytic reactions. The absence of bulk water, in nearly anhydrous solvents, allows the reverse reactions to occur. This has led to a variety of syntheses in high yields, including esterification[80-84], transesterification[85-88] and lactonisation[89,90].

In 1984, Gatfield first noted that when certain hydroxy acids were exposed to the lipase of *Mucor miehei* , lactones were formed[80]. This observation was subsequently confirmed by Yamada[89] who reported the enzymically-catalysed lactonisation of ω-hydroxy acid methyl esters. More recently still, Gutman *et al.* [90] found that porcine pancreatic lipase, in anhydrous solvents, catalysed the lactonisation of a number of esters of γ-hydroxy acids with high degrees of stereospecificity.

These intramolecular esterifications can be generally represented as follows:-

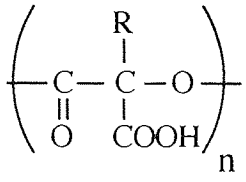


It should be noted, however, that these observed lactonisations were carried out in very dilute solutions. This condition is designed to promote an intramolecular esterification reaction. No data was provided for reactions completed in more concentrated solutions, where it would seem likely that intermolecular reactions would take precedence.

It is postulated that enzymically-catalysed intermolecular esterification reactions, involving hydroxy acids, should lead to oligomeric linear ester structures and may ultimately lead to polyester formation. Extending previous observations that have been made on biocatalytic esterifications in anhydrous solvents these reactions should also be highly stereo- and regiospecific.

It was highlighted in previous sections of this chapter, that existing methods for producing poly(α-hydroxy acid)s, other than poly (glycolic acid) or poly(lactic acid), relied on the initial formation of a cyclic anhydride monomer. The monomer was then subsequently converted, either thermally or catalytically, into the relevant polyester by ring-opening polymerisation. The difficulties encountered in the preparation and purification of these cyclic monomers, and in controlling the final polymerisation, were evident. Biocatalysis may have the potential to provide an alternative to these existing methods. Polymer products obtained enzymically would be particularly useful in biomedical applications as they would theoretically be easier to purify and hence ultimately free of physiologically harmful by-products.

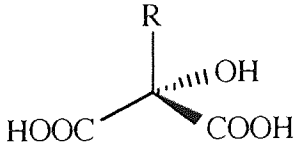
The work described in this thesis is primarily concerned with the development of novel alkyltartronic acids **3** and their derivatives. The ultimate goal is to be able to synthesise linear polyesters from these parent acids such that the repeat unit of the polymer would have the following structure:-



4

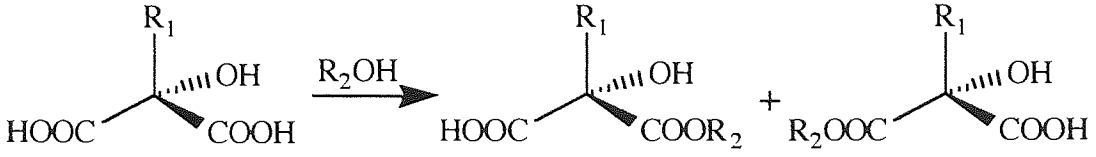
As was suggested earlier, these polymers would be extremely interesting because of the functionality that the pendant carboxyl groups would introduce *per se* . This functionality could be exploited by attaching other molecules, for example drug moities, to the polymer backbone. To achieve linearity (avoid branching) in any poly(alkyltartronic acid) **4** synthesised it is imperative that only one carboxyl group per acid molecule reacts with the hydroxyl of another. It is considered that this could be achieved enzymatically because of the stereochemical properties of alkyltartronic acid molecules.

Consider the structure of any alkyltartronic acid:-



3

The molecule is achiral. However, the two carboxyl groups can be made to differ if only one undergoes reaction. That will lead to enantiomeric products:-



The carboxyl functions are therefore said to be enantiotopic and the whole alkyltartronic acid molecule is described as being prochiral. Previous discussion in this section has highlighted the stereospecificity of biocatalysed ester synthesis in anhydrous solvents. The stereochemical inequivalence of the carboxyl groups, in alkyltartronic acids, should therefore be distinguished by an enzyme, in a successful polyesterification, such that only one of the carboxyl functions should react per acid molecule. This would lead to polyester products with desired linearity and pendant carboxyl functionality.

Studies on the biocatalysed reactions of a variety of α-hydroxy acids are currently in progress in these laboratories. In these studies the researchers will investigate the effect on the reactions of parameters such as solvent selection and reactant solution concentration, as well as investigating the activity and stereoselectivity of a variety of enzymes.

1.13 AIMS OF PROJECT WORK.

The aims of this project were to:-

1) repeat Grandjean's[59] synthesis of alkyltartronic acids **3** introducing the experimental detail that was absent in the initial publication. It materialised that this method is only suitable for synthesis on a very small scale.

2) develop a novel general synthesis for alkyltartronic acids **3** that would allow the creation of a range of products in bulk quantities and in good yields.

3) synthesise 'tailored' derivatives of alkyltartronic acids **3** that would be potentially more useful as precursors to poly(alkyltartronic acid)s **4**, with the polymerisation techniques currently available. More specifically, this 'tailoring' had as its ultimate goal the development of a cleaner, and more productive, synthesis of the cyclic anhydride monomers discussed earlier in this Chapter.

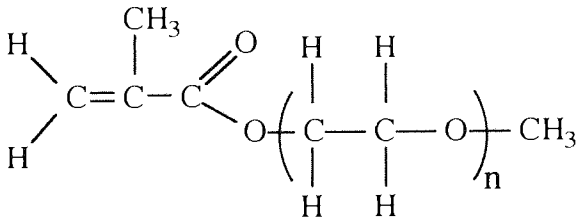
CHAPTER 2

THE SYNTHESIS OF A RANGE OF LINEAR POLYETHER ACRYLATES.

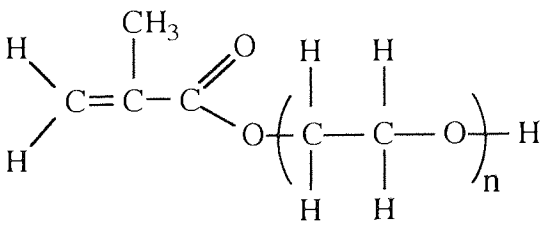
2.1 GENERAL INTRODUCTION.

As illustrated in Chapter 1, linear polyethers have been shown to inhibit the absorption of blood proteins onto alkyl methacrylate/ poly(ethylene glycol) monomethacrylate devices[50]. One theory postulated to explain this observation, is that the polyether side chains produce an excluded volume, which is a barrier to such protein absorption.

Oxley[91], a former colleague at this University, embraced these observations and proceeded to incorporate a range of methoxy poly(ethylene glycol) methacrylates (MPEGMAs) and poly(ethylene glycol) methacrylates (PEGMAs) into poly(2-hydroxyethyl methacrylate) (poly(HEMA)) based hydrogel networks:-



Methoxy poly(ethylene glycol) methacrylate



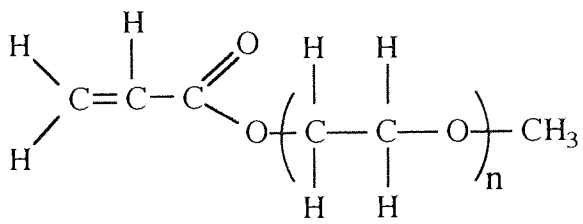
Poly(ethylene glycol) monomethacrylate

The incorporation of both MPEGMAs and PEGMAs individually, into hydrogel networks, allowed her not only to investigate the general effect of introducing

polyether side-chains into such networks, but also enabled her to make a comparison in behaviour between chains that were methoxy terminated and those that were hydroxy terminated.

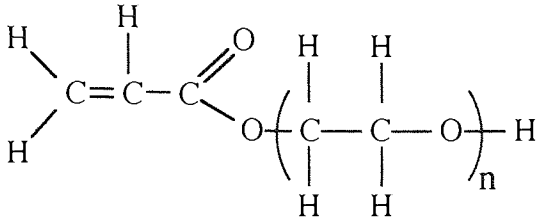
It was hoped that the anti-thrombogenicity observed when using hydrogels, with long polyether side-chains, at a blood interface could mean that related materials might be of interest in an ocular environment. One of the major goals in soft contact lens development, is the reduction to a minimum of protein and lipid absorption onto the lens surface. As explained earlier in Chapter 1, such absorption can cause discomfort to the wearer, and with time may impair the quality of the lens. Oxley demonstrated that the incorporation of linear polyethers into poly(HEMA) based hydrogels, seemingly improved the properties of those hydrogels with a view to soft contact lens applications.

As a continuation of the investigations of Oxley, it was considered that the behaviour of methoxy poly(ethylene glycol) acrylates (MPEGAs) **1** and poly(ethylene glycol) acrylates (PEGAs) **2** would also be of interest. These compounds are the acrylate analogues of the polyether methacrylates used by Oxley.



1

Methoxy poly(ethylene glycol) acrylate

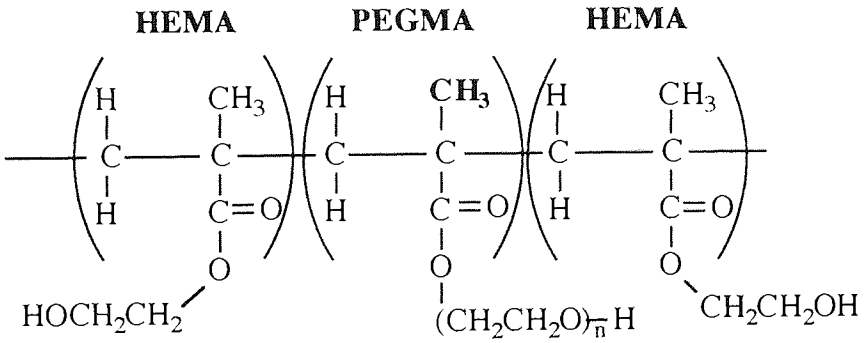


2

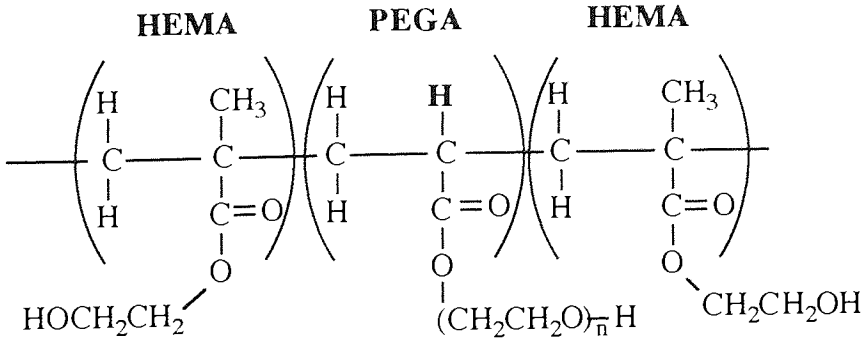
Poly(ethylene glycol) monoacrylate

These acrylate esters provide a different method of incorporating pendant polyether chains into a vinylic-based hydrogel network. Although similar to the methacrylate, subtle differences in behaviour may be observed when the acrylate is copolymerised with HEMA.

In the case of PEGMAs, for example, a typical section of the copolymer may be represented as:-



A typical section of the analogous copolymer containing a PEGA **2** may be represented by:-



Comparing the two it can be seen that the polyether methacrylate introduces an additional methyl group (highlighted), pendant to the carbon-carbon backbone of the polymer, that is not present in the case of the acrylate. This additional methyl group will create a steric barrier to rotation, about the carbon-carbon backbone, for the polymer unit containing the pendant polyether group. This barrier would not be present in the case of a polyether acrylate **1**, **2**. An increased freedom of rotation about the backbone of the polymer for the polyether acrylate unit should lead to a greater exclusion volume to protein absorption being produced by the polyether chain.

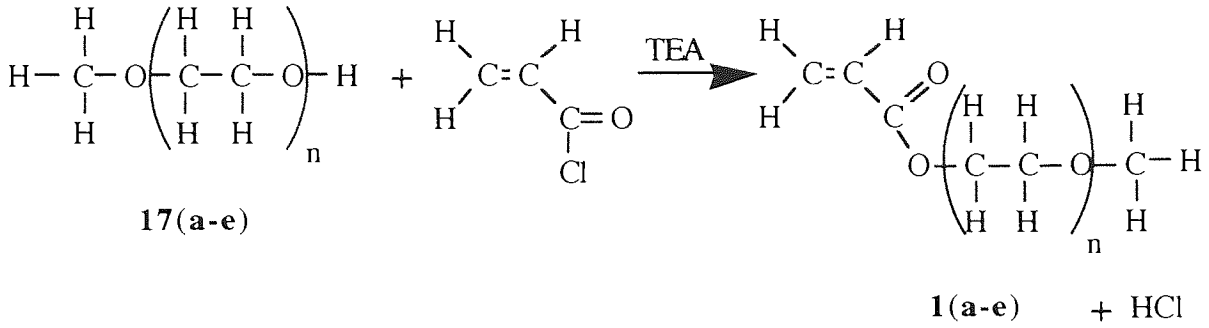
Although MPEGMAs and PEGMAs can be bought commercially, and were so by Oxley, no manufacturers of MPEGAs **1** and PEGAs **2** could be found. A synthesis for both methoxy- and hydroxy-terminated polyether acrylates had to be developed. Unfortunately it was found to be impossible to buy poly(ethylene glycol) samples of the required molecular weight, to form a series of polyether acrylates that would be directly comparable with the polyether methacrylates bought and employed by Oxley.

The incorporation of polyether acrylate monomers **1,2** into poly(HEMA) based hydrogel networks will be discussed in subsequent chapters. The novel synthesis of these monomers will be discussed in the remainder of this chapter.

2.2 THE SYNTHESIS OF METHOXY POLY(ETHYLENE GLYCOL) ACRYLATES (MPEGAs).

2.2.1 Introduction

A novel one step synthesis of MPEGAs can be represented using the scheme:-



17(a-e)

1(a-e) + HCl

Where:-

17(a) = Methoxy poly(ethylene glycol)-350 (MPEG-350)

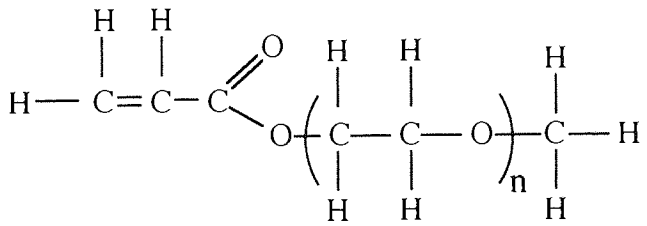
17(b) = MPEG-550

17(c) = MPEG-750

17(d) = MPEG-2000

17(e) = MPEG-5000

This process was used to synthesise the following range of MPEGAs **1**:-



MPEG350-A **1(a)** n= 7-8

MPEG550-A **1(b)** n= 11-12

MPEG750-A **1(c)** n= 16-17

MPEG2000-A **1(d)** n= 44-45

MPEG5000-A **1(e)** n= 112-113

2.2.2 Synthesis of Methoxy Poly(ethylene glycol) Acrylates **1(a-e)**.

A methoxy poly(ethylene glycol) (MPEG) **17(a-e)** and triethylamine were dissolved in dichloromethane. The solution was then cooled to -78°C using a dry ice/acetone bath. Under continuous stirring, acryloyl chloride was added to the mixture dropwise over a thirty minute period. The temperature of the reaction mixture was then allowed to rise to room temperature and the mixture was stirred for a further twenty four hours.

The triethylamine hydrochloride precipitate formed was filtered off. The filtrate was then washed with 1M hydrochloric acid to remove any excess triethylamine, and with 1M aqueous sodium hydroxide and 10% aqueous sodium hydrogen carbonate to remove any excess acryloyl chloride as sodium acrylate and any excess methoxy poly(ethylene glycol) **17(a-e)**. The organic layer was then dried over anhydrous magnesium sulphate.

Removal of the dichloromethane, under vacuum, gave the methoxy poly(ethylene glycol) acrylate (MPEGA) **1(a-e)**. The products obtained using this process were analysed using conventional techniques, and also by Electrospray Mass Spectrometry (ESMS). This relatively new technique will be discussed in detail in Section 2.4.

The products were shown to be essentially pure and were obtained in excellent yields:-

MPEG350-A **1(a)**	85%
MPEG550-A **1(b)**	87%
MPEG750-A **1(c)**	86%
MPEG2000-A **1(d)**	86%
MPEG5000-A **1(e)**	93%

2.3 THE SYNTHESIS OF POLY(ETHYLENE GLYCOL) ACRYLATES (PEGAs).

2.3.1 Introduction

Poly(ethylene glycol)s, or PEGs **18**, have the general formula:-

$HO\text{-}(CH_2CH_2O)_n\text{-}H$

18

They possess two terminal hydroxyl functions.The aim was eventually to introduce such systems of varying molecular weight and hence chain length, into poly(HEMA) based hydrogels as acrylate derivatives. Further to this, the derivatives would be monoacrylates which would enable the analysis of the effect of a free polyether pendant group on hydrogel properties, and at the same time allow a comparison to be made between hydroxy-terminated polyether pendant chains and the methoxy-terminated analogues discussed in Section 2.2.

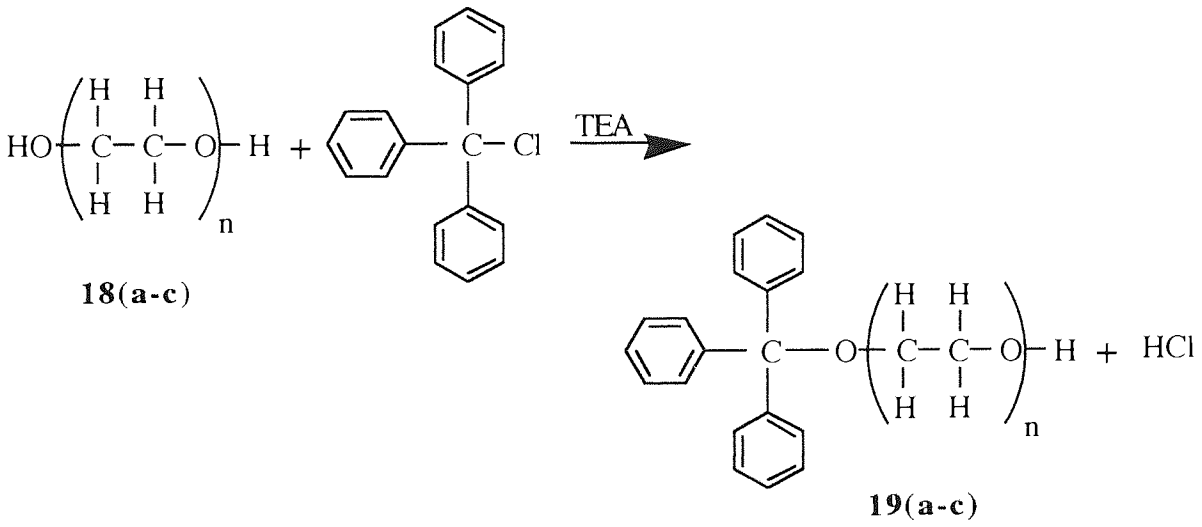
A problem arises when mono-substituted derivatives of PEGs are to be prepared since, except for low molecular weight oligomers (MW< 200), it is not possible to use a large stoichiometric excess of PEG in the reaction mixture to avoid formation of disubstituted products. In fact, final products would not be sufficiently differentiated from the starting materials, and thus would be impossible to isolate.

Dal Pozzo *et al.* [92] produced a solution to this problem. They suggested that reaction of a large stoichiometric excess of PEG-1000, where 1000 is the M_r of the PEG, with trityl chloride, a commonly used protecting group for alcoholic functions in the synthesis of carbohydrates[93,94], would produce a monotrityl ether derivative. Separation was possible because the trityl group was sufficiently large and different in properties to the polyether chain. A variety of functional groups were reacted with the other hydroxyl group. Then by removing the trityl group, they were able to produce a variety of 'monofunctional PEGs'.

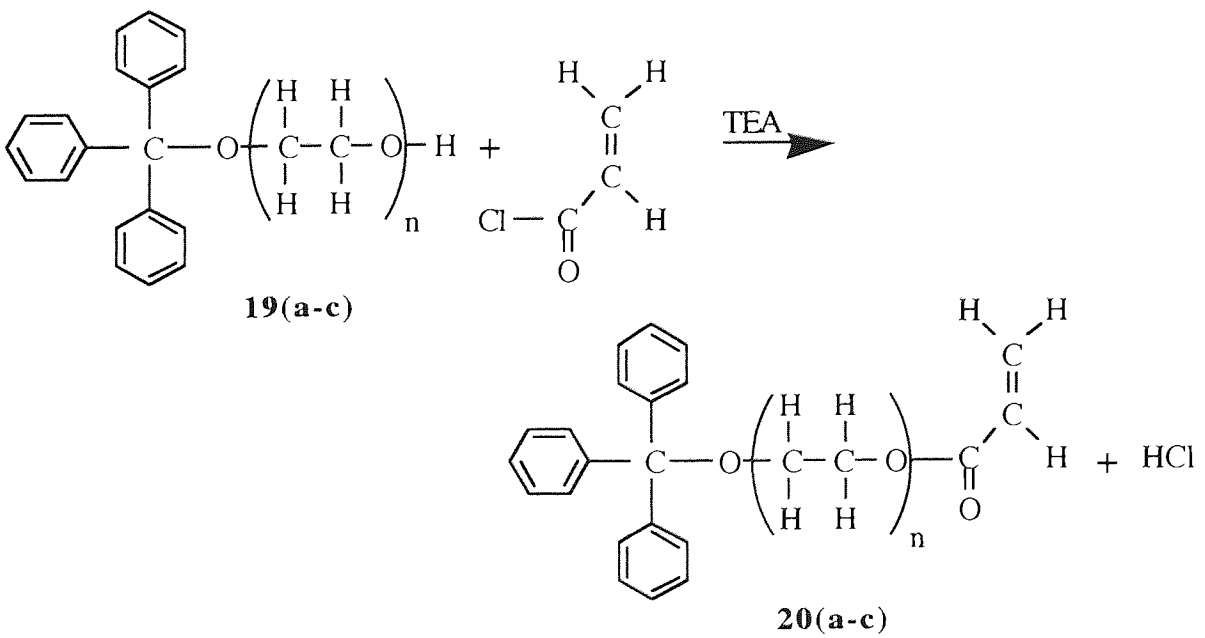
Initial work in producing poly(ethylene glycol) acrylates (PEGAs) **2** therefore, involved the use of a trityl protecting group. Although monotritylethers were successfully isolated **19(a-c)**, and an acrylate group attached to the remaining hydroxyl-group **20(a-c)**, problems were encountered in attempting to cleave the trityl

group. Dal Pozzo used 1M HCl to cleave the trityl group. When this method was applied to our systems, it was found that although cleavage was successful, simultaneous hydrolysis of the acrylate ester linkage occurred which was highly undesirable.

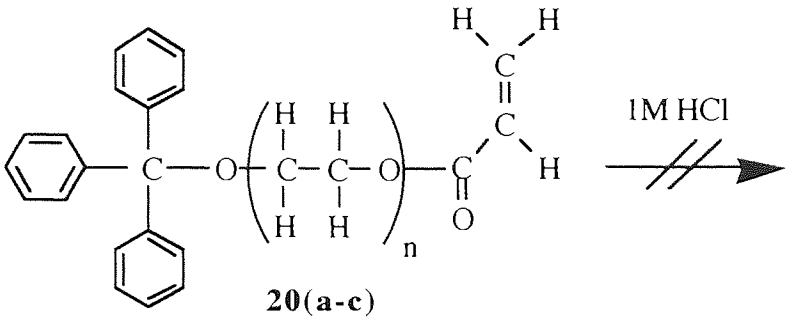
This can be illustrated using the following scheme:-



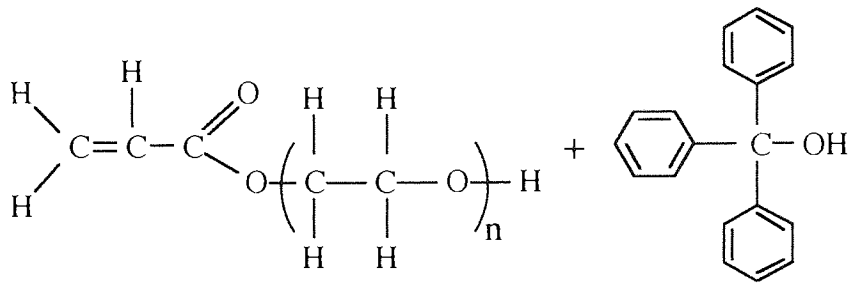
18(a-c) **19(a-c)**



19(a-c) **20(a-c)**



20(a-c)



2(a-c)

Where:-

18(a) = Poly(ethylene glycol)-200 (PEG-200)

18(b) = PEG-400

18(c) = PEG-1000

Lehrfield[95] reported the detritylation of some carbohydrate derivatives using a silica gel column. He observed that cleavage occurred on the column and that the carbohydrate and triphenylcarbinol could be eluted separately using different solvent mixtures. This method was found to cause trityl cleavage but that again some cleavage of the acrylate ester occured.

Although Dal Pozzo's system is basically sound, a more efficient protecting group was sought. There were three criteria for this protecting group:-

1) it must be stable to mildly acidic and basic conditions to avoid cleavage during aqueous work-up.

2) cleavage must occur under milder conditions than for the trityl group.

3) it must alter the properties of the polyether chain sufficiently, relative to the poly(ethylene glycol) starting material, to enable effective purification of the product to take place.

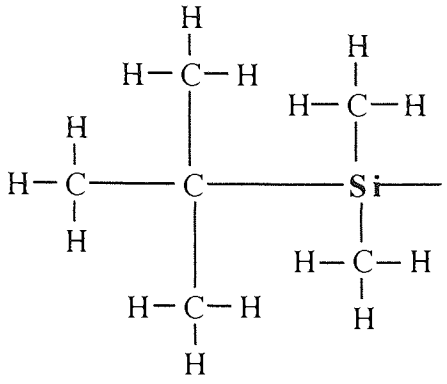
A number of hindered triorganosilyl groups have been employed for the purpose of protecting hydroxyl functions[96-99]. Among the more well known of these are the *tert* -butyldimethylsilyl (TBDMS)[96], triisopropylsilyl (TIP)[97] and the *tert* -butyldiphenylsilyl (TBDPS)[98] groups. The following table contains data on the relative ease of removal of TBDMS, TIPS and TBDPS groups protecting the primary butanol (data (**a**)) and the secondary cyclohexanol (data (**b**))[97].

R_3	(a) H^+	(a) OH^-	(b) H^+	(b) OH^-	(b) F^-
TBDM	1 min	1 h	4 min	26 h	76 min
TIP	18 min	14 h	100 min	44 h	137 min
TBDP	244 min	4 h	360 min	14 h	-

Acid hydrolysis: 1% HCl / 95% Ethanol / 22.5°C.
Base hydrolysis: 5% NaOH / 95% Ethanol / 90°C.
Fluoride ion cleavage: 2 equivalents of Bu_4NF / Tetrahydrofuran / 22.5°C.

Table 2.1 : Half-Life of Silyl Ethers R_3SiOR' Under Desilylation Conditions.

The table shows that the *tert* -butyldimethylsilyl group **21**:-

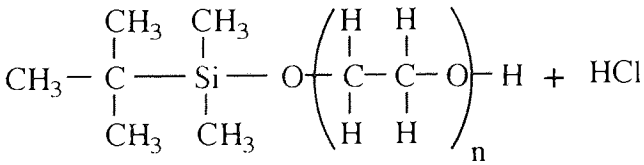


21

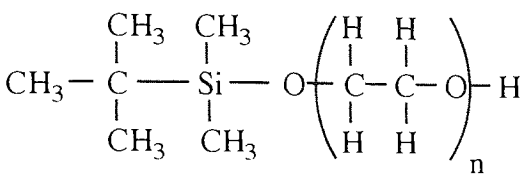
is not only satisfactorily stable to both acid and base, but also that the silyl ethers will be cleaved readily to alcohols by treatment with 2-3 equivalents of tetra-butylammonium fluoride in THF at 25°C[96].

The TBDMS group **21** was applied to Dal Pozzo's system. The successful synthesis of poly(ethylene glycol) acrylates **2(a-c)** can be represented by the following reaction scheme:-

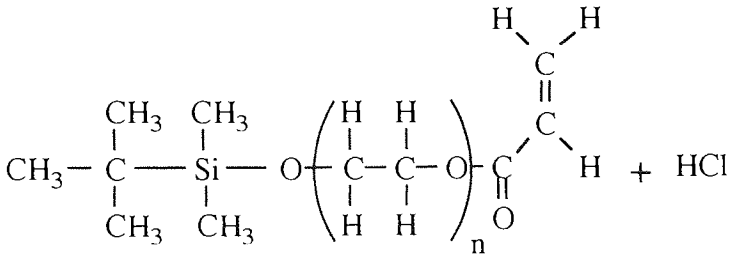
18(a-c)



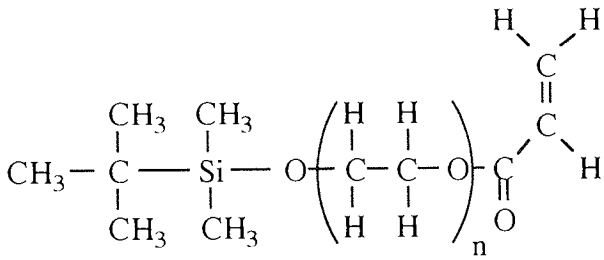
22(a-c)



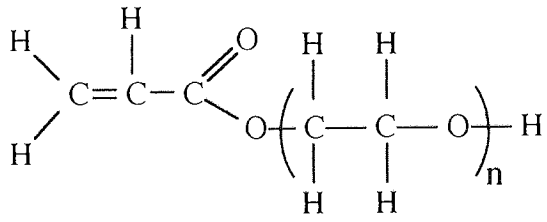
22(a-c)



23(a-c)

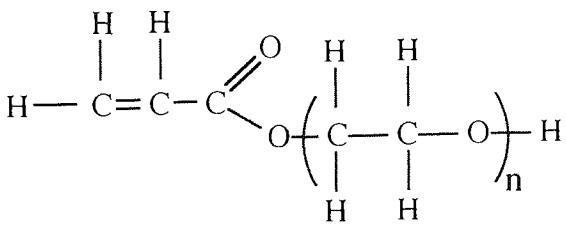


23(a-c)



2(a-c)

This process was used to synthesise the following range of PEGAs:-



PEG200-A **2(a)** n= 4-5

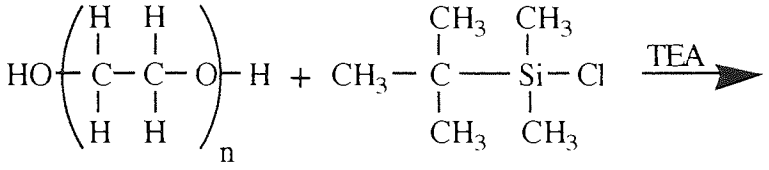
PEG400-A **2(b)** n= 8-9

PEG1000-A **2(c)** n= 22-23

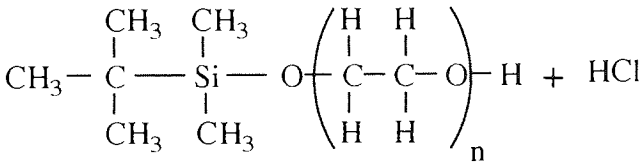
Cleavage of the silyl function from the silylether acrylates **23(a-c)** formed, was achieved using tetra-butylammonium fluoride in tetrahydrofuran.

Synthesis of PEGA adducts of 2000 and 5000 molecular weight was also attempted. These were unsuccessful as it was found to be impossible to separate excess poly(ethylene glycol) from the poly(ethylene glycol)*tert* -butyldimethylsilyl ether formed in the first stage of the synthesis.

2.3.2 Synthesis of Poly(ethylene glycol)*tert* -Butyldimethylsilyl Ethers **22(a-c)**.



18(a-c)



22(a-c)

To ensure that only the mono-substitution product was formed, on addition of the *tert* -butyldimethylsilyl chloride to the poly(ethylene glycol) **18(a-c)**, a thirty-fold excess of poly(ethylene glycol) to silyl chloride was used.

The poly(ethylene glycol) **18(a-c)** was dissolved in dichloromethane. To the solution was added triethylamine. The mixture was cooled to 0°C and *tert* -butyldimethylsilyl chloride, dissolved in dichloromethane, added dropwise with stirring. On completion of the addition, the mixture was stirred for a further twenty four hours during which time the mixture was allowed to reach room temperature.

Any triethylamine hydrochloride precipitate formed during the reaction was then filtered off, and the resultant mixture was then, extracted with phosphate buffer solution (pH7.2). This extraction was found to remove any excess poly(ethylene

glycol) **18(a-c)** starting material, without removing the mono-hydroxy-terminated poly(ethylene glycol)*tert* -butyldimethylsilyl ether product **22(a-c)**.

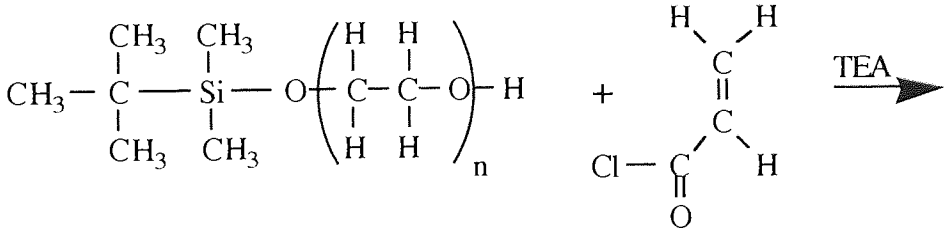
The organic layer was then dried over anhydrous magnesium sulphate, after which the solvent was removed under vacuum to give the poly(ethylene glycol)*tert* -butyldimethylsilyl ether **22(a-c)**. The products were obtained in excellent yields and were shown spectroscopically to have good purity.

Poly(ethylene glycol)-200*tert* -butyldimethylsilyl ether **22(a)** 92%

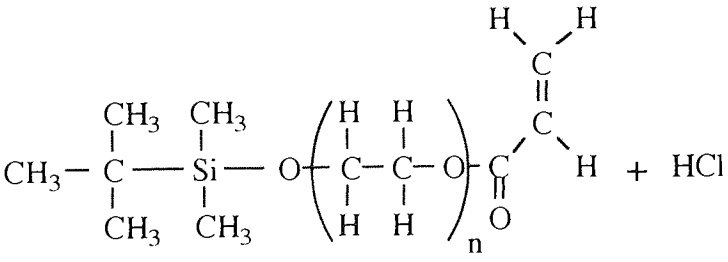
Poly(ethylene glycol)-400*tert* -butyldimethylsilyl ether **22(b)** 78%

Poly(ethylene glycol)-1000*tert* -butyldimethylsilyl ether **22(c)** 75%

2.3.3 Synthesis of Poly(ethylene glycol)*tert* -Butyldimethylsilylether Acrylates **23(a-c)**.



22(a-c)



23(a-c)

The poly(ethylene glycol)*tert* -butyldimethylsilyl ether **22(a-c)** was dissolved in dichloromethane. To the solution was added triethylamine. The mixture was cooled to -78°C, using a dry ice/acetone bath, and acryloyl chloride was added dropwise.

The mixture was stirred for twenty-four hours during which time the mixture was allowed to reach room temperature.

The triethylamine hydrochloride precipitate, formed in the reaction, was filtered from the mixture, and the remaining solution was extracted with 0.1M aqueous HCl, to remove any excess triethylamine, and 1M aqueous sodium hydroxide, to remove any excess acryloyl chloride.

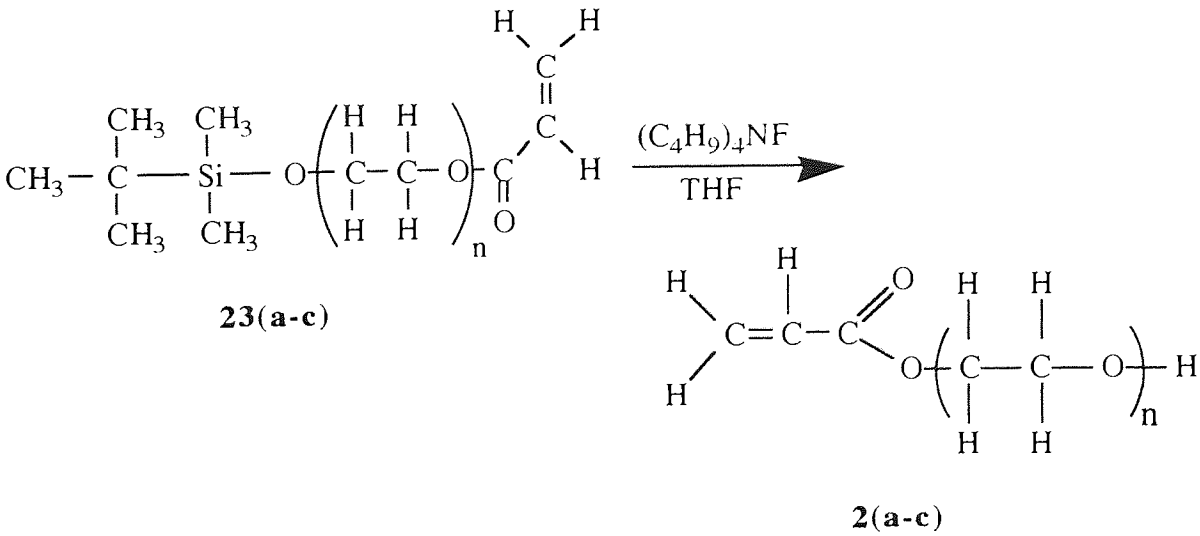
The organic layer was dried over anhydrous magnesium sulphate. Removal of the solvent under vacuum gave the poly(ethylene glycol)*tert* -butyldimethylsilylether acrylate **23(a-c)**. The products were obtained in excellent yields and were shown spectroscopically to have good purity.

Poly(ethylene glycol)-200*tert* -butyldimethylsilylether acrylate **23(a)** 90%

Poly(ethylene glycol)-400*tert* -butyldimethylsilylether acrylate **23(b)** 73%

Poly(ethylene glycol)-1000*tert* -butyldimethylsilylether acrylate **23(c)** 79%

2.3.4 Synthesis of Poly(ethylene glycol) Acrylates **2(a-c)**.



23(a-c)

2(a-c)

As described earlier, it was found that when trityl protected poly(ethylene glycol) acrylate was deprotected using aqueous 1M HCl, that the acrylate ester was cleaved simultaneously. The apparent susceptibility of the acrylate ester to acid catalysed hydrolysis led to the search for a potentially milder method.

The use of tetra-butylammonium fluoride, in tetrahydrofuran, as a method for the unmasking of silyl ethers under mildly basic conditions, has been employed for many systems[96-99]. It is generally obtained from chemical suppliers as a 1.0M solution in THF containing 5% wt/wt water. The use of this reagent in the cleavage of **23(a-c)** is now described.

A sample of poly(ethylene glycol)*tert* -butyldimethylsilyl acrylate **23(a-c)** was dissolved in THF. The mixture was stirred and cooled to 0°C. Two molar equivalents of tetra-butylammonium fluoride, in THF, was then added dropwise over a ten minute period. The mixture was allowed to reach room temperature and was then stirred for a further two hours.

The THF was then removed under vacuum, and the residue redissolved in dichloromethane. This solution was then washed with water thoroughly, to remove any excess tetra-butylammonium fluoride. The organic layer was then dried over anhydrous magnesium sulphate, before the dichloromethane was removed under vacuum.

The crude product was then shaken in petroleum ether (40-60°C) for one hour. This was found to remove any *tert* -butyldimethylsilanol formed during the cleavage of the silyl ether.

Removing the spirit gave the desired poly(ethylene glycol) acrylate **2(a-c)** product. The products obtained using this process were analysed using conventional

techniques, and also using Electrospray Mass Spectrometry (ESMS). This relatively new technique will be discussed in detail in the following section.

The products were shown to be essentially pure and were obtained in excellent yields:-

PEG200-A **2(a)**	88%
PEG400-A **2(b)**	85%
PEG1000-A **2(c)**	84%

2.4 ELECTROSPRAY MASS SPECTROMETRY (ESMS).

The MPEGA and PEGA derivatives were synthesised by the addition of acryloyl chloride (MW=90.5) to a series of MPEGs (MW=350-5000) and PEGs (MW=200-1000) respectively. Elimination of HCl during esterification means that the difference in molecular weight between starting material and product is 54 Daltons. The percentage of the molecular weight of the final products that this change constitutes is

MPEG350-A **1(a)**	13.3%
MPEG550-A **1(b)**	8.9%
MPEG750-A **1(c)**	6.7%
MPEG2000-A **1(d)**	2.7%
MPEG5000-A **1(e)**	1.1%

PEG200-A **2(a)**	21.3%
PEG400-A **2(b)**	11.9%
PEG1000-A **2(c)**	5.1%

The usual methods for analysing product purity for polymers are elemental analysis and Gel Permeation Chromatography (GPC). However due to the small percentage change in molecular weight between starting material and product in this instance, especially for higher molecular weight products, it would be impossible to accurately distinguish between product and starting material, and hence to ultimately confirm the success of the syntheses, using either of the above techniques.

ESMS is a new technique that enables the deduction of molecular weights to an accuracy of 0.01 Da, for molecules of molecular weight up to 100 kDa.Thus far, it has been used mainly in biological systems for the resolution of protein mixtures and in analysing globins[100-102].

The essentials of the system are illustrated in Figure 2.1.

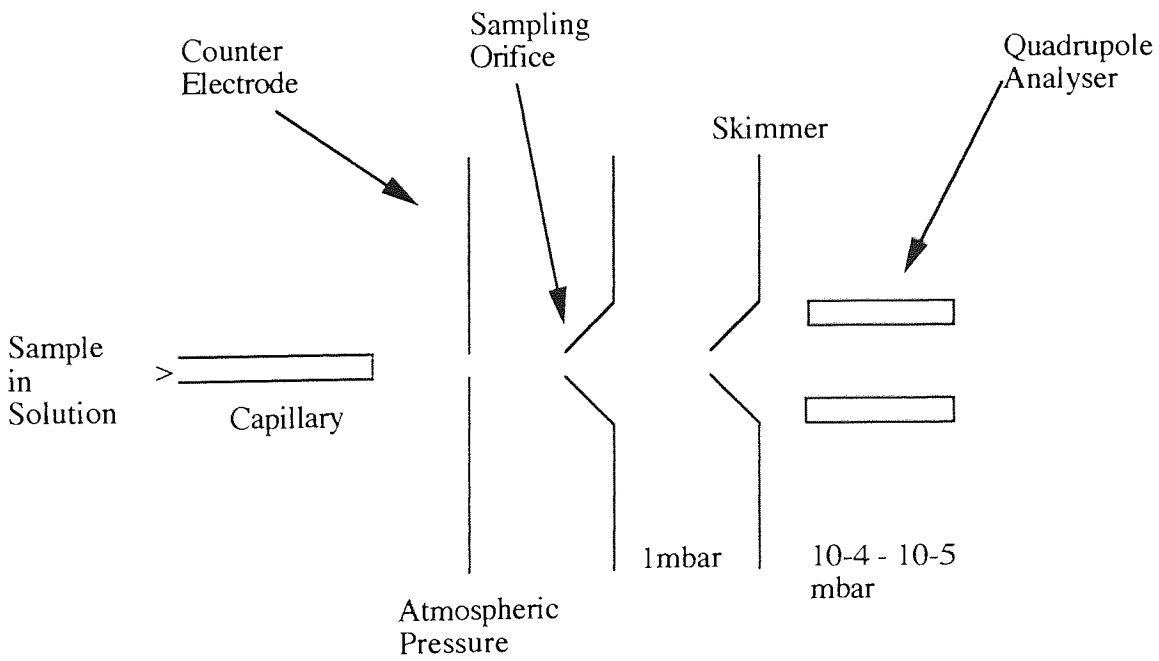


Figure 2.1: The Essentials of an Electrospray Mass Spectrometer.

The sample is introduced in solution into the atmospheric pressure ion source of the spectrometer through a silica capillary tube. Typical solvents are 50/50 water/methanol or water/acrylonitrile, containing 1-5% acetic or formic acid. The concentration of the sample used will be 1-10 mmol/cm^3 .

As a consequence of the strong electric field between the capillary (4kV) and the counter electrode (1kV), the sample solution emerging from the capillary is dispersed into an aerosol of highly charged droplets:- the electrospray. These droplets, assisted by a flow of warm gas flowing through the source, diminish in size by evaporation until a point is reached where multiply-charged ions of individual sample molecules, free of solvent, are released. Some of the ions pass through a small hole (the sampling orifice) into a pumped region at 1mbar pressure and then through a second hole (the skimmer) into the quadrupole analyser, where their mass to charge ratios are measured.

Note, therefore, the difference between this technique and conventional Mass Spectrometry. In this technique, detectable ions are not created by sample bombardment with electrons. Instead ions are produced by association of the sample with ions in the initial solution. Hence fragmentation of parent ions is less likely to occur, and that is why ESMS can detect the molecular weight of samples up to 100kDa.

A typical positive ion spectrum consists of a series of peaks, each of which represents an ion of the molecule of the sample plus a specific number of protons donated from the initial acidic solution.

The ions have the general form :-

$$(M+ nH)^{n+}$$

M= Molecular weight of the sample.
n= Integer number of protons (charges).
H= Mass of the proton (1.00794 Da).

The mass spectrometer measures the mass to charge ratio of each peak where:-

$$m = (M+ nH) / n$$

The molecular weight (M = n (m-H)) can be calculated from the mass to charge ratio, if n can be found. To determine n, any two consecutive peaks differing by one proton in the series may be used.

i.e.. $m_2 = (M+ nH) / n$ $m_1 = \{M + (n+1)H\} / (n+1)\}$

By solving these simultaneously, the charge (n) on m_2 is determined as :-

$$n = (m_1 - H) / (m_2 - m_1)$$

Using a data system therefore, the molecular weight of the sample can be calculated from all consecutive peaks by finding n for each value of m. An accurate average value of the molecular weight can then be found.

Such deductions are hence straight forward for a single species sample and indeed mixtures of say proteins that all have no molecular weight distribution, as 'peak patterns' can be observed allowing resolution into individual components.

Difficulty arises in the analysis of polymers with a molecular weight distribution, as each molecular weight will give rise to its own ion peaks. As in polyether derivatives, protons are likely to associate with the ether linkages to form the detectable ions, problems arise particularly in higher molecular weight adducts where the greater number of repeat units will give a greater range of multiply-charged ions to be resolved. However,the power of the latest data-base systems and also an experienced eye can resolve these problems.

Another potential problem is that as well as $(M+ nH)^{n+}$ ions being formed, ions such as $(M+nNa)^{n+}$ and $(M+ nNH_4)^{n+}$ may also be seen. By altering the spectrometer conditions, these peaks can be eliminated in most cases. For samples containing greater than trace amounts of sodium, the problem can be overcome by adding NaCl(aq) instead of acid to the initial sample solution so as to observe $(M+ nNa)^{n+}$ peaks as opposed to $(M+ nH)^{n+}$.

The five MPEG **17(a-e)** and three PEG **18(a-c)** starting materials and the five MPEGA **1(a-e)** and three PEGA **2(a-c)** products were analysed by first dissolving in water at a concentration of 10 μg/μl. These solutions were then diluted to 1μg/μl in 50/50 water/acrylonitrile containing 5% acetic acid. 10μl aliquots were used for each analysis.

Figures 2.2 to 2.5 are examples of the spectra obtained using this technique. The figures shown are for MPEG350 **17(a)**, MPEG350-A **1(a)**, MPEG550 **17(b)** and MPEG550-A **1(b)** respectively. By comparing the spectra obtained, the addition of 54 Daltons to the starting material to obtain the product can be clearly seen. Note that the dominant spectrum in each case is for the $(M + H)^+$, except for Figure 2.5 where the $(M+ Na)^+$ spectrum is most well defined. For MPEG 750-5000 derivatives **1(c-e)**, results again show successful addition of 54 Da to the starting material. A trace of starting material is only evident in MPEG2000-A **1(d)**. For all of

the hydroxy-terminated PEG derivatives **2(a-c)**, the addition of 54 Da to the starting material is also be clearly seen. In the case of PEG1000-A **2(c)**, however, there is some evidence to suggest that although the vast majority of the product is the monoacrylate, that some addition of 108 Da has occured. This in turn suggests the formation of an acrylate ester at both terminal hydroxyl groups. The diacrylate is therefore a small impurity in the PEG1000-A **2(c)** product. This can be seen on inspection of Figures 2.6 and 2.7.

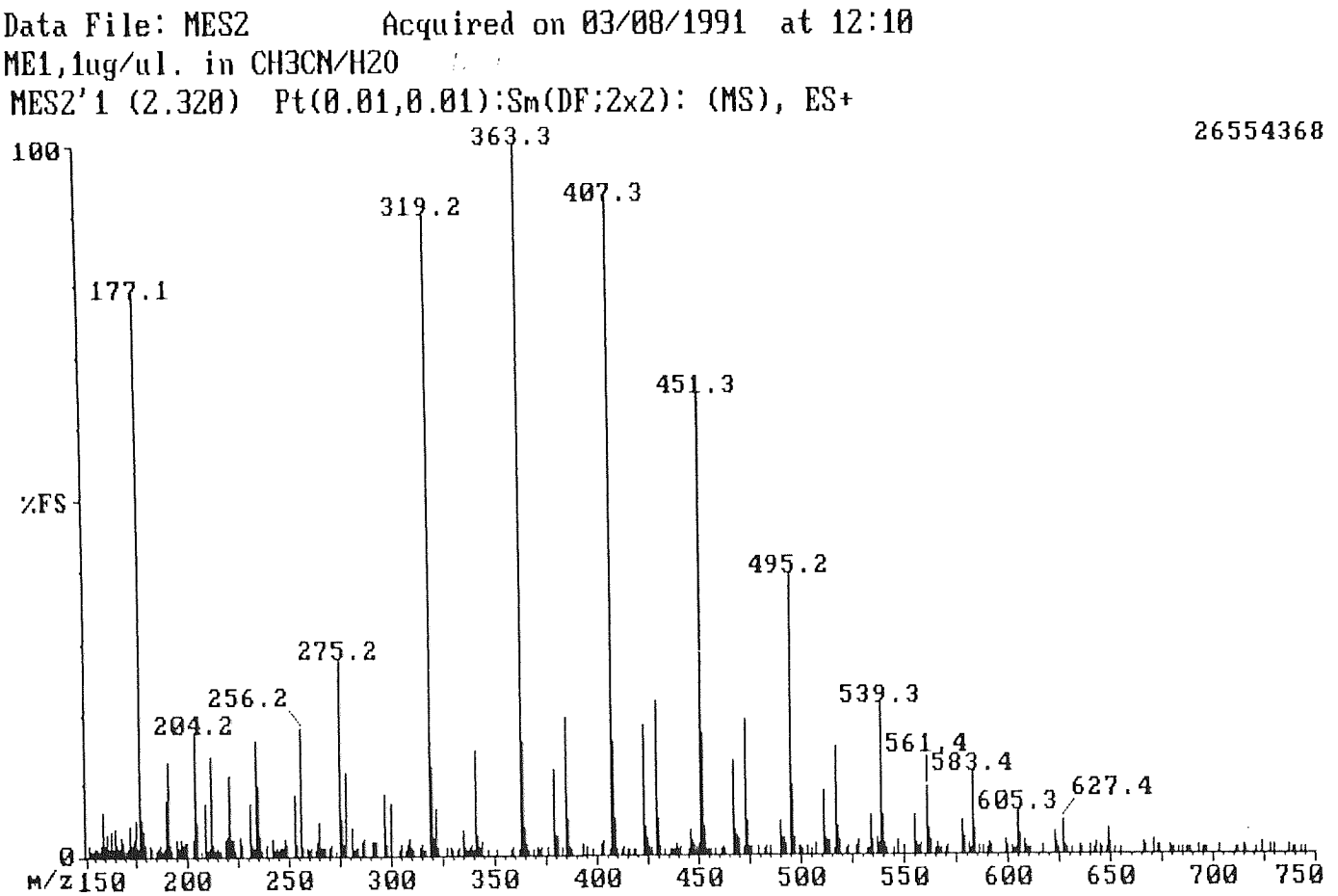


Figure 2.2 : Electrospray Mass Spectrum of MPEG350.

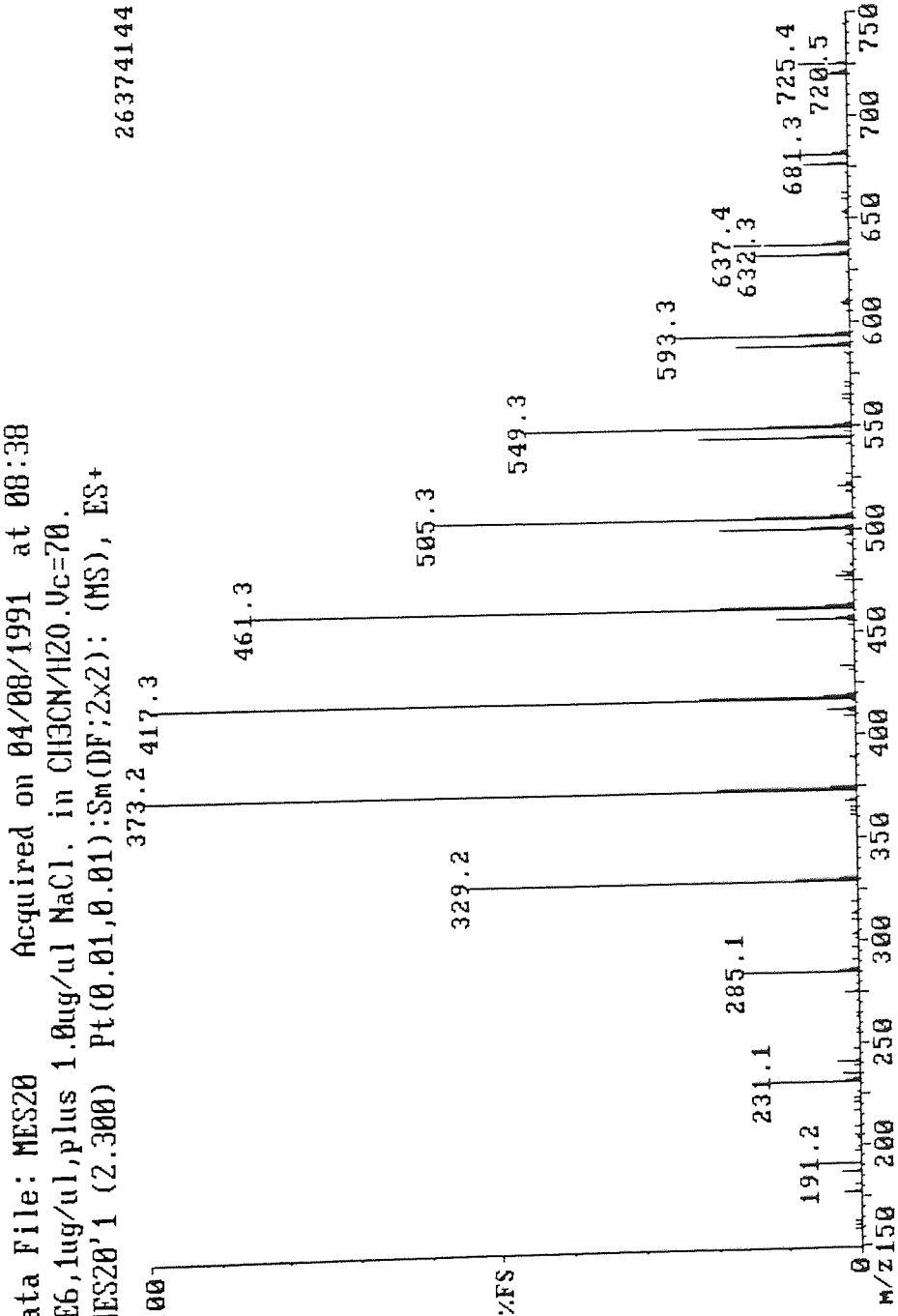


Figure 2.3 : Electrospray Mass Spectrum of MPEG350-A.

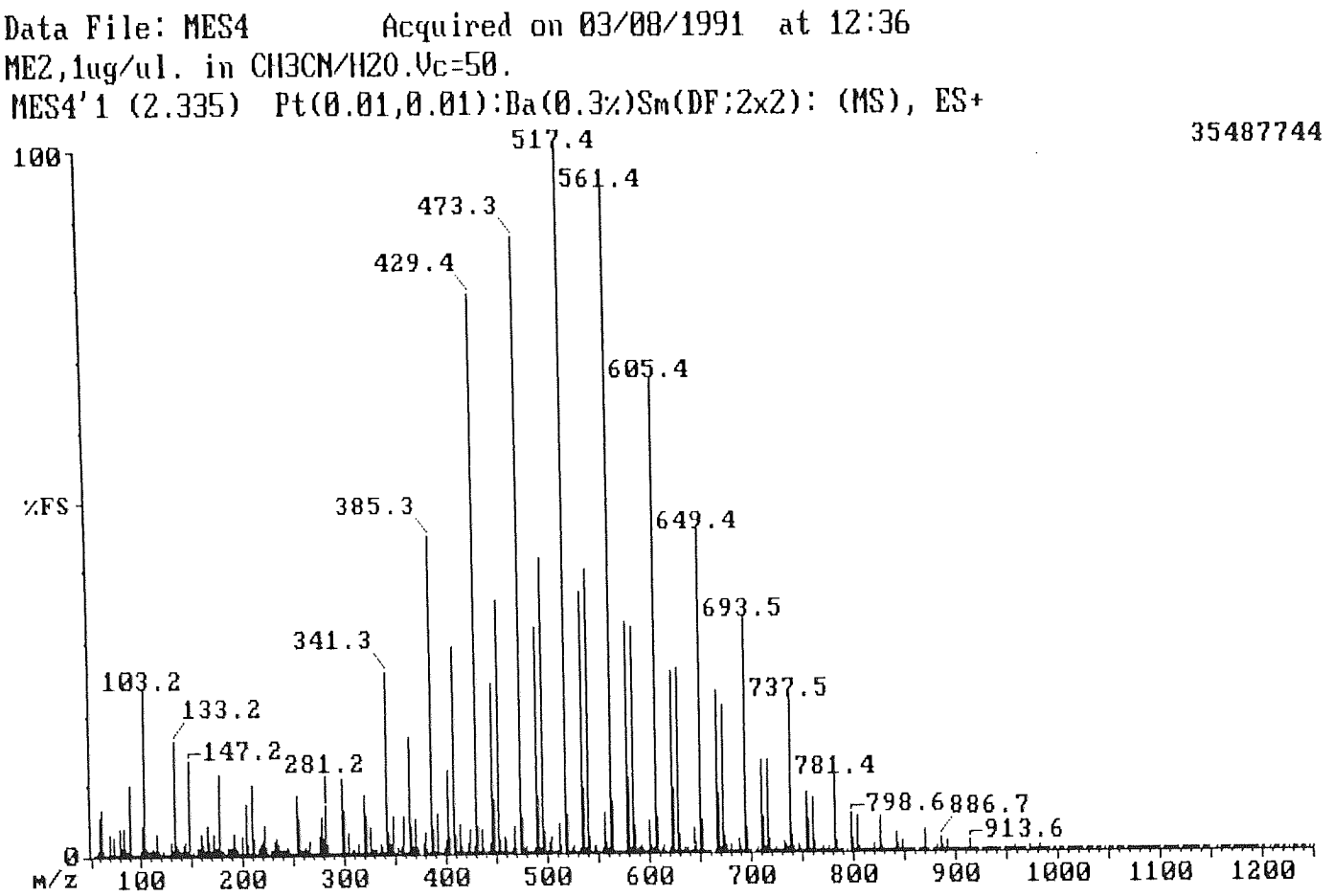


Figure 2.4 : Electrospray Mass Spectrum of MPEG550.

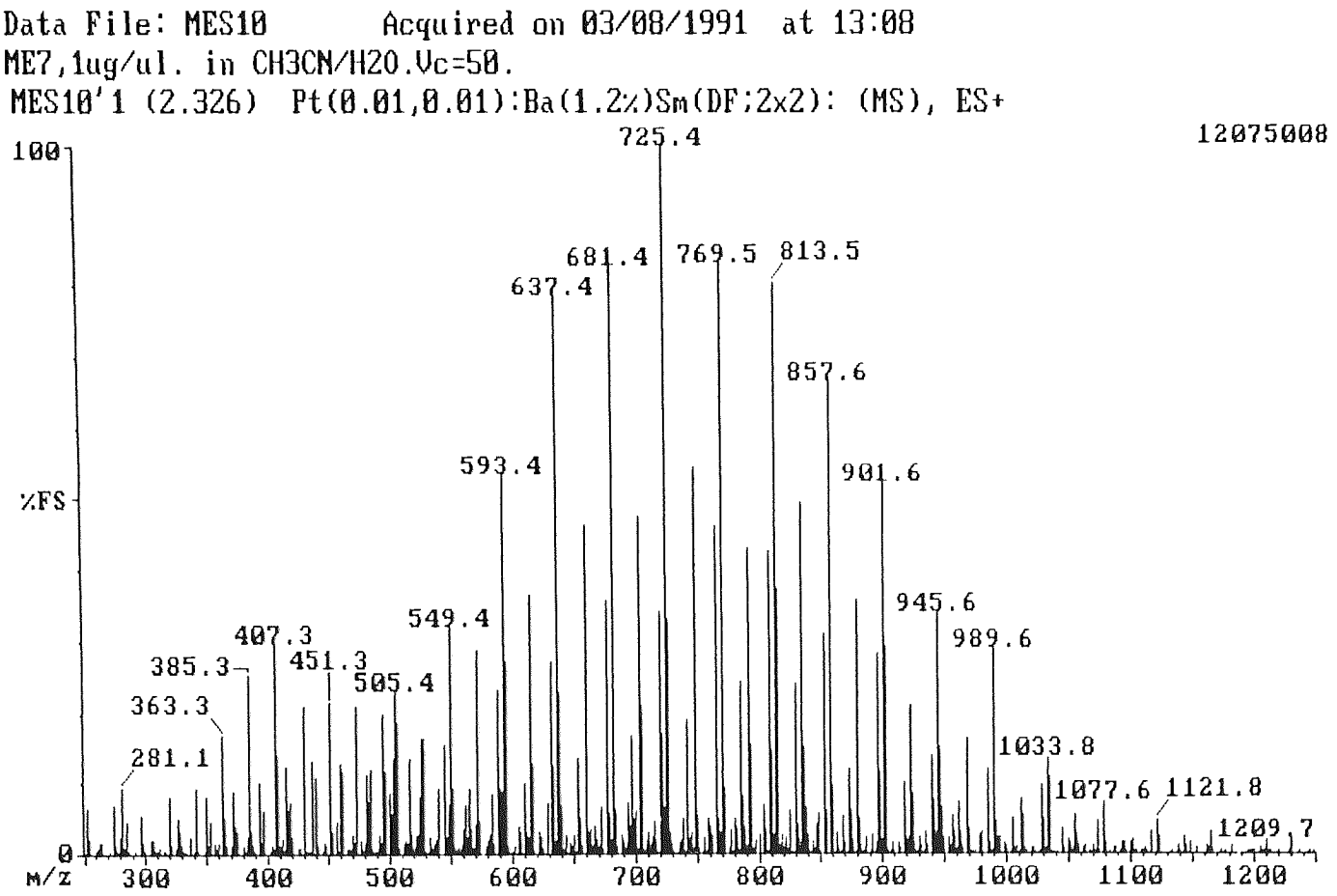


Figure 2.5 : Electrospray Mass Spectrum of MPEG550-A.

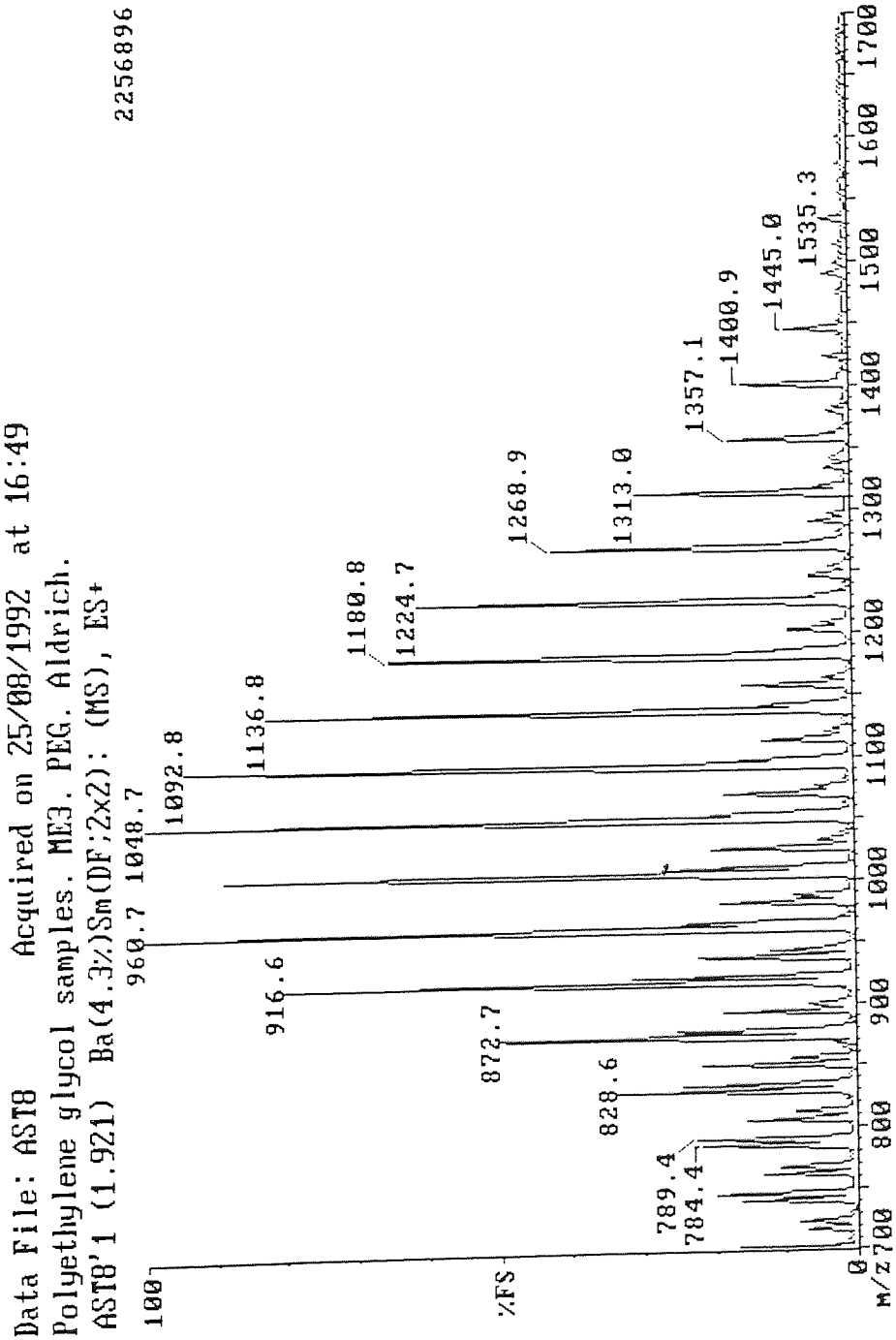


Figure 2.6 ; Electrospray Mass Spectrum of PEG1000.

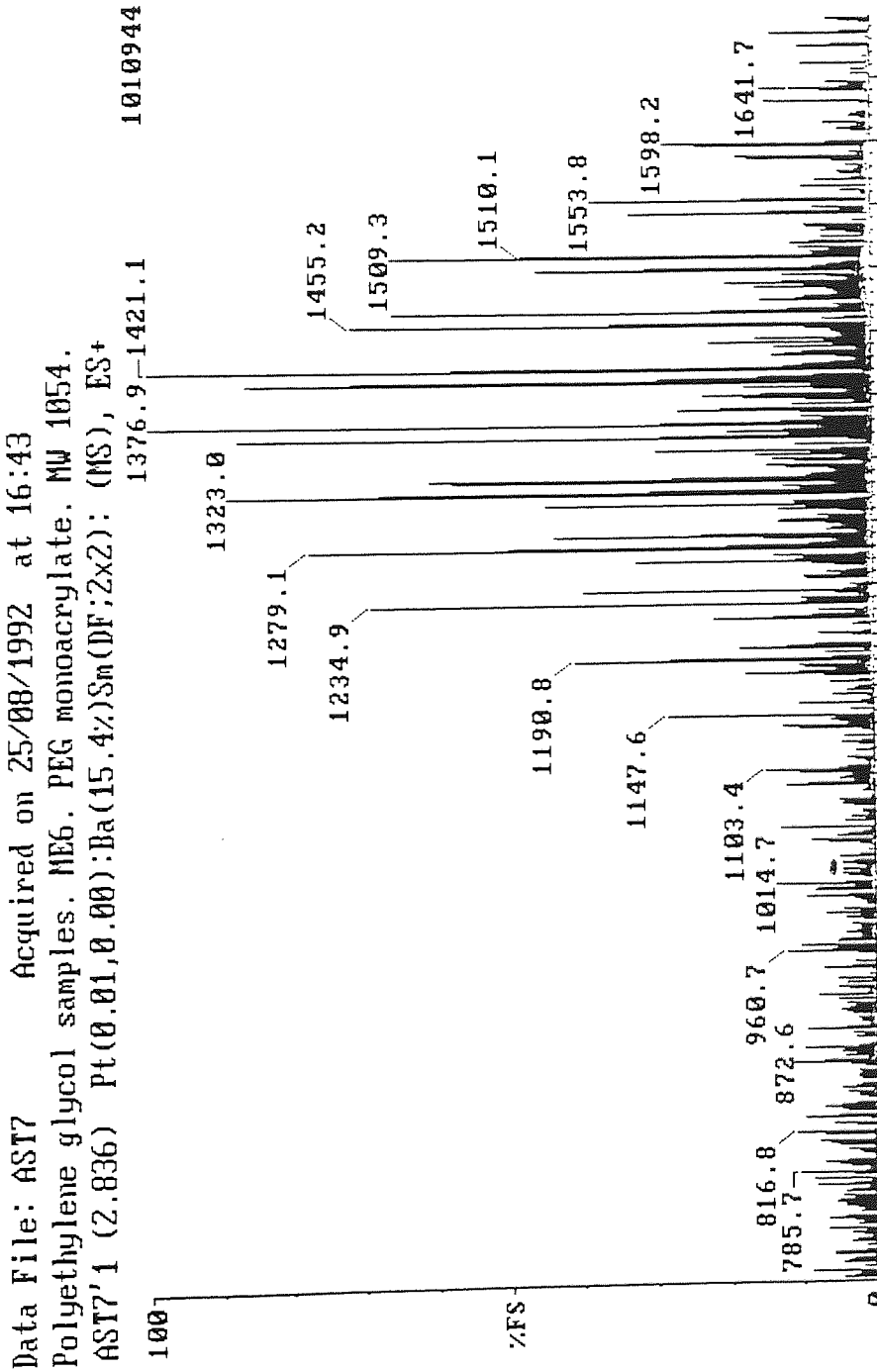
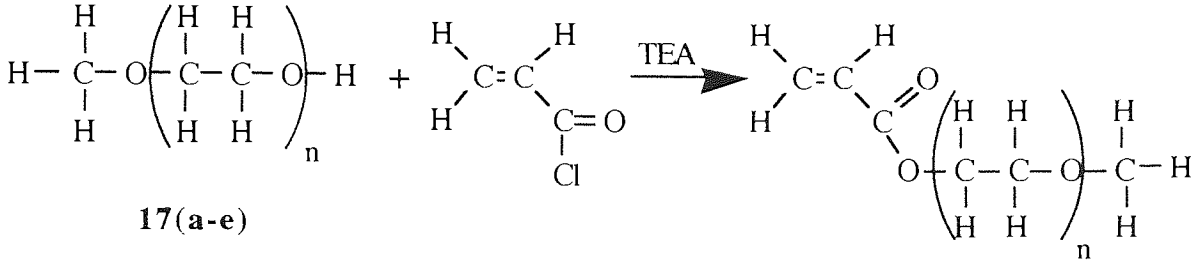


Figure 2.7 : Electrospray Mass Spectrum of PEG1000-A.

2.5 CONCLUSIONS

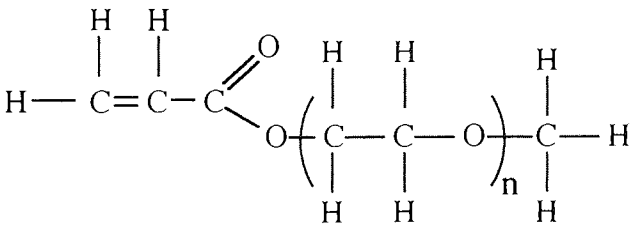
The synthesis of methoxy poly(ethylene glycol) acrylates **1(a-e)** was successfully achieved using a one step method that can be represented using the scheme:-



17(a-e)

1(a-e) + HCl

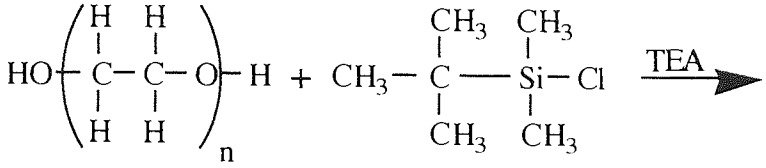
This scheme was used to synthesise the following range of MPEGAs:-



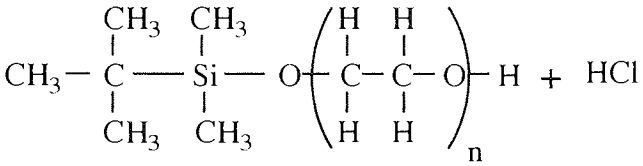
MPEG350-A	**1(a)**	n= 7-8
MPEG550-A	**1(b)**	n= 11-12
MPEG750-A	**1(c)**	n= 16-17
MPEG2000-A	**1(d)**	n= 44-45
MPEG5000-A	**1(e)**	n= 112-113

The synthesis of hydroxy-terminated poly(ethylene glycol) acrylates **2(a-c)** proved to be more complicated. Initial attempts to follow the ideas of Dal Pozzo *et al.* [92] were unsuccessful. He suggested that monofunctional poly(ethylene glycol)s could be produced by initial protection of one terminal hydroxyl function with a trityl group, addition of the desired function to the other terminal hydroxyl group and subsequent deprotection of the trityl ether to give the desired product. It was found that in the case of this system that the first two of these stages were successful, but that the deprotection of the trityl ether, using 1M aqueous HCl, caused the simultaneous cleavage of the acrylate ester.

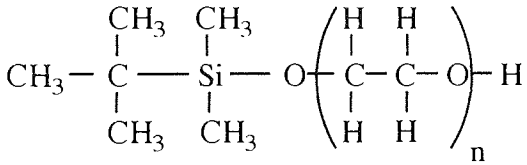
Using the the *tert* -butyldimethylsilyl group **21** to protect one hydroxy group, adding the acrylate function to the other terminal hydroxy group and finally deprotecting the silyl ether allowed the successful synthesis of poly(ethylene glycol) acrylates **2(a-c)**. This can be represented by the following scheme:-



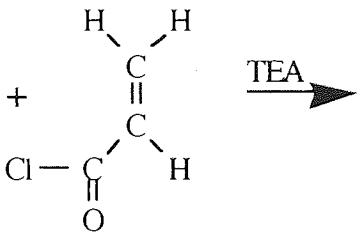
18(a-c)

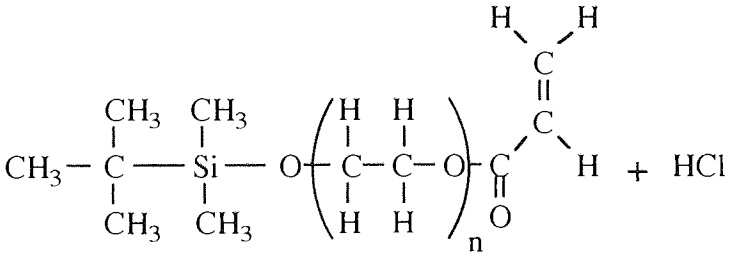


22(a-c)

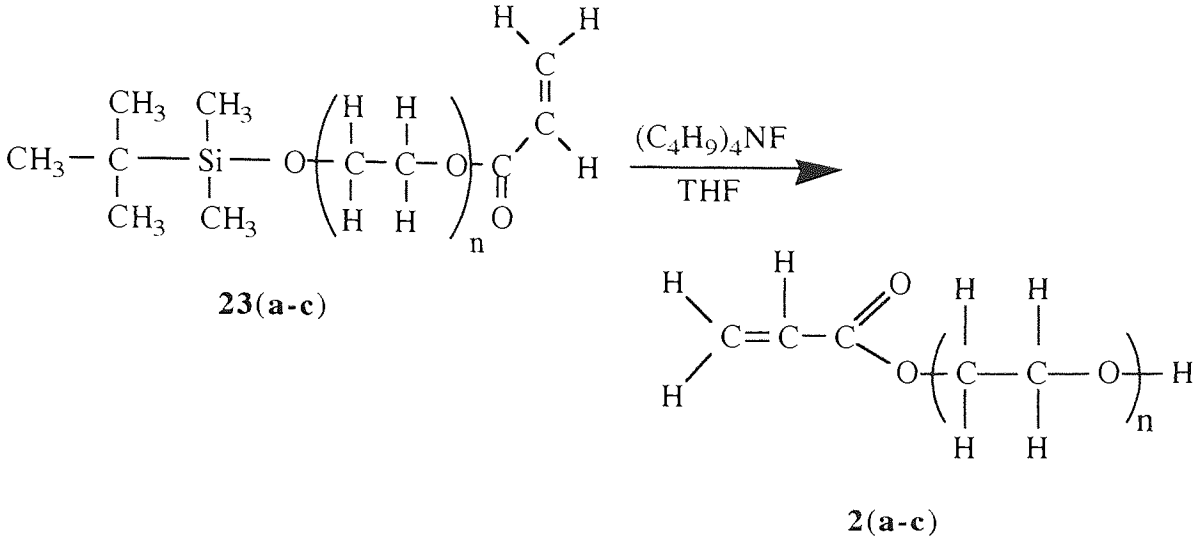


22(a-c)





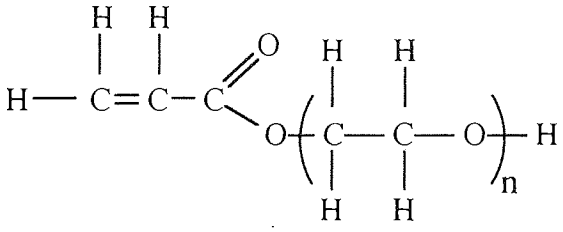
23(a-c)



23(a-c)

2(a-c)

This three stage synthesis was used to produce the following range of PEGAs:-



PEG200-A **2(a)** n= 4-5

PEG400-A **2(b)** n= 8-9

PEG1000-A **2(c)** n= 22-23

Both the methoxy- and hydroxy-terminated polyether acrylate products **1(a-e)** and **2(a-c)** formed, using these syntheses, were analysed using the conventional techniques of NMR and IR and also using the relatively new technique of Electrospray Mass Spectrometry (ESMS). Using ESMS to analyse the products formed, and comparing them with their respective starting materials, it was possible to further assess product purity. From the spectra obtained, it is possible to see the addition of 54 Daltons (the mass of one acrylate function) in all of the products. This absolutely affirmed the success of the syntheses. Minor impurities were only detected in MPEG2000-A **1(d)** and PEG1000-A **2(c)** samples. In the case of **1(d)** a small amount of MPEG2000 **17(d)** starting material was apparent, whilst for **2(c)** small amounts of the diacrylate were suggested by a series of peaks consistent with an addition of 108 Daltons to the starting material PEG1000.

CHAPTER 3

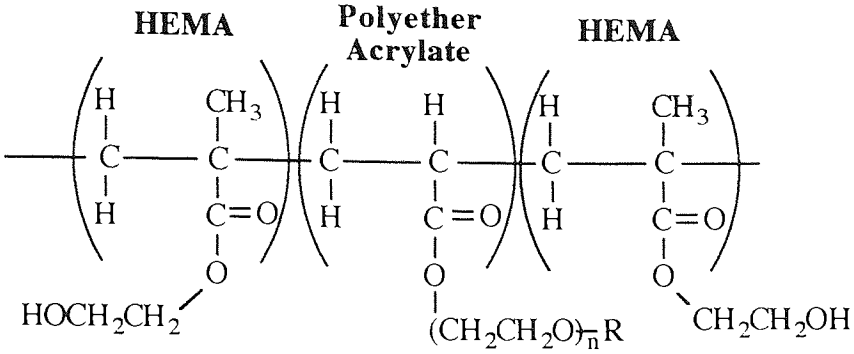
THE BULK AND SURFACE PROPERTIES OF POLY(2-HYDROXYETHYLMETHACRYLATE) COPOLYMERS CONTAINING LINEAR POLYETHERS.

3.1 GENERAL INTRODUCTION.

Linear polyethers have been shown to be relatively inert towards biological species[39]. An example of this is that linear polyethers have been shown to inhibit the absorption of blood proteins onto alkyl methacrylate/ poly(ethylene glycol) monomethacrylate devices[50]. One theory postulated is that the polyether side chains produce an excluded volume, a barrier to such protein absorption. It was hoped, therefore, that inclusion of the polyether acrylates, discussed in Chapter 2, might enhance the biocompatability of poly(2-hydroxyethyl methacrylate) (poly(HEMA)) based hydrogel membranes.

Many properties of hydrogels are influenced by the water maintained within the polymer matrix. The water allows the transport of many water soluble materials through the gel. For example an extremely important factor, when considering the development of new soft contact lens materials, is the rate of oxygen diffusion through the lens. The rate of diffusion of oxygen through the gel will be enhanced by an increased equilibrium water content. It was anticipated that incorporating polyether side-chains into a poly(HEMA) based gel might cause an increase in the EWC of the gel. This effect would be due to the influence of the polar $-CH_2CH_2O-$ repeat units.

As hydrogel surfaces are often in intimate contact with biological fluids, it is important to study the surface properties of these materials. The indications that thrombogenic reactions to synthetic polymers are significantly reduced by the presence of polyether side chains, would in turn suggest that the surface properties of such polymers are manifestly controlled by the polyether side chains. When the polyether acrylates, synthesised as described in Chapter 2, are copolymerised with HEMA, a typical section of the resulting copolymer would be expected to look as follows:-



Where n= 4 - 113

This highlights the fact that, if the polyether chains do dictate the surface properties of polyether acrylate/HEMA copolymers, the surface of the copolymer should show the polar characteristics of the -CH_2CH_2O- repeat unit. Measurement of the surface free energy of polyether acrylate/HEMA hydrogels is possible. Furthermore the surface free energy values obtained can be resolved into both polar and dispersive components . A comparison of the data obtained for such systems, with that for a pure poly(HEMA) gel, should allow conclusions to be drawn on the surface effects of the polyether chains. An increase in the polar component of surface free energy, would be consistant with a polyether acrylate:HEMA based gel whose surface properties are being controlled by the polyether side chains.

In summary, therefore, both the equilibrium water content and the surface free energy of a hydrogel are fundamental parameters to be considered when assessing the prospective biocompatability of that material. Measurement of these properties allows an insight into the bulk and surface properties of hydrogels.

The remainder of this chapter focuses on the measurement of EWC and surface free energy for hydrogels consisting of HEMA and the polyether acrylates synthesised as described in Chapter 2.

3.2 PREPARATION OF HYDROGELS.

A range of copolymers were prepared by adding 5 to 20% weight of each of the eight polyether acrylate monomers to HEMA:EGDM (99:1). Ethylene glycol dimethacrylate (EGDM) was employed as a crosslinking agent. The full experimental procedure for the synthesis of these hydrogels may be found in Chapter 8.

3.3 THE EFFECT OF POLYETHER ACRYLATES ON THE EWC OF HEMA:EGDM (99:1) HYDROGELS.

The experimental method used in calculating EWC values for the prepared hydrogels can be found in Chapter 8. The definition of the EWC value for a hydrogel is:-

$$EWC = \frac{\text{Weight of water present in the hydrated gel}}{\text{Total weight of hydrated gel}} \times 100$$

The EWC values of all HEMA:EGDM (99:1) based hydrogels prepared are given in Table 3.1.

A statistical treatment of the error involved in EWC determination by this technique gives a standard deviation of $\sigma_{n-1} = 0.4$, an error of approximately 3% [103].

Membrane Composition	EWC (%)
HEMA:EGDM (99:1)	37.3
HEMA:EGDM (99:1) + 5% MPEG350-A	40.1
HEMA:EGDM (99:1) + 10% MPEG350-A	41.7
HEMA:EGDM (99:1) + 20% MPEG350-A	46.9
HEMA:EGDM (99:1) + 5% MPEG550-A	40.4
HEMA:EGDM (99:1) + 10% MPEG550-A	42.7
HEMA:EGDM (99:1) + 20% MPEG550-A	47.6
HEMA:EGDM (99:1) + 5% MPEG750-A	40.6
HEMA:EGDM (99:1) + 10% MPEG750-A	43.5
HEMA:EGDM (99:1) + 20% MPEG750-A	48.2
HEMA:EGDM (99:1) + 5% MPEG2000-A	42.3
HEMA:EGDM (99:1) + 10% MPEG2000-A	45.3
HEMA:EGDM (99:1) + 20% MPEG2000-A	53.4
HEMA:EGDM (99:1) + 5% MPEG5000-A	46.0
HEMA:EGDM (99:1) + 10% MPEG5000-A	50.5
HEMA:EGDM (99:1) + 20% MPEG5000-A	58.6
HEMA:EGDM (99:1) + 5% PEG200-A	38.9
HEMA:EGDM (99:1) + 10% PEG200-A	40.4
HEMA:EGDM (99:1) + 20% PEG200-A	41.3
HEMA:EGDM (99:1) + 5% PEG400-A	41.1
HEMA:EGDM (99:1) + 10% PEG400-A	42.2
HEMA:EGDM (99:1) + 20% PEG400-A	44.1
HEMA:EGDM (99:1) + 5% PEG1000-A	41.4
HEMA:EGDM (99:1) + 10% PEG1000-A	44.4
HEMA:EGDM (99:1) + 20% PEG1000-A	48.2

Table 3.1 : The Effect of Polyether Content on the EWC of HEMA:EGDM (99:1) Hydrogels.

These results suggest that the EWC of HEMA:EGDM (99:1) based hydrogels is affected in the following ways on inclusion of polyether acrylates:-

1) increasing the concentration of the same polyether acrylate within the hydrogel network, causes an increase in EWC.

2) for two HEMA:EGDM (99:1) hydrogels containing the same concentration of different polyether acrylates, the gel with the highest EWC value will be the one containing the polyether acrylate of highest molecular mass i.e. the acrylate with the longest polyether chain.

These results, although promising and interesting, are not particularly suprising. What would be more interesting would be to determine whether the EWC value for a gel is affected more by a large number of small polyether chains, or by a small number of longer polyether chains. To deduce such an effect, the concentration of $-CH_2CH_2O-$ ether repeat units must be a constant between two comparative gels i.e. that the only variable being analysed is the change in EWC with chain length. Producing plots of EWC versus the concentration of $-CH_2CH_2O-$ ether repeat units in wt% should give a graph that should allow the relationship between EWC and chain length to be investigated.

Figure 3.1 shows a plot of EWC (wt%) versus [$-CH_2CH_2O-$] (wt%) for all synthesised hydrogels containing HEMA:EGDM (99:1) and MPEGA derivatives. Figure 3.2 shows the analogous plot for the PEGA derivatives.

Analysis of Figure 3.1 shows the marked affect of polyether chain length on EWC at a constant concentration of ether repeat units. The EWC values observed for all of the MPEG derivatives at 10% weight of polyether repeat units are listed as follows:-

MPEG Derivative	EWC (wt%)
MPEG350-A	~43
MPEG550-A	~43.5
MPEG750-A	~44
MPEG2000-A	~46
MPEG5000-A	~51.5

These results show for a constant concentration of ether repeat units that observed EWC values increase with increasing polyether chain length. This observation is probably due to the fact that the hydrogel matrix is essentially made up of a hydrophobic backbone with hydrophilic side-chains. For the longer polyether side-chains, a continually increasing proportion of ether repeat units exist an increasing distance away from the hydrophobic polymer backbone. The further the repeat units are away from this hydrophobic influence, the greater will be their ability to structure water leading to the results observed above. Analysis of Figure 3.2 allows similar deductions to be made for the hydroxy-terminated PEGA derivatives.

More generally, comparing the EWC values displayed in Table 3.1, Figure 3.1 and Figure 3.2 establishes that inclusion of polyether side-chains in a poly(HEMA) based hydrogel increases the water content of the gel with respect to a pure poly(HEMA) gel.

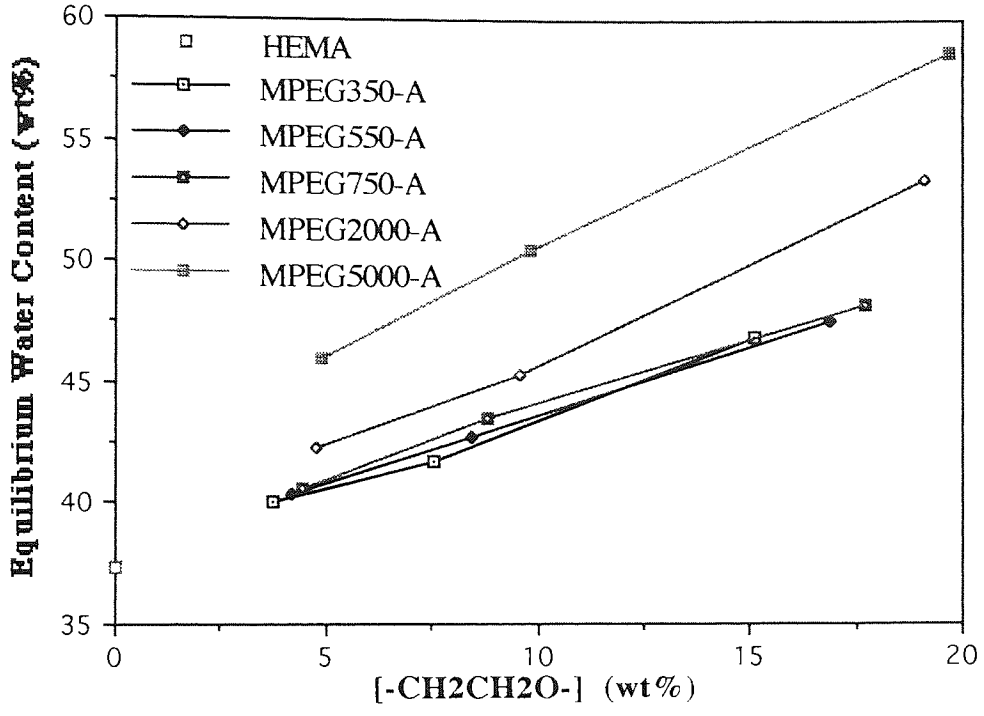


Figure 3.1: Effect of the Methoxy-Terminated MPEGAs on the EWC of HEMA:EGDM(99:1) Hydrogels.

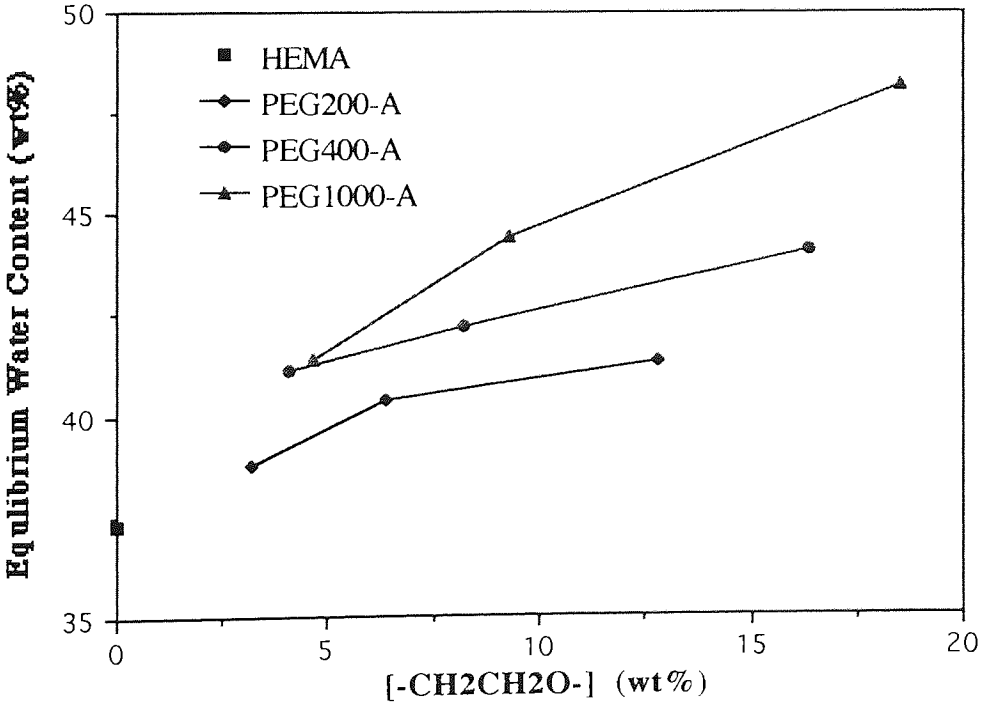


Figure 3.2: Effect of the Hydroxy-Terminated PEGAs on the EWC of HEMA:EGDM(99:1) Hydrogels.

3.4 THE EFFECT OF POLYETHER ACRYLATES ON THE SURFACE ENERGIES OF HEMA:EGDM (99:1) HYDROGELS.

3.4.1 Introduction.

Contact angle goniometry was used to determine the surface energy of these hydrogel copolymers. The practical details associated with this technique are discussed in Chapter 8. The application of contact angle theory allows the surface energies of hydrogels to be calculated in either their hydrated or dehydrated states. Consideration had therefore to be given to which alternative would be most appropriate in this case.

It is thought that polyether side chains resist protein absorption by providing an excluded volume about the polymer surface. If contact angles were to be measured in the dehydrated state then it is considered that removal of the aqueous environment might cause the side chains to 'collapse' against the surface of the copolymer. This would significantly alter the characteristics of the copolymer surface, and would not allow a surface study of the effects of mobile polyether side chains to be made. In addition to this, surface energies would be difficult to measure, in the dehydrated state, where hydrophilic samples absorb water rapidly. Measurement of contact angles in the hydrated state would provide the ability to study the effect of mobile polyether side chains on the hydrogel surface.

The measurement of contact angles, and eventually surface energies, has been achieved in the past by first creating a three phase interface in the following ways, using:-

1) a drop of wetting liquid, the hydrogel surface and air. This constitutes the sessile drop technique used for measuring dehydrated contact angles. This system is shown in Figure 3.3.

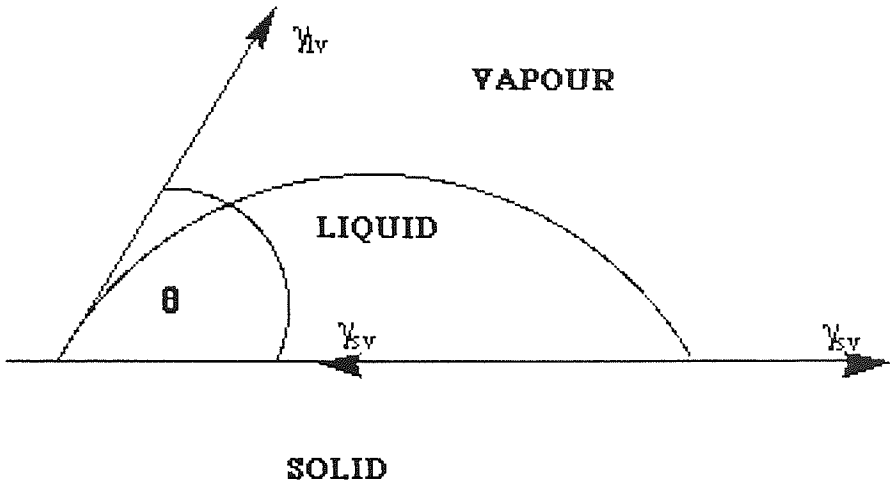


Figure 3.3 : Interfacial Energies of a Sessile Drop on a Solid Surface.

where:-

γ_{sv} = the solid/vapour interfacial free energy.
γ_{sl} = the solid/liquid interfacial free energy.
γ_{lv} = the liquid vapour interfacial free energy.

2) a drop of octane, the hydrogel surface and water in which the system is immersed. This constitutes Hamilton's method[104] used, in conjunction with the captive bubble technique, to measure hydrated contact angles. This system is shown in Figure 3.4.

3) a bubble of air, the hydrogel surface and water in which the system is immersed. This constitutes the captive bubble technique used, in conjunction with Hamilton's method, to measure hydrated contact angles. This system is shown in Figure 3.5.

Hamilton's method[104] and the captive bubble technique can be combined to give valuable information on the hydrated surface properties, of the polyether acrylate :HEMA copolymers being studied, by allowing values for the total- (γ_S^t), polar- (γ_S^P) and dispersive- (γ_S^d) surface free energies to be calculated.

3.4.2 Measurement of the Surface Energies of Hydrated Surfaces.

Hamilton[104] found that both octane and water have the same dispersive component to their free energies, 21.8 mN/m. He also noted that the polar component of octane is zero.

Consider a sample S suspended under water whist being wetted with a drop of octane:-

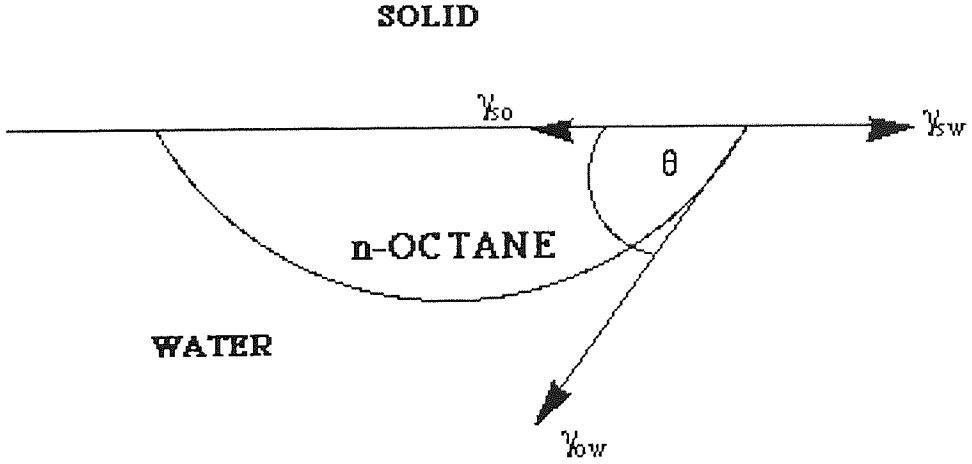


Figure 3.4 : Components of Surface Free Energy Using Hamilton's Method.

Where:-

γ_{sw} = solid-water interfacial free energy.
γ_{so} = solid-octane interfacial free energy.
γ_{ow} = octane-water interfacial free energy.

A relationship describing the work of adhesion at a solid-liquid interface was developed by Fowkes[105]. He assumed that there was no polar interaction across the interface:-

$$\gamma_{sl} = \gamma_s + \gamma_{lv} - 2(\gamma_{lv}^d \gamma_s^d)^{0.5} \quad (3.1)$$

There is no term within this expression to account for stabilisation from non-dispersive forces. A modified form of this equation was introduced by Tamai et al.[106] that accounted for this omission:-

$$\gamma_{sl} = \gamma_s + \gamma_{lv} - 2(\gamma_{lv}^d \gamma_s^d)^{0.5} - I_{sl} \quad (3.2)$$

where:-

$$I_{sl} = 2(\gamma_{lv}^p \gamma_s^p)^{0.5} \quad (3.3)$$

Young[107] resolved the forces at the point of contact of a sessile drop and a solid and proposed the following relationship:-

$$\gamma_{sv} = \gamma_{sl} + \gamma_{lv} . \mathrm{Cos}\theta \quad (3.4)$$

As octane has no polar component, and the dispersive components of octane and water (γ_{lv}^d) are identical, combining equations (3.2) and (3.4) leads to the following expression for I_{sw}, the polar stabilisation energy between water and the solid:-

$$I_{sw} = \gamma_{w'v} - \gamma_{ov} - \gamma_{ow}.\mathrm{Cos}\theta \quad (3.5)$$

$\gamma_{w'v}$, the surface tension of octane saturated water, γ_{ov} and γ_{ow} have all been determined experimentally. Hence I_{sw} can be determined. Therefore, γ_{sp}, the polar component of the surface free energy of the hydrogel, may be deduced using equation (3.3).

The captive air bubble technique is similar to that of Hamilton's method experimentally in that the hydrogel sample is placed under water and a bubble of air is blown onto the surface from below. The contact angle θ' is measured as shown in Figure 3.5:-

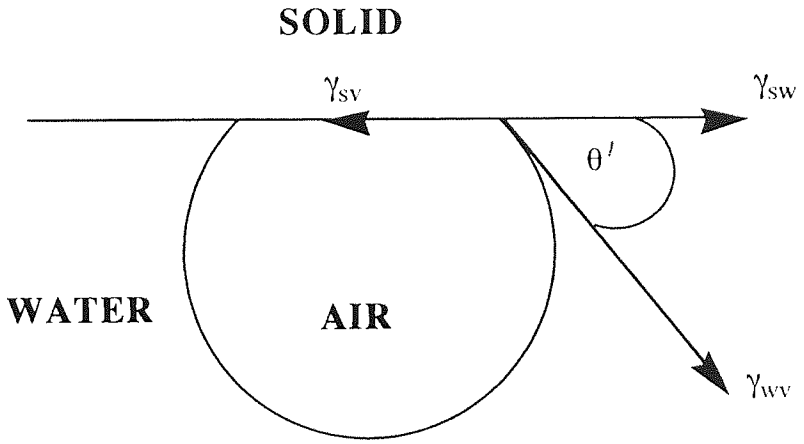


Figure 3.5: Components of Surface Free Energy for the Captive Air Bubble Technique.

Where:-

γ_{sw} = solid-water interfacial free energy.

γ_{wv} = water-vapour interfacial free energy (surface tension of the water).

γ_{sv} = solid-vapour interfacial free energy or approximately γ_s the surface energy of the solid.

The forces acting at the three phase interface present can again be resolved using the Young equation:-

$$\cos\theta' .\gamma_{wv} = \gamma_{sv} - \gamma_{sw} \quad (3.6)$$

γ_{wv} is the surface tension of water and is known to be 72.8 mN/m. θ' is measured as the contact angle. This allows a value for $\gamma_{sv} - \gamma_{sw}$ to be calculated. An equation describing the polar stabilisation parameter (I_{sw}) was defined previously as equation (3.5):-

$$I_{sw} = \gamma_{w'v} - \gamma_{ov} - \gamma_{ow}.\mathrm{Cos}\theta' \quad (3.5)$$

As γ_{wv} = 72.8 mN/m, γ_{ov} = 21.8 mN/m and γ_{ow} = 51.0 mN/m this equation may be rewritten as:-

$$I_{sw} = 51.0\ (1 - \mathrm{Cos}\theta') \quad (3.7)$$

allowing I_{sw} to be determined. Combining equations (3.2) and (3.6) gives:-

$$(\gamma_{sv} - \gamma_{sw}) = 2(\gamma_{wv}{}^{d}\gamma_{sv}{}^{d})0.5 + I_{sw} - \gamma_{wv} \quad (3.8)$$

Rearranging this equation gives an expression gives an equation for the dispersive component ($\gamma_{sv}{}^{d}$) of the hydrogel:-

$$\gamma_{sv}{}^{d} = [\ \{(\gamma_{sv} - \gamma_{sw}) - I_{sw} + \gamma_{wv}\}\ /\ 2(\gamma_{wv}{}^{d})^{0.5}]^{2} \quad (3.9)$$

Values of $\gamma_{sv}{}^{d}$, $\gamma_{sv}{}^{p}$ and $\gamma_{sv}{}^{t}$ were calculated using the Macintosh Works™ package for the hydrogels prepared in Section 3.2.

The contact angles obtained, using both Hamilton's method and the captive air bubble technique, were recorded as the average of six readings. It was found that readings obtained had values within $\pm 1^{\circ}$ of the mean value. This range of contact angle readings gave a general error, for all surface free energy values obtained, of ± 1.5 mN/m for γ_S^d, ± 0.5 mN/m for γ_S^p and ± 2 mN/m for γ_S^t.

3.4.2 Discussion of Surface Energy Studies.

Figures 3.8 and 3.11 describe the total surface free energies observed for gels containing MPEGA and PEGA derivatives respectively. They show the effect on surface energy of increasing the concentration of one particular derivative, and also show an inter-comparison between hydrogels containing all derivatives and a pure poly(HEMA) hydrogel.

Figures 3.6 and 3.9 show the dispersive component of surface free energy, and Figures 3.7 and 3.10 the polar component of surface free energy, for hydrogels containing MPEGA and PEGA derivatives respectively.

Figures 3.8 and 3.11 demonstrate that the total free energy of a poly(HEMA) based hydrogel increases with ether repeat unit concentration for gels containing both methoxy- and hydroxy-terminated polyether derivatives. Figures 3.7 and 3.10 show that this increase in surface free energy, with respect to a pure poly(HEMA) gel, is manifestly controlled by an increase in the polar surface energy component.

Combining the above observations tends to suggest that a polar influence is present at the surface of hydrogels containing polyether derivatives that is not present in a pure poly(HEMA) hydrogel. From these deductions it is postulated that the surface properties of hydrogels containing such derivatives are as anticipated dictated by the polar polyether side-chains.

In addition to these analyses reference to Figures 3.6 and 3.9 shows, in the case of hydrogels containing either methoxy- or hydroxy- terminated derivatives, that the dispersive surface energy component is suppressed. As the dispersive component arises predominantly from contributions to the surface energy by non-polar groups, in this case the hydrophobic polymer backbone, it is reasonable to deduce that the observed suppression results from a shielding effect by the polyether chains at the hydrogel surface.

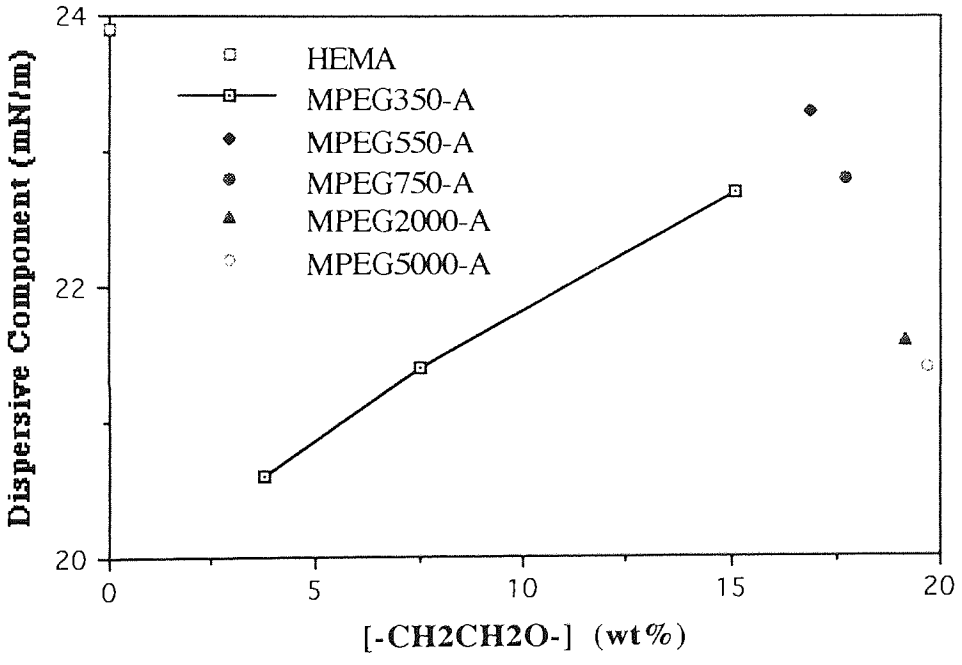


Figure 3.6: Effect of the Methoxy-Terminated MPEGAs on γ_s^d of HEMA:EGDM(99:1) Hydrogels.

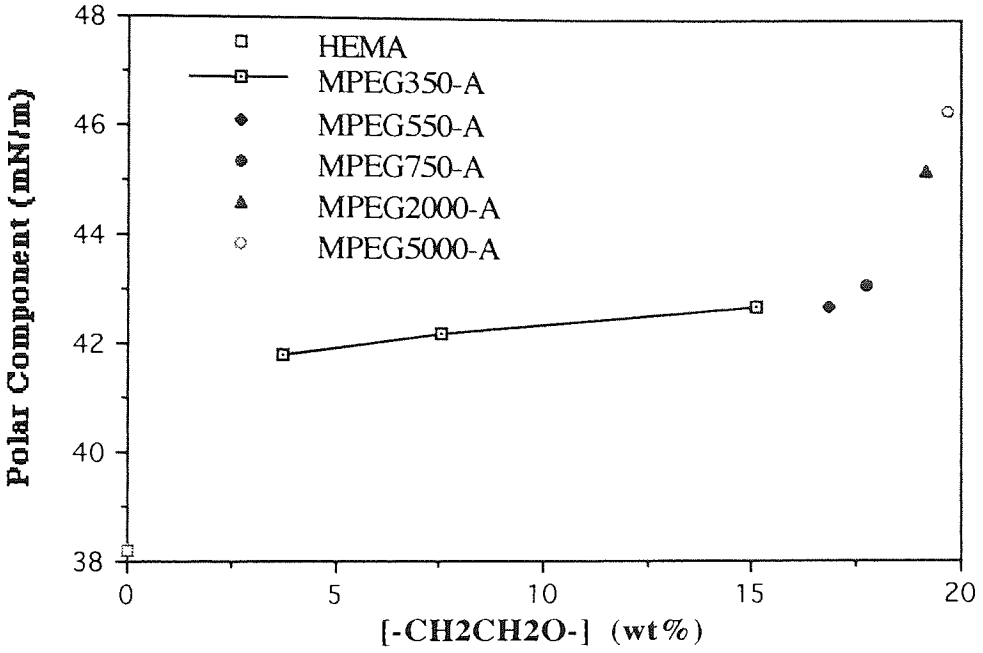


Figure 3.7: Effect of the Methoxy-Terminated MPEGAs on γ_s^p of HEMA:EGDM(99:1) Hydrogels.

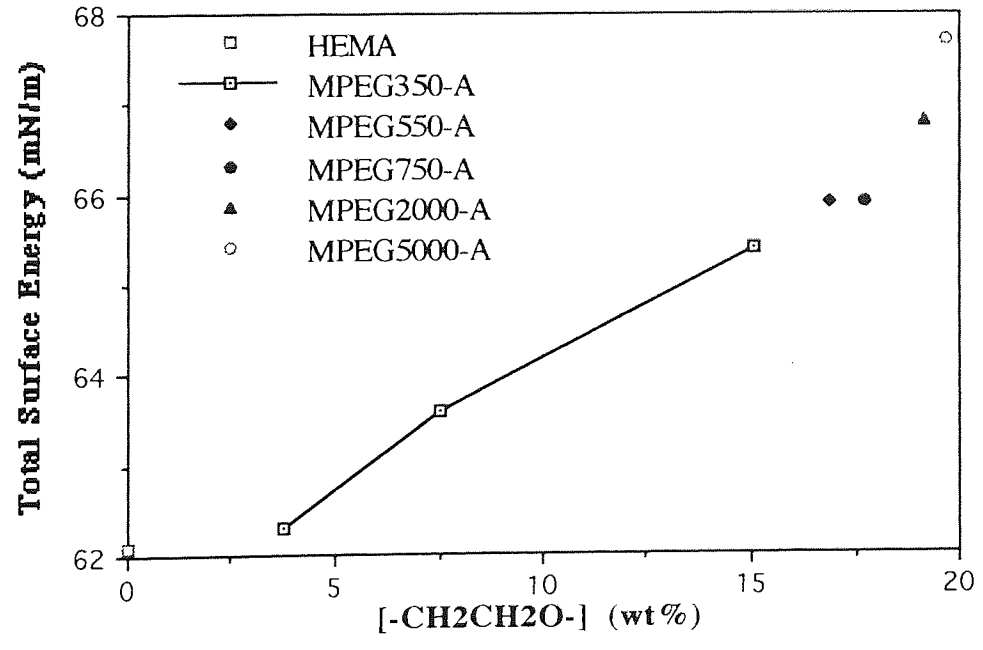


Figure 3.8: Effect of the Methoxy-Terminated MPEGAs on γ_s^t of HEMA:EGDM(99:1) Hydrogels.

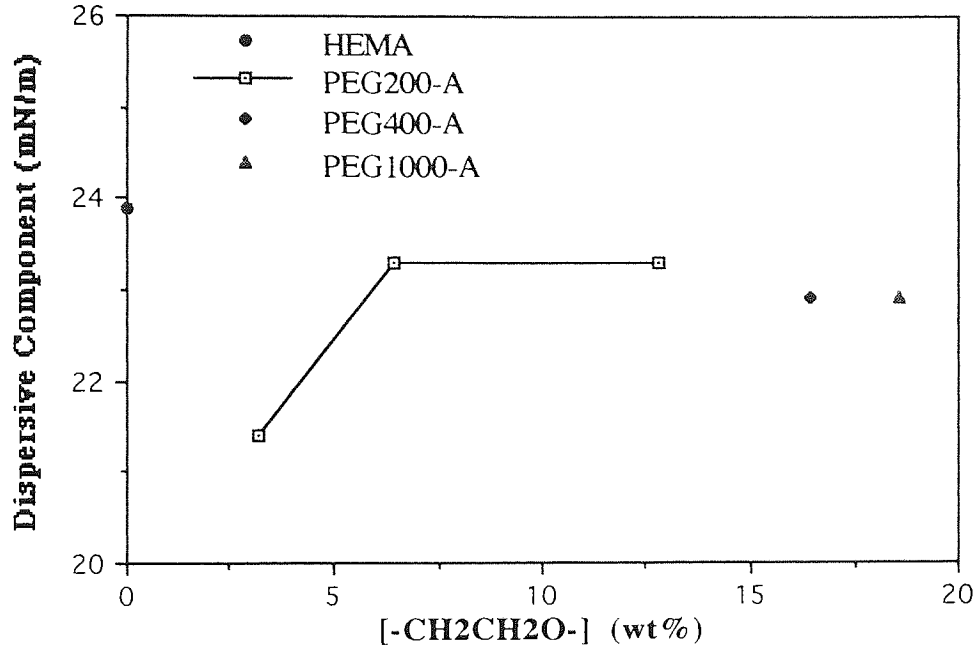


Figure 3.9: Effect of the Hydroxy-Terminated PEGAs on γ_s^d of HEMA:EGDM(99:1) Hydrogels.

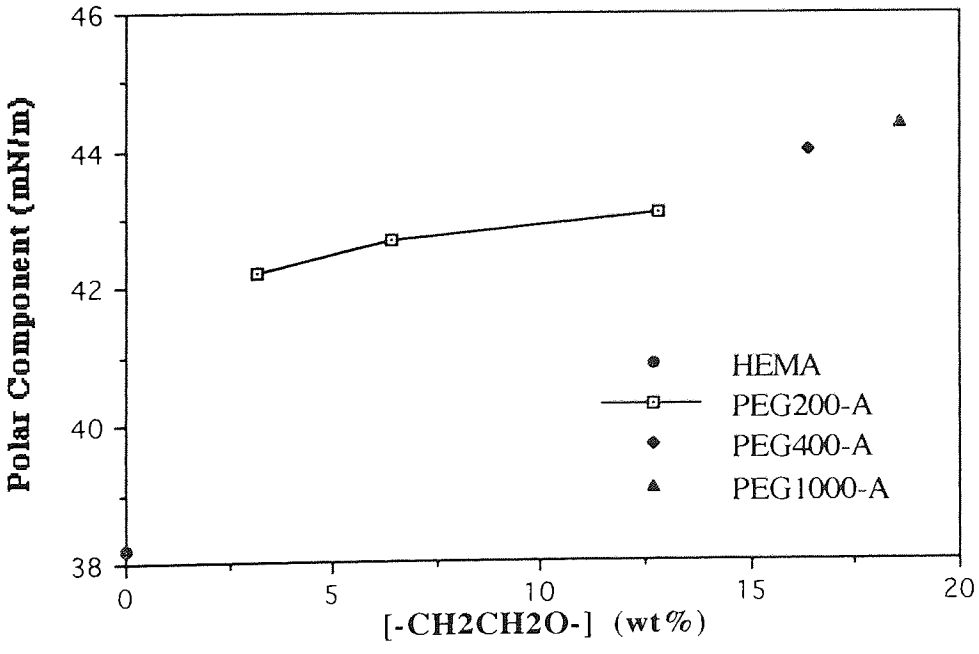


Figure 3.10: Effect of the Hydroxy-Terminated PEGAs on γ_s^p of HEMA:EGDM(99:1) Hydrogels.

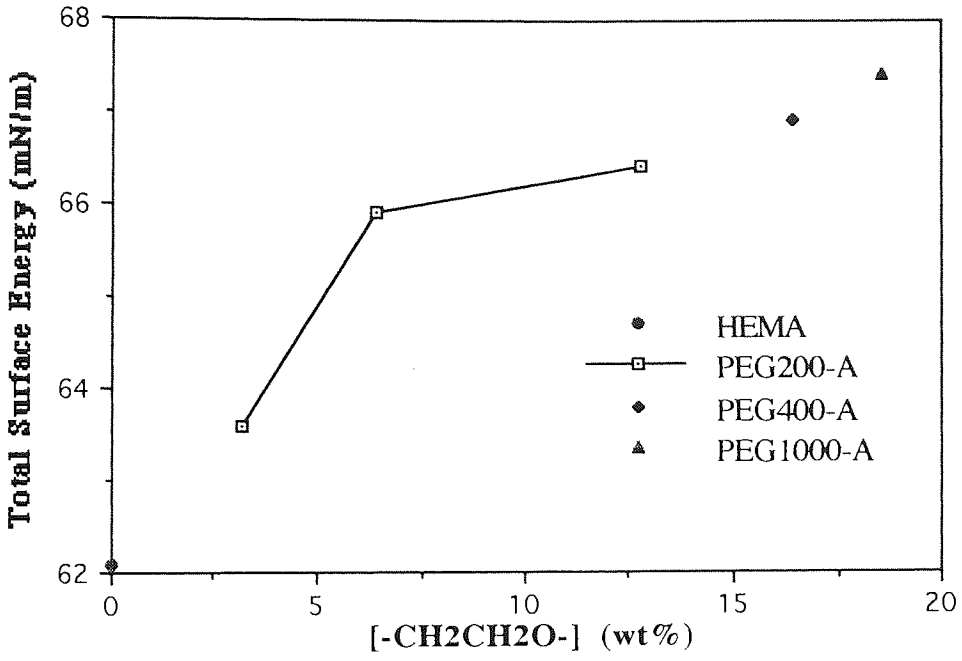


Figure 3.11: Effect of the Hydroxy-Terminated PEGAs on γ_s^t of HEMA:EGDM(99:1) Hydrogels.

3.5 CONCLUSIONS.

Several conclusions may be drawn from studies of the bulk and surface properties of poly(HEMA) based hydrogels, containing the polyether derivatives synthesised as described in Chapter 2.

Results, from studies of the bulk properties of hydrogels studied, show for gels containing either methoxy- or hydroxy-derivatives that:-

1) introducing polyether acrylates into a poly(HEMA) based hydrogel increases the equilibrium water content (EWC) of the gel with respect to one containing only poly(HEMA).

2) increasing the concentration of the same polyether acrylate, within the hydrogel network, causes an increase in EWC with respect to a gel containing only poly(HEMA).

3) for two hydrogels containing different polyether acrylates, but with the same concentration of ether repeat units, the gel with the highest EWC value will be the one containing the polyether acrylate of highest molecular mass i.e. the acrylate with the longest polyether chain.

The first two of these observations highlight that incorporating polyether chains into the hydrogel matrix increases the hydrophilicity of the gel. This effect is due to the influence of the polar $-CH_2CH_2O-$ ether repeat units. The third of these observations is probably due to the fact that the hydrogel matrix is essentially made up of a hydrophobic backbone with hydrophilic side-chains. For the longer polyether side-chains, a continually increasing proportion of ether repeat units exist an increasing distance away from the hydrophobic polymer backbone. The further the repeat units are away from this hydrophobic influence, the greater will be their ability to structure water.

Results of the surface properties of hydrogels studied, show for gels containing either methoxy- or hydroxy-derivatives that:-

1) the total surface free energy, of a poly(HEMA) based hydrogel, increases with ether repeat unit concentration.

2) the increase in total surface free energy is controlled by an specific increase in the polar surface energy component.

3) a supression in the dispersive surface free energy component is observed.

The first two of these observations support very strongly the idea that the surface properties of the hydrogels are dictated by the polar polyether side-chains. The third of these observations is probably due to the fact that the dispersive free energy component arises predominantly from contributions to the surface energy by non-polar groups, in this case the hydrophobic polymer backbone. It is reasonable to deduce, therefore, that the observed suppression results from a shielding effect by the polyether chains at the hydrogel surface.

Combining these conclusions, made on analysis of both bulk and surface properties, suggests that polyether acrylates may be potentially suitable biomaterials when copolymerised with HEMA. The observed increase in EWC suggests polyether modified materials could have superior oxygen permeability, that may make them potentially useful in soft contact lens applications. Additionally it would appear that the surface properties of these polyether modified materials are largely controlled by the polar polyether side-chains. This would be consistent with the theory that devices containing pendant polyether side-chains resist protein deposition because the chains provide an excluded volume about the surface of the material[50].

CHAPTER 4

CELL ADHESION AND PROPERTY STUDIES OF POLY(2-HYDROXYETHYLMETHACRYLATE) :POLY(METHYL METHACRYLATE) TERPOLYMERS CONTAINING LINEAR POLYETHERS.

4.1 GENERAL INTRODUCTION.

One of the major applications of hydrogels is in biomedical applications[108]. These applications include contact lenses[109], liver support systems[22], and replacement blood vessels[110] among a variety of other related and potential uses.

Hydrogels, when used in *in vivo* applications, are usually well tolerated in comparison to other polymers. There are a number of attributes that hydrogels possess that make this so. Hydrogels bear a resemblance to tissue in that their water contents are relatively high (20-99% depending on the degree of cross-linking). The water maintained within a hydrogel matrix acts as a plasticiser, a transport medium for water soluble species (e.g. oxygen) and a 'bridge' across any difference in surface energies between the hydrogel matrix and the physiological system. Hydrogels, because of their chemical fabric, often show low interfacial tensions with aqueous environments. This is especially important when considering their compatability as replacement blood vessels where minimal interfacial tension has been related to thromboresistance, or blood compatability[111]. This observed phenomenon also results in the minimal amount of frictional irritation of the surrounding tissues[112]. Summarising, therefore, one of the most exciting aspects of synthetic hydrogels is that they show favourable interfacial properties in physiological environments.

As stated before, workers have recently indicated that thrombogenic reactions to synthetic polymers are reduced by the presence of linear polyether side chains. The incorporation of polyether side chains into hydrogel matrices using methoxy poly(ethylene glycol) acrylates and poly(ethylene glycol) monoacrylates as was demonstrated in Chapter 2 of this thesis. The polyether acrylate:HEMA:EGDM (99:1) hydrogels formed were studied in Chapter 3 where equilibrium water content (EWC) and surface energy values were obtained. It was demonstrated that the inclusion of polyether side chains significantly increased the water content of the hydrogels.

Further to this the increased polar component of surface energy, observed on increasing the concentration of [-CH_2CH_2O-] ether repeat units, suggested that the surface properties of the hydrogels were increasingly dictated by the polyether chains. This would be consistent with previous theoretical postulations that the observed reduction in thrombogenic reactions, noted above, is attributable to the presence of flexible hydrated polyether chains which provide an excluded volume to protein absorption at the polymer surface. It would be extremely interesting to know, in addition to the above, how polyether acrylate modified hydrogels interacted with proteins. This information would enable estimates to be made of how these novel materials may interact at an *in vivo* biological interface.

In order to answer these questions, cell adhesion studies were carried out in this laboratory. These studies helped to ascertain the tolerance of polyether acrylate modified hydrogels to protein deposition. A more detailed description of the biological implications of these particular studies is considered to be beyond the scope of this thesis, but such a description can be found in the Ph.D. thesis of Dr J.H. Fitton[113], to whom I am indebted. Descriptions of the experimental techniques used in cell adhesion studies are also well documented by Thomas[114].

When a foreign surface is placed in contact with a biological environment, either *in vivo* or *in vitro*, there is an almost immediate active deposition from the biological system[115]. This process can irreversibly alter the surface of the foreign material and in fact constitutes, when *in vivo*, one of the first stages of physiological rejection. The necessity of potential biomaterials to interact reversibly in a biological environment is therefore of great importance if rejection is to be avoided. This is true of applications as diverse as soft contact lenses through to synthetic replacement blood vessels. The development of synthetic hydrogels has produced a number of materials that potentially overcome these difficulties, for reasons stated earlier in this section. Substantial research is in progress within the Speciality Materials Research Group

here at Aston, concerned with *in vitro* protein deposition onto hydrogel materials. One such method involves the study of cell adhesion to the hydrogels.

If hydrogels are allowed to interact with a biological interface for a period of time, then the gels will interact with the proteins in that medium absorbing the proteins reversibly or irreversibly (deposition). After a period of time washing of the hydrogel samples will leave only proteins that have been permanently deposited on the hydrogel surface. These proteins will have within them specific anchorage sites. It is possible to grow anchorage dependent cells, in cultures, in controlled conditions. These cells have receptors that will recognise specific proteins on the hydrogel surface. The cells will therefore be able to adhere to these proteins. After staining the adhered cells, counting them under a microscope gives a cell count proportionate to the degree of protein deposition that has occurred on the hydrogel surface.

An interesting overview of the cell adhesion behaviour of hydrogels is available[116]. Within this report it was highlighted that hydrogels containing between 5% and 35% water were responsive to cell adhesion studies. At water contents >35%, the cell response decreased suddenly producing very slow adhesion that was impossible to study over a short time span. These observations have been substantiated here and are demonstrated in the work of Oxley[91] and Fitton[113]. Together, they attempted to analyse the cell adhesion properties of some polyether:HEMA based hydrogels all of which had EWC values in excess of 35%. The results they obtained showed this 'cut-off point' for cell adhesion, which meant that inter-sample comparison was impossible and heavily restricted the technique.

For reasons stated it was considered that the hydrogels synthesised as described in Chapter 3 of this report would be unsuitable for cell adhesion studies. However, by creating terpolymers, incorporating the non-hydrophilic methyl methacrylate (MMA) in 50:50 HEMA:MMA (wt%), it was possible to produce a

series of hydrogels that had EWC values of between 16% and 34%, optimum for cell adhesion study. As these hydrogels are significantly different in chemistry from those discussed in Chapter 3, the remainder of this chapter not only contains details of cell adhesion studies but also of the water content and surface energy characteristics of this series.

4.2 PREPARATION OF HYDROGELS.

A range of copolymers were prepared by adding 5 to 20% weight of each of the eight monomers, prepared as described in Chapter 2, to {HEMA:MMA(50:50)}: EGDM(99:1). Ethyleneglycol dimethacrylate (EGDM) was employed as a crosslinking agent. The full experimental procedure for the synthesis of these hydrogels, may be found in Chapter 8.

4.3 THE EFFECT OF POLYETHER ACRYLATES ON THE EWC OF {HEMA:MMA(50:50)}:EGDM (99:1) HYDROGELS.

The experimental method used in calculating EWC values for the prepared hydrogels can be found in Chapter 8. The definition of the EWC value for a hydrogel is:-

$$EWC = \frac{\text{Weight of water present in the hydrated gel}}{\text{Total weight of hydrated gel}} \times 100$$

The EWC values of all HEMA:EGDM (99:1) based hydrogels prepared are given in Table 4.1. A statistical treatment of the error involved in EWC determination by this technique gives a standard deviation of $\sigma_{n-1} = 0.4$, an error of approximately 3% [103].

Membrane Composition	EWC (%)
{HEMA:MMA (50:50)}:EGDM (99:1)	16.4
{HEMA:MMA (50:50)}:EGDM (99:1) + 5% MPEG550-A	16.9
{HEMA:MMA (50:50)}:EGDM (99:1) + 10% MPEG550-A	17.1
{HEMA:MMA (50:50)}:EGDM (99:1) + 20% MPEG550-A	21.6
{HEMA:MMA (50:50)}:EGDM (99:1) + 5% MPEG2000-A	20.1
{HEMA:MMA (50:50)}:EGDM (99:1) + 10% MPEG2000-A	23.2
{HEMA:MMA (50:50)}:EGDM (99:1) + 20% MPEG2000-A	33.7
{HEMA:MMA (50:50)}:EGDM (99:1) + 5% PEG400-A	17.2
{HEMA:MMA (50:50)}:EGDM (99:1) + 10% PEG400-A	21.4
{HEMA:MMA (50:50)}:EGDM (99:1) + 20% PEG400-A	27.1
{HEMA:MMA (50:50)}:EGDM (99:1) + 5% PEG1000-A	21.4
{HEMA:MMA (50:50)}:EGDM (99:1) + 10% PEG1000-A	24.7
{HEMA:MMA (50:50)}:EGDM (99:1) + 20% PEG1000-A	29.6

Table 4.1 : The Effect of Polyether Content on the EWC of {HEMA:MMA (50:50)}:EGDM (99:1) Hydrogels.

These results initially suggest that the EWC of {HEMA:MMA(50:50)}:EGDM (99:1) based hydrogels is affected as follows on the inclusion of polyether acrylates:-

1) increasing the concentration of the same polyether acrylate within the hydrogel network, causes an increase in EWC.

2) for two HEMA:EGDM (99:1) hydrogels containing the same concentration of different polyether acrylates, the gel with the highest EWC value will be the

one containing the polyether acrylate of highest molecular mass i.e. the acrylate with the longest polyether chain.

These observations hold for both the methoxy- and hydroxy- terminated series of polyether acrylates.

A further note should be made of the generally lower EWC values of these hydrogels relative to those discussed in Chapter 3. This is obviously due to the inclusion of hydrophobic methyl methacrylate within the hydrogel network.

These results, although promising and interesting, are not particularly surprising. What would be more interesting would be to determine whether the EWC value for a gel is affected more by a large number of small polyether chains, or by a small number of longer polyether chains. To deduce such an effect, the concentration of $-CH_2CH_2O-$ ether repeat units must be constant between two comparative gels i.e. the only variable being analysed is the change in EWC with chain length. Producing plots of EWC versus the concentration of $-CH_2CH_2O-$ ether repeat units in wt% will give a graph that should allow the relationship between EWC and chain length to be investigated. Such a relationship can be deduced for hydrogels containing both methoxy- and hydroxy- terminated polyether acrylates. The two relationships can then be compared.

Figure 4.1 shows a plot of EWC (wt%) versus [$-CH_2CH_2O-$] (wt%) for all synthesised hydrogels containing {HEMA:MMA(50:50)}:EGDM (99:1) and MPEGA derivatives. Figure 4.2 shows the analogous plot for the PEGA derivatives.

Analysis of Figure 4.1 shows the affect of polyether chain length on EWC at a constant concentration of ether repeat units. This effect is similar to that observed whilst studying the hydrogels prepared as described in Chapter 3. The EWC values

observed for the MPEG derivatives at 10% weight of polyether repeat units are listed as follows:-

MPEG Derivative	**EWC (wt%)**
MPEG550-A	~18
MPEG2000-A	~24

These results show for a constant concentration of ether repeat units that observed EWC values increase with increasing polyether chain length. As was observed in Chapter 3, this is probably due to the fact that the hydrogel matrix is essentially made up of a hydrophobic backbone with hydrophilic side-chains. For the longer polyether side-chains, a continually increasing proportion of ether repeat units exist an increasing distance away from the hydrophobic polymer backbone. The further the repeat units are away from this hydrophobic influence, the greater will be their ability to structure water leading to the results observed above. Analysis of Figure 4.2 allows similar deductions to be made for the hydroxy-terminated PEGA derivatives

More generally, comparing the EWC values displayed in Table 4.1, Figure 4.1 and Figure 4.2 highlights that inclusion of polyether side-chains in a poly(HEMA:MMA) based hydrogel increases the water content of the gel with respect to a pure poly(HEMA:MMA) gel.

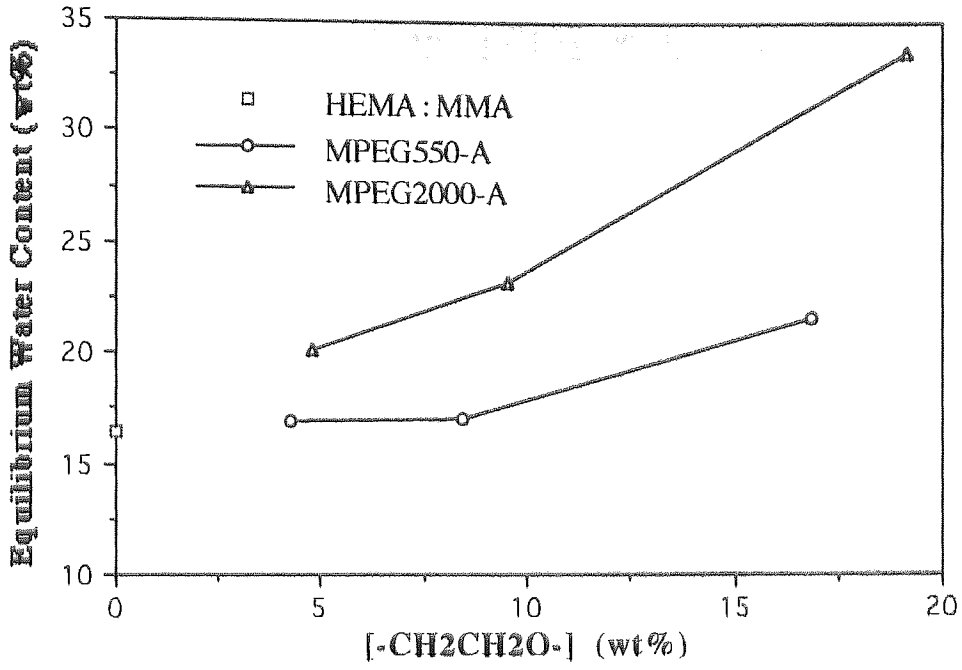


Figure 4.1: Effect of the Methoxy-Terminated MPEGAs on the EWC of {HEMA:MMA(50:50)}:EGDM(99:1) Hydrogels.

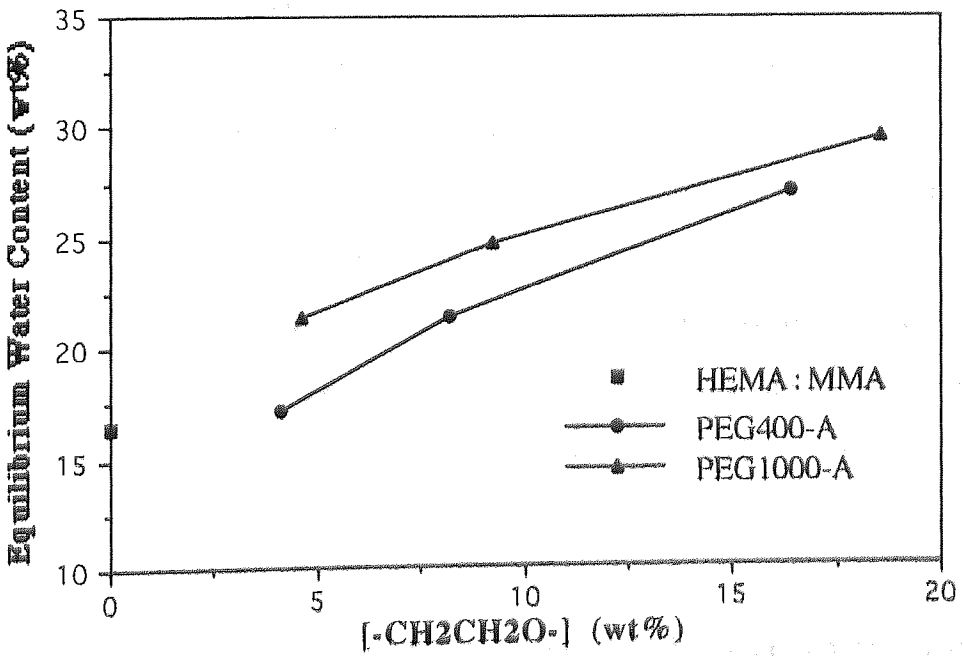


Figure 4.2: Effect of the Hydroxy-Terminated PEGAs on the EWC of {HEMA:MMA(50:50)}:EGDM(99:1) Hydrogels.

4.4 THE EFFECT OF POLYETHER ACRYLATES ON THE SURFACE ENERGIES OF {HEMA:MMA(50:50)}:EGDM (99:1) HYDROGELS.

4.4.1 Introduction.

Contact angle goniometry was used to determine the surface energy of these hydrogel copolymers. The practical details associated with this technique are discussed in Chapter 8. The application of contact angle theory allows the surface energies of hydrogels to be calculated in either their hydrated or dehydrated states. Consideration had therefore to be given to which alternative would be most appropriate in this case.

As discussed previously, the mechanism by which polyether side chains resist protein absorption is thought to be by providing an excluded volume about the polymer surface. If contact angles were to be measured in the dehydrated state then it is considered that removal of the aqueous environment might cause the side chains to 'collapse' against the surface of the copolymer. This would significantly alter the characteristics of the copolymer surface, and would not allow a surface study of the effects of mobile polyether side chains to be made. In addition to this, surface energies are difficult to measure, in the dehydrated state, where hydrophilic samples absorb water rapidly. Measurement of contact angles in the hydrated state, however, provides the ability to study the effect of mobile polyether side chains on the hydrogel surface.

The theory and method behind the measurement of contact angles in the hydrated state, and the application of these results to find values for the total- (γ_s^l), polar- (γ_s^p) and dispersive- (γ_s^d) surface free energies, may be found in Chapter 3.

The contact angles obtained, using both Hamilton's method[104] and the captive air bubble technique, were recorded as the average of six readings. Due to the increased hydrophobicity of the surfaces, it was more difficult to obtain consistant contact angle measurements in comparison to those analysed as reported in Chapter 3. This was due to the increased affinity of the surfaces to both octane and air. It was found, however, that readings obtained had values within $\pm 2^{\circ}$ of the mean value. This range of contact angle readings gave a general error, for all surface free energy values obtained, of ± 3 mN/m for γ_s^d, ± 1 mN/m for γ_s^p and ± 4 mN/m for γ_s^l.

4.4.2 Discussion of Surface Free Energy Studies.

Figures 4.5 and 4.8 show the total surface free energies observed for gels containing MPEGA and PEGA derivatives respectively. They show the effect, on surface energy, of increasing the concentration of one particular derivative, and also show an inter-comparison between hydrogels containing all derivatives and a pure poly(HEMA:MMA) hydrogel.

Figures 4.3 and 4.6 show the dispersive component of surface free energy, and Figures 4.4 and 4.7 the polar component of surface free energy, for hydrogels containing MPEGA and PEGA derivatives respectively.

Initial analysis of these figures, as a whole, suggests that the relationship between surface free energy and ether repeat unit concentration is not quite as straight forward or obvious as for those poly(HEMA):polyether acrylate hydrogels studied in Chapter 3. As was mentioned earlier, there were considerably more difficulties encountered in measuring the contact angles of hydrogels prepared as described in this chapter. This was due to the introduction of the hydrophobic methyl methacrylate as a copolymer. The resultant series of hydrogels had considerably lower water contents and greater affinities for the non-polar octane, making the consistent measurement of

contact angles difficult on occasion. This is considered to be why occasional rogue points were observed, particularly on analysis of hydrogels containing MPEG2000-A and PEG400-A. However similar trends are observed to those noted for the poly(HEMA):polyether acrylate hydrogels, which allow a similar a treatment of results to be made.

Figures 4.5 and 4.8 suggest that the total free energy of a poly(HEMA:MMA) based hydrogel increases, with ether repeat unit concentration, for gels containing both methoxy- and hydroxy-terminated polyether derivatives. Figures 4.4 and 4.7 show that this increase in surface free energy is predominantly controlled by an increase in the polar surface energy component.

Combining the above observations tends to suggest that a polar influence is present at the surface of hydrogels containing polyether derivatives, that is not present in a pure poly(HEMA) hydrogel. From these deductions it is postulated that the surface properties of hydrogels containing such derivatives are as anticipated dictated by the polar polyether side-chains.

Reference to Figures 4.3 and 4.6 shows that the dispersive surface energy component is surpressed, for hydrogels containing either methoxy- or hydroxy-terminated derivatives. As the dispersive component arises predominantly from contributions to the surface energy by non-polar groups, in this case the hydrophobic polymer backbone, it is reasonable to deduce that the observed suppression results from a shielding effect by the polyether chains at the hydrogel surface.

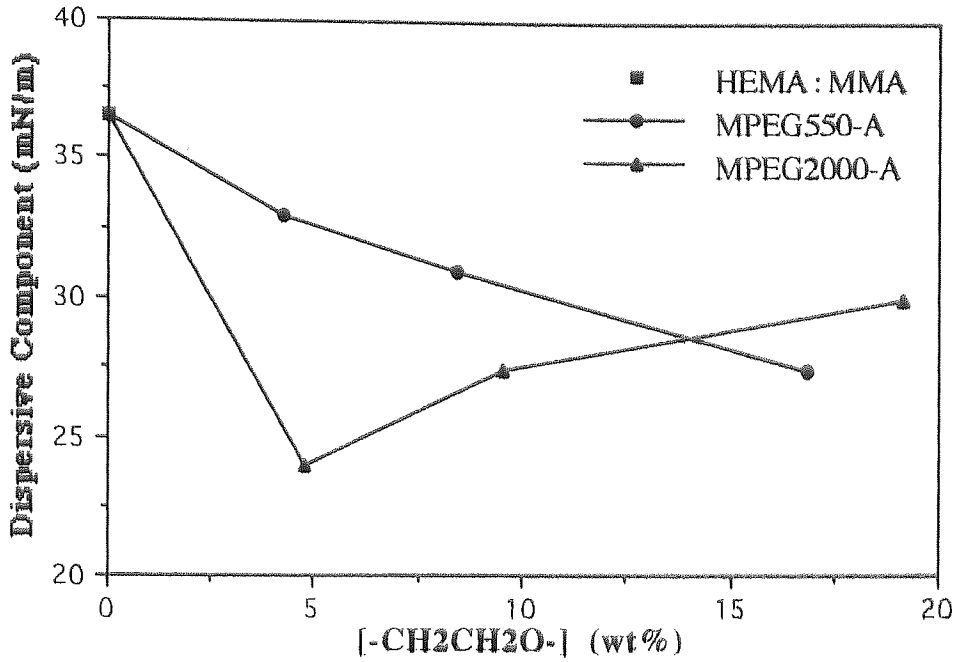


Figure 4.3: Effect of the Methoxy-Terminated MPEGAs on γ_s^d of {HEMA:MMA(50:50)}:EGDM(99:1) Hydrogels.

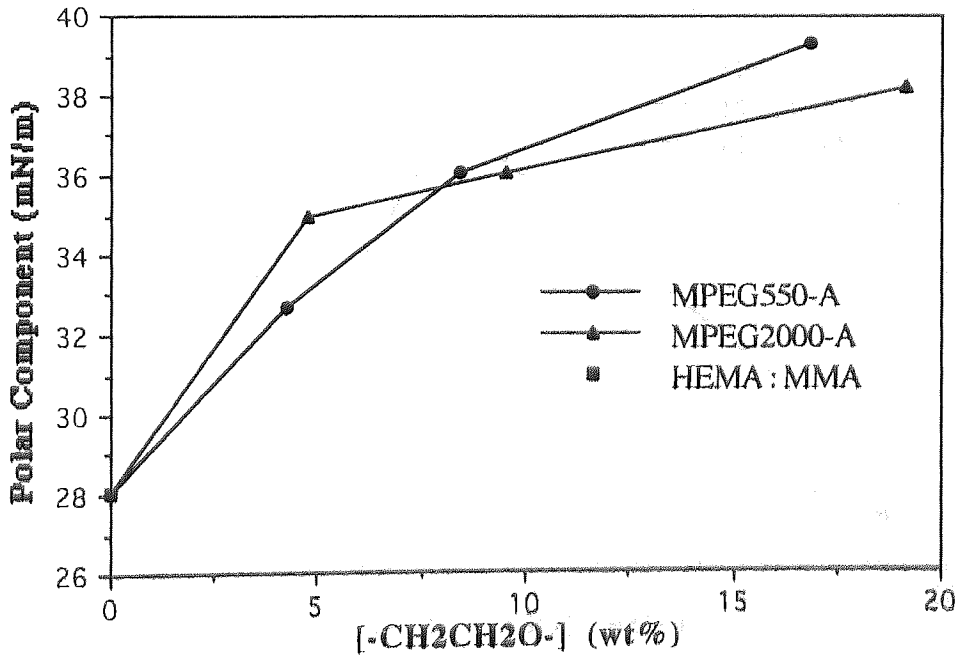


Figure 4.4: Effect of the Methoxy-Terminated MPEGAs on γ_s^p of {HEMA:MMA(50:50)}:EGDM(99:1) Hydrogels.

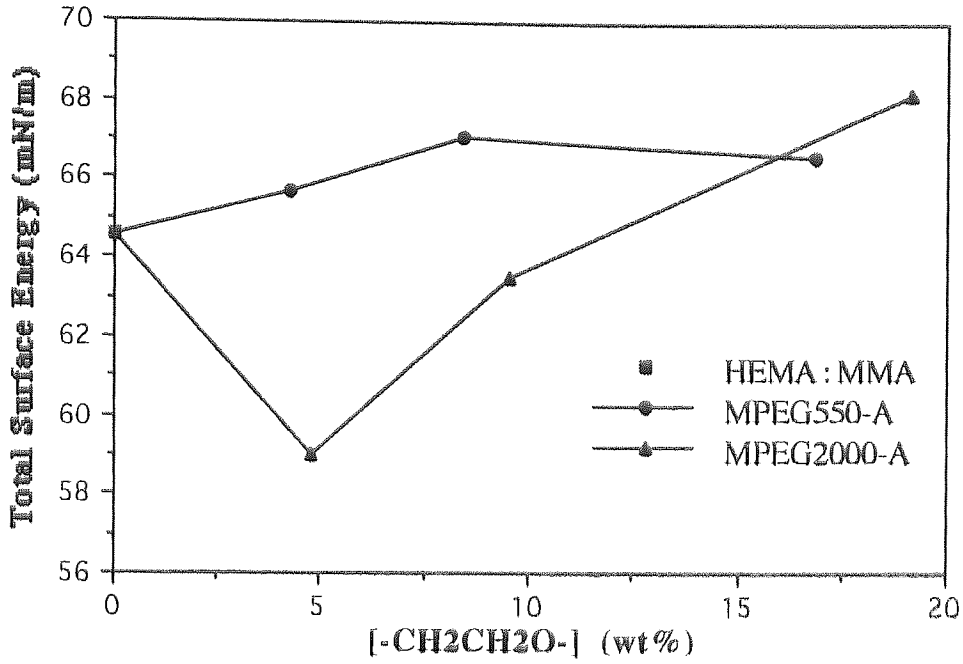


Figure 4.5: Effect of the Methoxy-Terminated MPEGAs on γ_s^l of {HEMA:MMA(50:50)}:EGDM(99:1) Hydrogels.

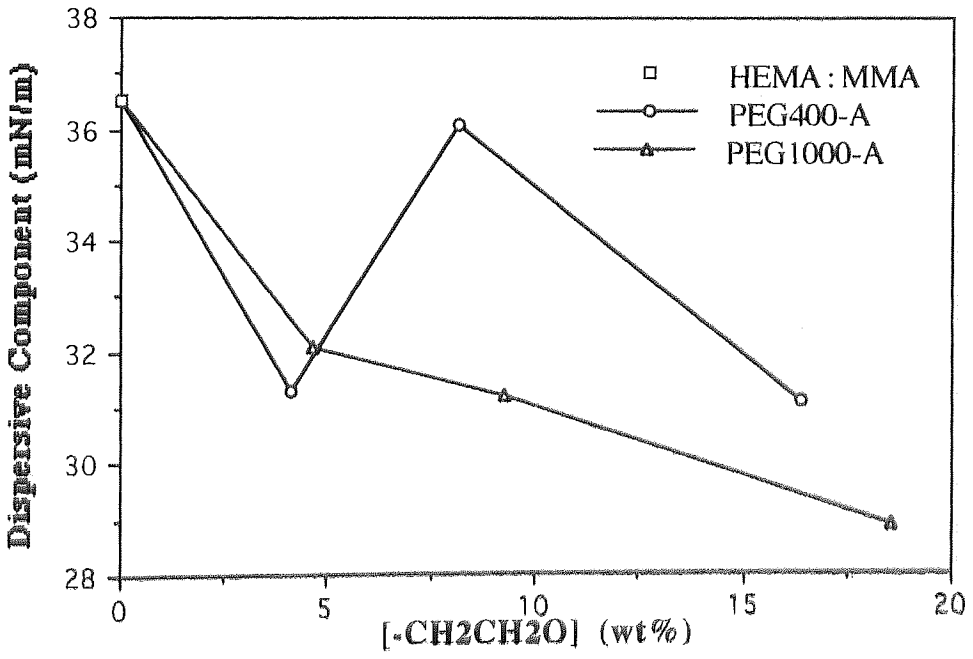


Figure 4.6: Effect of the Hydroxy-Terminated PEGAs on γ_s^d of {HEMA:MMA(50:50)}:EGDM(99:1) Hydrogels.

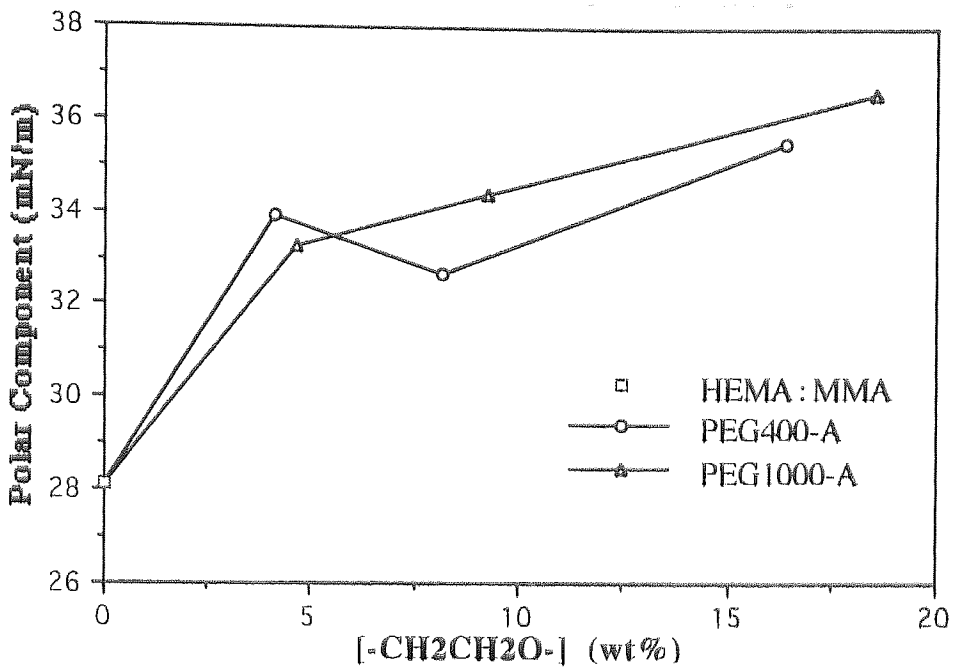


Figure 4.7: Effect of the Hydroxy-Terminated PEGAs on γ_s^p of {HEMA:MMA(50:50)}:EGDM(99:1) Hydrogels.

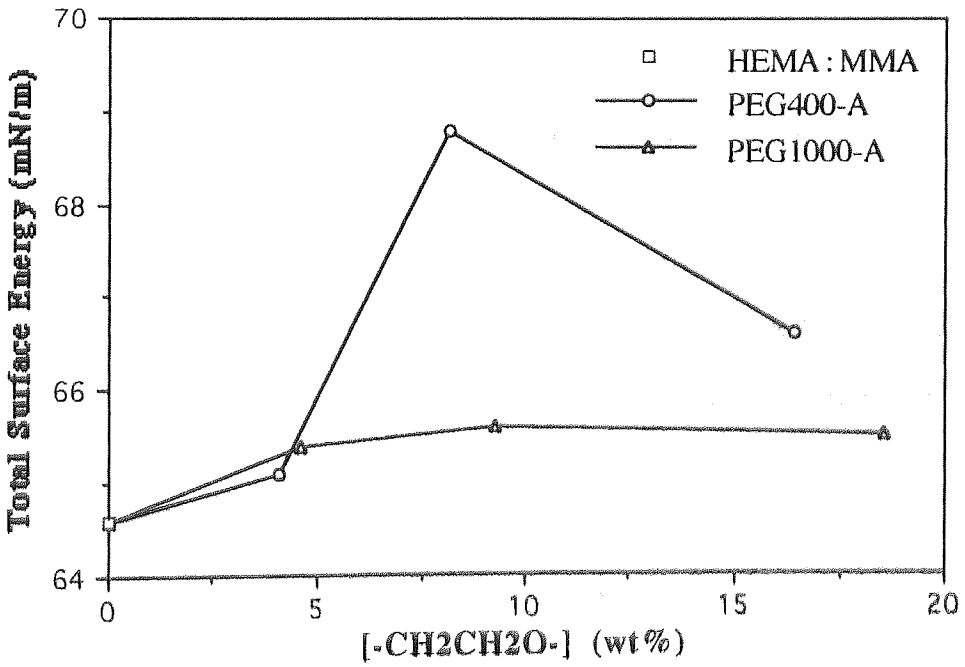


Figure 4.8: Effect of the Hydroxy-Terminated PEGAs on γ_s^l of {HEMA:MMA(50:50)}:EGDM(99:1) Hydrogels.

4.4 CELL ADHESION TO POLYETHER ACRYLATE MODIFIED {HEMA:MMA(50:50)}:EGDM (99:1) HYDROGELS.

4.4.1 Introduction.

Cell culture is a technique that makes use of the fact that, under strict conditions, cells can be kept alive *in vitro*. Such cells can then be used to investigate the surface properties of materials such as hydrogels. This section is therefore concerned with the results of preliminary investigations to assess the effects, of MPEGA and PEGA comonomers, on the adhesion of 3T3 cells to poly(HEMA) based hydrogels. A thorough description of the experimental techniques used in these studies is considered to be beyond the scope of this thesis. A more complete description can however be found in the thesis of Dr J.H. Fitton[113].

4.4.2 Discussion of Cell Adhesion Studies.

The effect of polyether chain length and polyether derivative concentration, on the adhesion of 3T3 cells, was studied for the hydrogels containing MPEG550-A, MPEG2000-A, PEG400-A and PEG1000-A, prepared as described in Section 4.2. For comparison, studies were also carried out on a poly(HEMA:MMA) hydrogel containing no polyether derivatives. On completion of each of the studies, the cell growth was determined.

The results from the analyses are documented in Figures 4.9 to 4.12.

The introduction to this chapter illustrated that the technique of cell adhesion allowed an assessment of irreversible protein interaction, between the hydrogel under analysis and the surrounding medium. The cell count obtained on conclusion of the studies will therefore be in some way proportional to the degree of protein deposition

that has occured at the hydrogel surface. The desire, therefore, is to observe low cell counts for materials which would be potentially useful at an *in vivo* biological interface.

Figures 4.9 and 4.10 show the results of cell adhesion studies on poly(HEMA:MMA) hydrogels containing MPEG550-A and MPEG2000-A respectively. For both additives the following trends are observed relative to a pure poly(HEMA:MMA) hydrogel:-

1) incorporating an MPEGA comonomer into the polymer matrix causes a decrease in observed cell count. A decrease in cell count suggests that the inclusion of MPEGA additives increases the resistance of the hydrogel to irreversible protein interaction (protein deposition).

2) increasing the concentration of a particular MPEGA comonomer in the polymer matrix causes a decrease in the observed cell count.

3) increasing the concentration of MPEG2000-A produces a greater decrease in the observed cell count than analogous increases in concentration of the MPEG550-A derivative.

These observations do suggest primarily that incorporating derivatives with methoxy-terminated polyether side chains into poly(HEMA:MMA) based hydrogels causes a significant decrease in protein deposition at the hydrogel surface. Furthermore, the results also display that the degree of protein deposition is in some way directly related to polyether chain length. This observation adds credence to the the theory that protein deposition is in fact inhibited by an excluded volume, produced by the polyether chains, at the hydrogel's surface.

Figures 4.11 and 4.12 show the results of cell adhesion studies on poly(HEMA:MMA) hydrogels containing PEG400-A and PEG1000-A respectively. These derivatives, which contain hydroxy- rather than methoxy-terminated polyether side chains, do not display the obvious consistent trends shown by their MPEGA analogues. However, interesting deductions can be made from these results, and hypotheses advanced to explain inconsistencies in the results obtained for hydroxy- versus methoxy-terminated derivatives. The following comments can be made:-

1) hydrogels containing PEG400-A comonomer show an irregular pattern as regards cell adhesion. The trend, however, would appear to be that protein deposition is generally greater, or at least very similar, to that observed for a pure poly(HEMA:MMA) hydrogel. For hydrogels containing PEG1000-A, a significant pattern does emerge showing that deposition is generally greater on the surface of gels containing this derivative relative to a pure poly(HEMA:MMA) hydrogel.

2) hydrogels containing PEG1000-A show that protein deposition increases, with increasing concentration of polyether derivative, although a similar pattern cannot be discerned from data obtained from gels containing PEG400-A.

3) the lack of pattern observed in studies using PEG400-A make inter-comparison, with the hydrogels containing PEG1000-A, impossible.

Combining this data, it is proposed that inclusion of the hydroxy-terminated PEGA products into poly(HEMA:MMA) based hydrogels caused either little difference, or an increase, in protein deposition relative to a pure poly(HEMA:MMA) gel. Results from studies on hydrogels containing PEG1000-A suggest that protein deposition increases with increasing comonomer concentration.

It would appear therefore that incorporation of hydroxy-terminated polyether chains has an opposite effect on protein deposition to that of their methoxy-analogues.

ESMS analysis of PEG1000-A, described in Chapter 2, showed there to be a small amount of diacrylate impurity in the final product. It may be that this could have altered the pattern observed in these cell adhesion studies. However, the small amount of diacrylate would effectively have acted as a cross-linker, when copolymerised, and would therefore have altered the bulk properties of any hydrogels prepared, to a greater degree than the surface properties that are generally considered to be manifestly controlled by free polyether chains. Analysis of the bulk properties of hydrogels containing PEG1000-A, prepared as described in both this Chapter and Chapter 3, by water content studies do not show anything else but the anticipated greater hydrophilicity compared with gels formed from its shorter chained analogues.

Assuming the above observations to be genuine, they are in agreement with results obtained by Oxley[91]. Using the 'Aston Tear Model'[117] she used a different technique to cell adhesion to measure the rate of lipid and protein deposition onto the surfaces of polyether methacrylate modified poly(HEMA) based hydrogels. Detection of deposited material was made via fluorescence rather than cell adhesion. She also found that hydroxy-terminated polyether side chains encouraged protein deposition whereas methoxy-terminated chains had the opposite effect. She postulated, very reasonably, that any potential excluded volume to protein deposition, that may be created by the polyether chains expressing themselves at the hydrogel surface, is in fact counteracted by hydrogen bonding interactions between protein molecules and the hydroxy-terminus of the polyether chain.

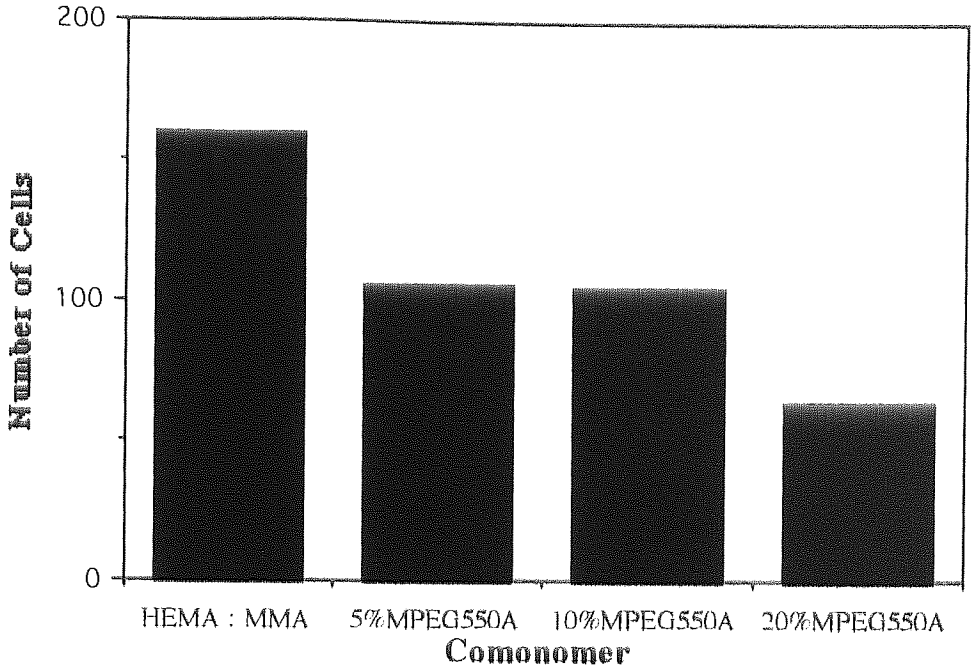


Figure 4.9:Adhesion of 3T3 Cells to Methoxy-Terminated MPEG550-A Modified {HEMA:MMA(50:50)}:EGDM(99:1) Hydrogels.

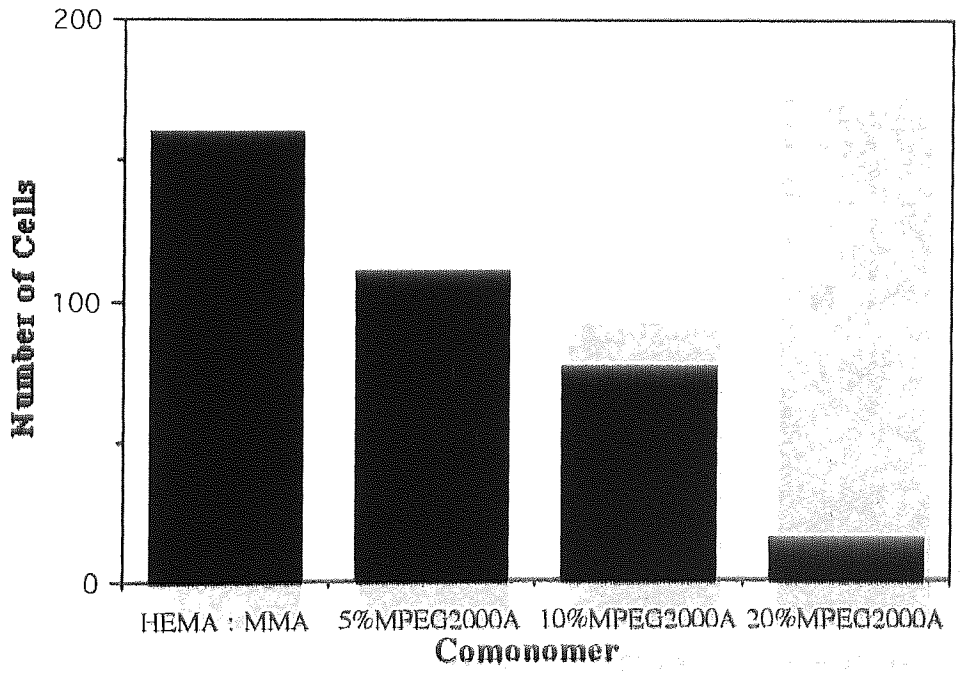


Figure 4.10: Adhesion of 3T3 Cells to Methoxy-Terminated MPEG2000-A Modified {HEMA:MMA(50:50)}:EGDM(99:1) Hydrogels.

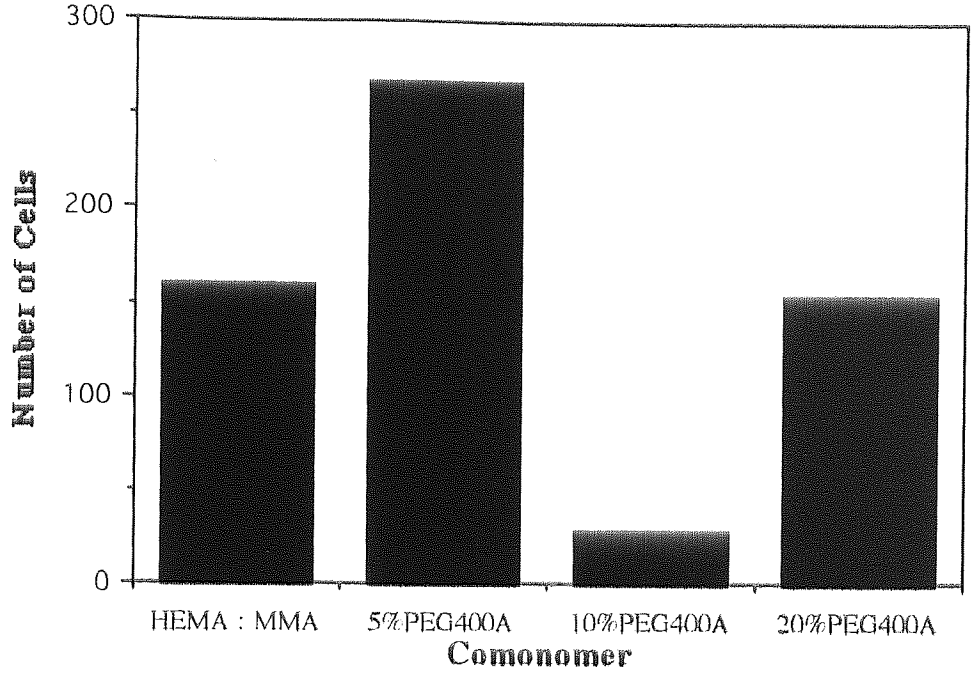


Figure 4.11: Adhesion of 3T3 Cells to Hydroxy-Terminated PEG400-A Modified {HEMA:MMA(50:50)}:EGDM(99:1).

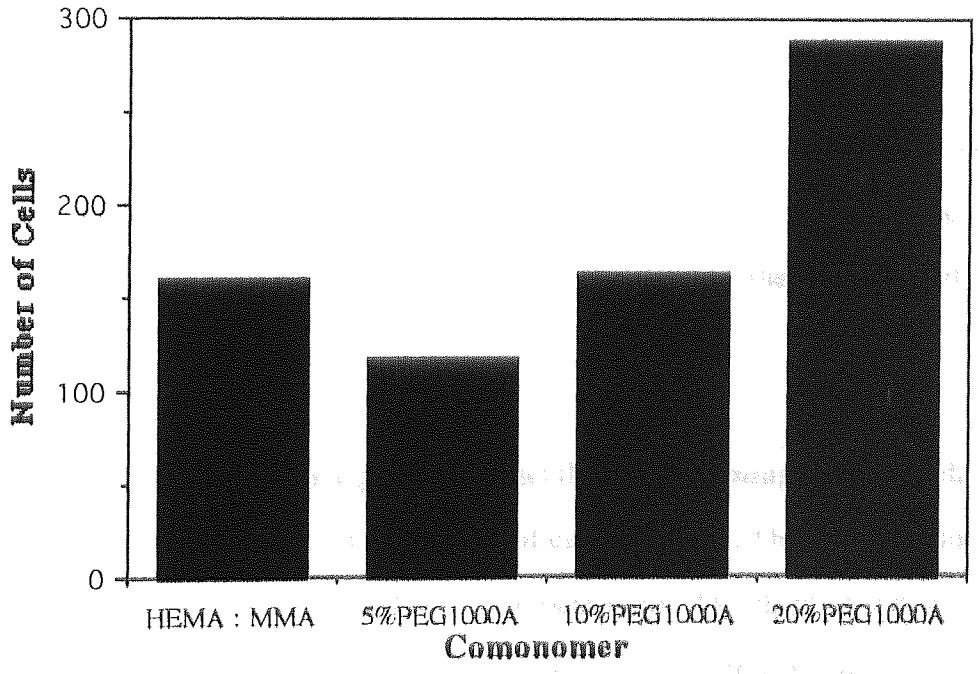


Figure 4.11: Adhesion of 3T3 Cells to Hydroxy-Terminated PEG1000-A Modified {HEMA:MMA(50:50)}:EGDM(99:1).

4.5 CONCLUSIONS.

Several conclusions may be drawn from studies of the bulk and surface properties of poly(HEMA:MMA) based hydrogels, containing polyether acrylate derivatives.

Results from studies of the bulk properties of hydrogels studied show for gels containing either methoxy- or hydroxy-derivatives that:-

1) introducing polyether acrylates into a poly(HEMA) based hydrogel increases the equilibrium water content (EWC) of the gel with respect to one containing only poly(HEMA).

2) increasing the concentration of the same polyether acrylate within the hydrogel network, causes an in some way proportionate increase in EWC with respect to a gel containing only poly(HEMA).

3) for two hydrogels containing different polyether acrylates, but with the same concentration of ether repeat units, the gel with the highest EWC value will be that containing the polyether acrylate of highest molecular mass, i.e. the acrylate with the longest polyether chain.

The first two observations highlight the fact that incorporating polyether chains into the hydrogel matrix increases the hydrophilicity of the gel. This effect is due to the influence of the polar $-CH_2CH_2O-$ ether repeat units. The third observation is probably due to the fact that the hydrogel matrix is essentially made up of a hydrophobic backbone with hydrophilic side-chains. For the longer polyether side-chains, a continually increasing proportion of ether repeat units exist an increasing distance away from the hydrophobic polymer backbone. The further the repeat units

are away from this hydrophobic influence, the greater will be their ability to structure water.

Results, from surface energy studies, show for gels containing either methoxy- or hydroxy-derivatives that:-

1) the total surface free energy, of a poly(HEMA) based hydrogel, increases with ether repeat unit concentration.

2) the increase in total surface free energy is controlled by an specific increase in the polar surface energy component.

3) a supression in the dispersive surface free energy component is observed.

The first two observations support very strongly the idea that the surface properties of the hydrogels are dictated by the polar polyether side-chains. The third observation is probably due to the fact that the dispersive free energy component arises predominantly from contributions to the surface energy by non-polar groups, in this case the hydrophobic polymer backbone. It is reasonable to deduce, therefore, that the observed suppression results from a shielding effect by the polyether chains at the hydrogel surface.

Cell adhesion studies, carried out *in vitro* using 3T3 cells, on the hydrogels prepared yielded extremely interesting results. The following observations and conclusions can be drawn:-

1) the incorporation of methoxy-terminated MPEGA comonomers into poly(HEMA:MMA) based hydrogels causes a decrease in observed cell count relative to a pure poly(HEMA:MMA) hydrogel. This decrease is seen to be

directly related to the concentration of a particular MPEGA additive and also directly related to the length of polyether side-chain of the additive chosen.These observations do suggest primarily that incorporating derivatives with methoxy-terminated polyether side chains, into poly(HEMA:MMA) based hydrogels, causes a significant decrease in protein deposition at the hydrogel surface.

2) results obtained from incorporating hydroxy-terminated PEGA comonomers into poly(HEMA:MMA) based hydrogels show that cell adhesion is generally greater, or at least very similar, to that observed for a pure poly(HEMA:MMA) hydrogel. Hydrogels containing PEG1000-A show increases in protein deposition, with increasing concentration of polyether derivative, although a similar pattern cannot be discerned from data obtained from gels containing PEG400-A. It is proposed that inclusion of the hydroxy-terminated PEGA products into poly(HEMA:MMA) based hydrogels caused either little difference, or an increase, in protein deposition relative to a pure poly(HEMA:MMA) gel. Results from studies on hydrogels containing PEG1000-A suggest that protein deposition increases with increasing comonomer concentration.

A combination of these conclusions made on analysis of both bulk and surface properties suggests that methoxy-terminated polyether acrylates may be potentially suitable biomaterials when copolymerised into HEMA based systems . The increase in EWC observed suggests that polyether-modified materials should have superior oxygen permeability, which may make them potentially useful in soft contact lens applications. Additionally it would appear that the surface properties of these polyether modified materials are largely controlled by the polar polyether side-chains. This suggestion is apparently confirmed by cell adhesion studies showing that the inclusion of polyether chains reduces protein deposition on the hydrogel surface. This

observation is presumably due to the chains providing an excluded volume about the surface of the material.

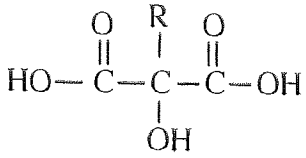
When copolymerised with HEMA:MMA, hydroxy-terminated polyether acrylates were seen to have excellent EWC and surface energy properties. However these hydrogels were seen to have a fairly poor resistance to protein deposition. This may preclude their use in HEMA based biomaterials. This seemingly opposing effect to that observed for their methoxy-terminated analogues could be due to the fact that any potential excluded volume to protein deposition, that may be created by the hydroxy-terminated polyether chains expressing themselves at the hydrogel surface, is in fact counteracted by hydrogen bonding interactions between protein molecules and the hydroxy-terminus of the polyether chain.

CHAPTER 5

THE SYNTHESIS OF ALKYLTARTRONIC ACIDS.

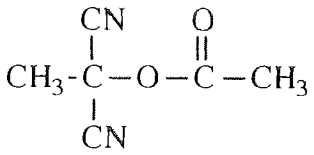
5.1 GENERAL INTRODUCTION.

The synthesis of alkyltartronic acids **3** is described in this chapter. The alkyltartronic acids **3** have the following general formula:-



3

It was discussed in Chapter 1 that although the synthesis of tartronic acid is well documented[53-57], the synthesis of alkyltartronic acids is not. Bardroff[58] describes the synthesis of methyltartronic acid (**3**; R=CH_3) using 1,1- dicyanoethyl acetate **5** as a starting material



5

By conversion of the cyano groups to amide functions and by subsequent hydrolysis of both the amide functions and the acetate ester, the desired product was obtained.

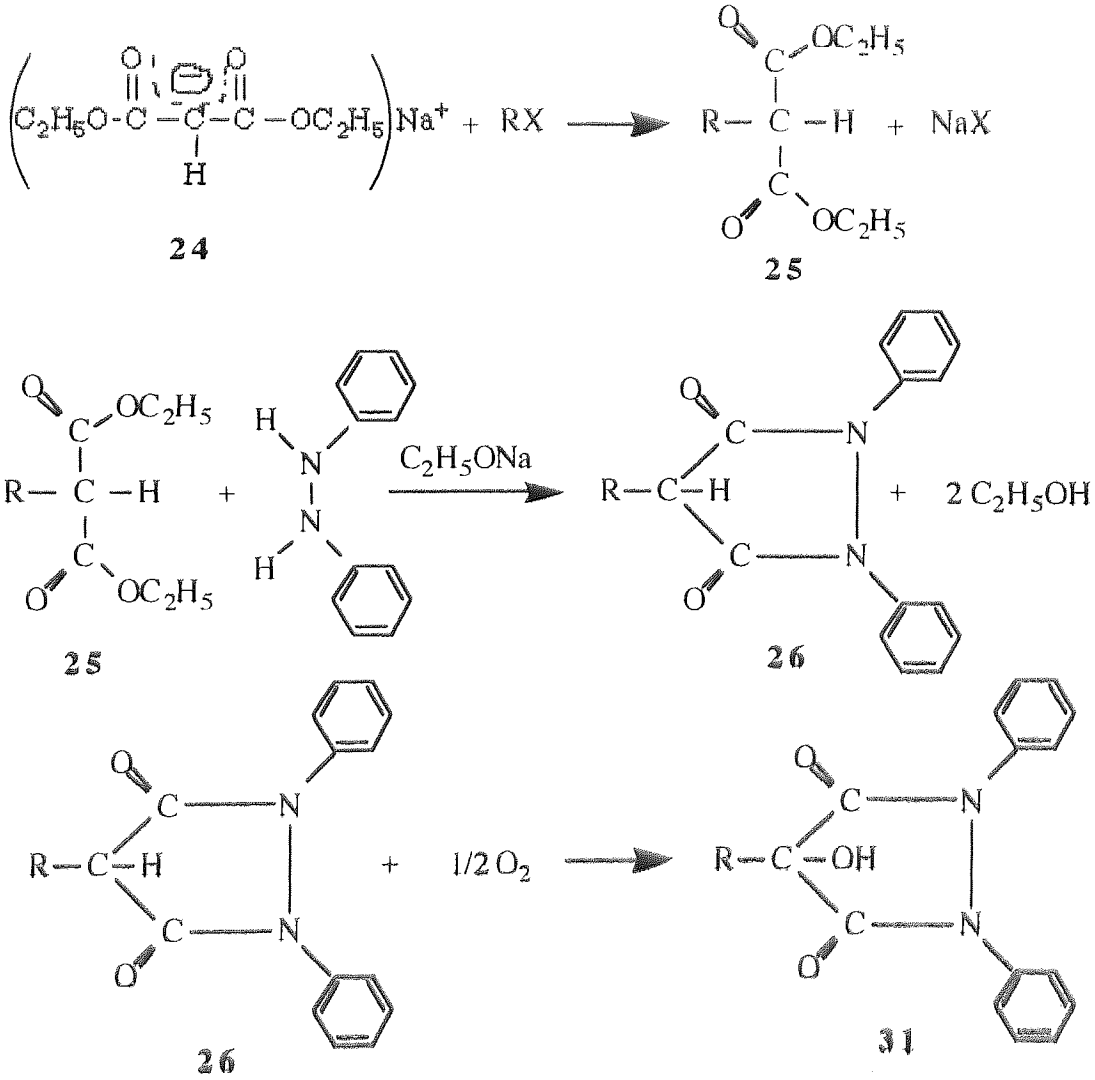
The only general synthetic pathway to alkyltartronic acids **3** was published by Grandjean[59] in 1969 who obtained alkyltartronic acids **3**, with R= ethyl, propyl, butyl, pentyl, hexyl, heptyl, isopentyl, cyclohexyl and benzyl, using a four step synthesis. The Grandjean report gives little experimental detail. Regardless of this we were able to produce an alkyltartronic acid following Grandjean's method. Our procedure, with the necessary experimental detail omitted by Grandjean, is given in Section 5.2 of this thesis.

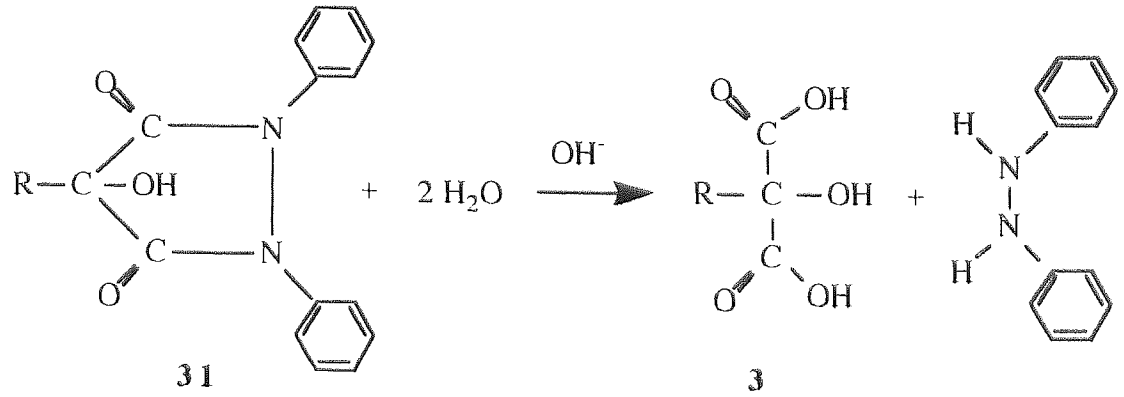
For reasons that will be discussed in Section 5.2, Grandjean's method is not practicable for producing sizable quantities of alkyltartronic acids **3**. A novel, elegant four stage synthesis of alkyltartronic acids **3** for R= pentyl, octyl, stearyl and isopropyl is detailed in Section 5.3. The synthesis is not only straightforward and considered to be a general route to any alkyltartronic acid, but it also allows the bulk synthesis of the desired acids in good yields.

5.2 GRANDJEAN SYNTHESIS OF ALKYLTARTRONIC ACIDS.

5.2.1 Introduction.

Grandjean's synthetic scheme[59] is outlined below:-

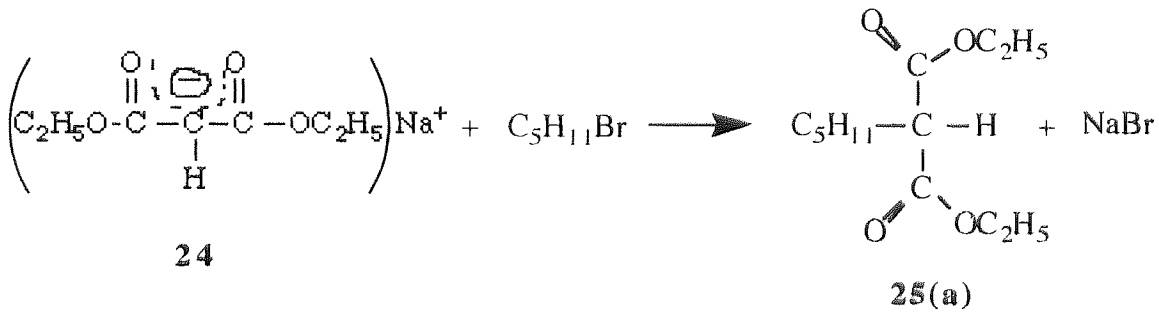




31 3

Although Grandjean's paper omits experimental detail, we were able to synthesise pentyltartronic acid **3(a)** following Grandjean's scheme by either applying already existing experimental knowledge or by invention. The stages in this successful synthesis are described in the following sub-sections.

5.2.2 Synthesis of Diethyl Pentylmalonate **25(a)**.



24 **25(a)**

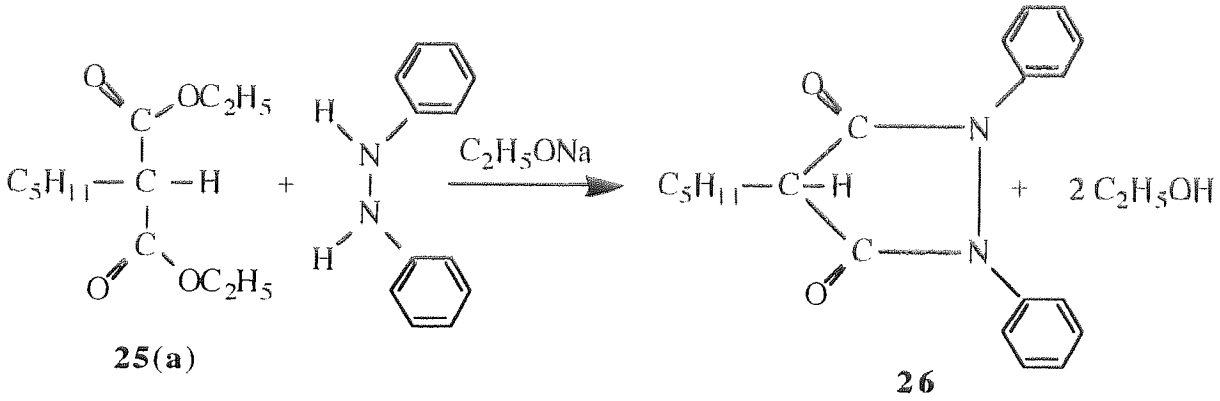
The alkylation of diethyl malonate has been extensively reported[118-122]. The general procedure involves the addition of diethyl malonate to an equimolar quantity of sodium ethoxide. The basic ethoxide removes one of the protons alpha to the two carbonyl functions generating the diethyl malonate carbanion **24**. The lability, and hence acidity, of the α-protons is due to the resonance stability of **24**.

By reacting pentyl bromide with **24**, diethyl pentylmalonate **25(a)** was obtained by nucleophilic substitution. Sodium bromide was also produced, the

majority of which precipitated out of the ethanolic solution. The reaction mixture was refluxed until neutral, and then the ethanol was removed under vacuum. The residue which remained was shaken with some water. The water dissolved the sodium bromide precipitate, allowing the insoluble crude ester to separate.

After distillation under vacuum, pure diethyl pentylmalonate **25(a)** was isolated in 81% yield.

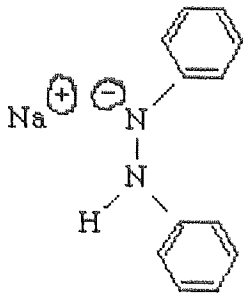
5.2.3 Synthesis of 1,2-Diphenyl-3,5-dioxo-4-pentyl Pyrazolidine **26**.



25(a) **26**

Two practical methods for the preparation of 3,5-dioxopyrazolidines exist in the literature.

The synthesis shown above, and employed by Grandjean, was originally published by Ruhkoph[123] in 1940. The malonic ester is heated with hydrazobenzene and sodium ethoxide at 150-200°C. The mechanism by which ring closure proceeds is via nucleophilic substitution. The nitrogen lone pairs of the hydrazobenzene attack at the δ^+ carbonyl carbons of the diethyl pentylmalonate eliminating ethanol which can be removed under vacuum as the reaction proceeds. Sodium ethoxide promotes ring closure as it acts as a binding agent forming species such as **27**.



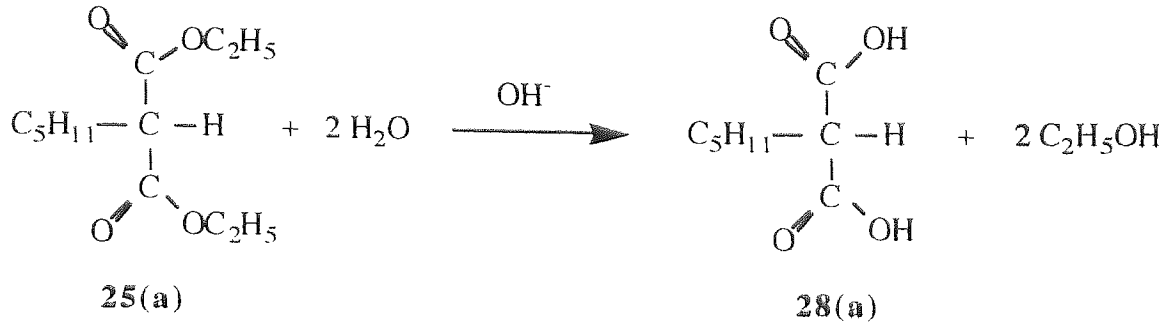
27

The formation of species such as **27** increases the nucleophilicity of the attacking species.

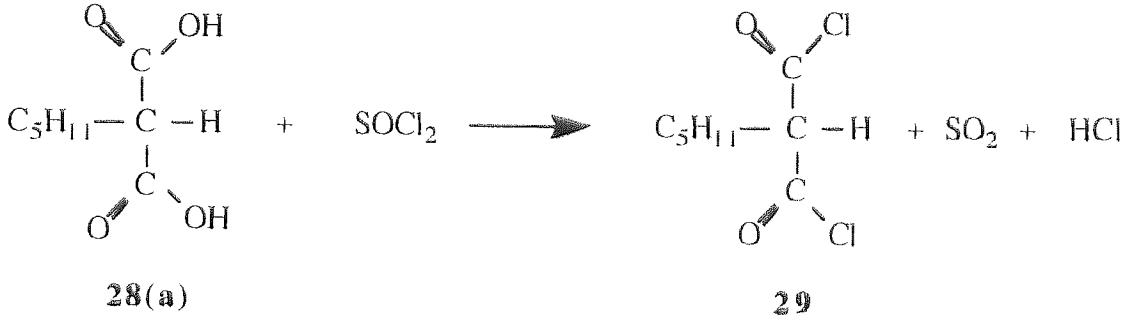
It was found however that when this condensation was attempted, it failed to give the desired product **26** in good yield (yield obtained=11%). Extensive decomposition of the reaction mixture was seen to occur.

The second practical method quoted in the literature involves the reaction of an alkylmalonyl chloride with hydrazobenzene either alone, as suggested by Tsumaki[124], or in the presence of pyridine at 0°C (B.P. 646,597)[125]. Budziarek *et al.* [126] explored the latter alternative. They found that the use of alkylmalonyl chlorides in ether-pyridine gave 3,5-dioxopyrazolidines in moderate yield. However, the use of chloroform-pyridine gave improved yields. They postulated that the higher yield was probably due to the increased solubility of the alkylmalonyl chloride - pyridine complex in chloroform.

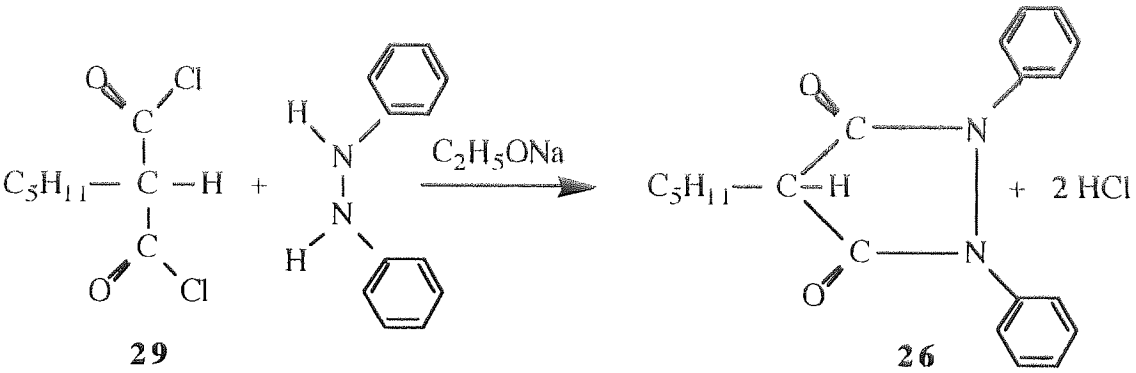
The synthesis of 1,2-diphenyl-3,5-dioxo-4-pentyl pyrazolidine **26** was carried out using the modified method of Budziarek et al.. This can be represented as follows:-



25(a) **28(a)**



28(a) **29**

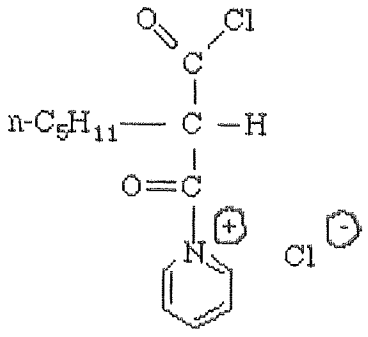


29 **26**

The first of these stages, conversion of diethyl pentylmalonate **25(a)** to pentylmalonic acid **28(a)**, is described in detail in Section 5.3.3.

The second step of Budziarek's method, when applied to this system, was the conversion of pentylmalonic acid **28(a)** to pentylmalonyl dichloride **29**. This was achieved[127] by stirring the acid with thionyl chloride for three days at 45°C-50°C, and then for five hours at 60°C. Any excess thionyl chloride was removed under vacuum and **29** was used immediately.

In the third stage, **29** was added dropwise to a mixture of pyridine and chloroform. The system was maintained at 0°C during the addition. The pyridine had the purpose of both combining with **29** to form complexes such as **30**



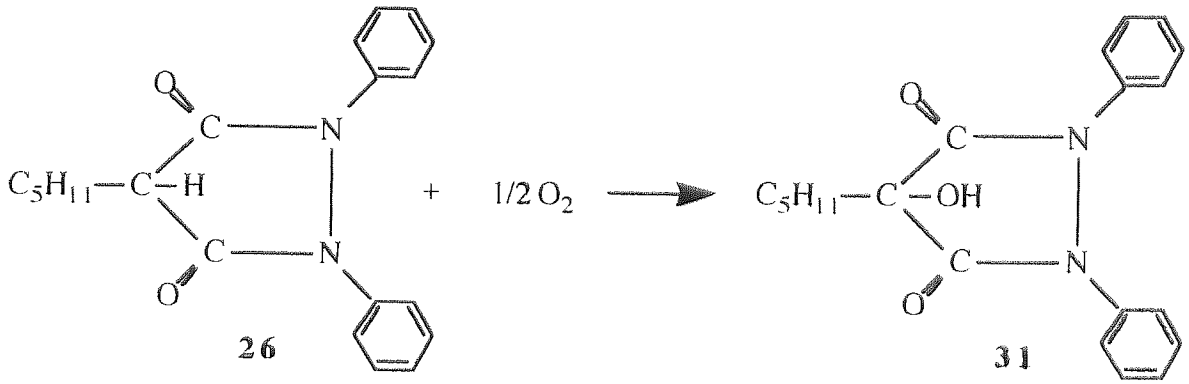
30

making **29** more susceptable to nucleophilic attack, and of trapping hydrogen chloride gas formed during the reaction to give pyridine hydrochloride.

The hydrazobenzene was then added, in chloroform, and stirred for two hours at room temperature. The mixture was then shaken with 2M hydrochloric acid to remove both existing pyridine hydrochloride and any remaining pyridine as the hydrochloride, and then with 2M sodium carbonate solution. The latter yielded the sodium salt of the desired product in solution. On acidification, the free pyrazolidine **26** precipitated out.

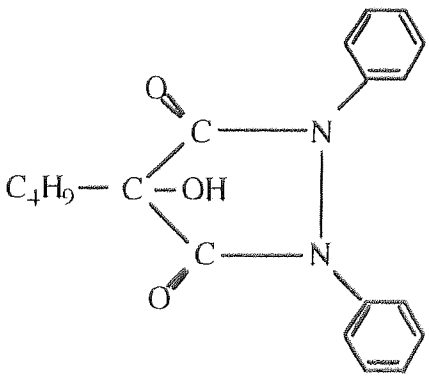
The synthesis was successful. After recrystallisation from ethanol/water the yield of 1,2-diphenyl-3,5-dioxo-4-pentyl pyrazolidine **26** was 87%.

5.2.4 Synthesis of 1,2-Diphenyl-3,5-dioxo-4-hydroxy-4-pentyl Pyrazolidine **31**.



26 **31**

It is known that 1,2-diphenyl-3,5-dioxo-4-hydroxy-4-butyl pyrazolidine (phenylbutazone) is of value in the treatment of rheumatoid arthritis and related conditions. Hence the drug's behaviour in an aqueous environment was of great interest. Jadot *et al.* [128] commented on how the drug, in aqueous conditions, was seen to oxidise progressively in air. They then proceeded to investigate possible oxidation of the drug at its melting point. Although they gave no experimental conditions, they created a thin film of molten phenylbutazone (m.p. 106°C) that was seen to resolidify, with time, to form a new compound with a melting point of 132°C. On the basis of IR and NMR data, they assigned the structure of the new compound as 1,2-diphenyl-3,5-dioxo-4-hydroxy-4-butyl pyrazolidine:-

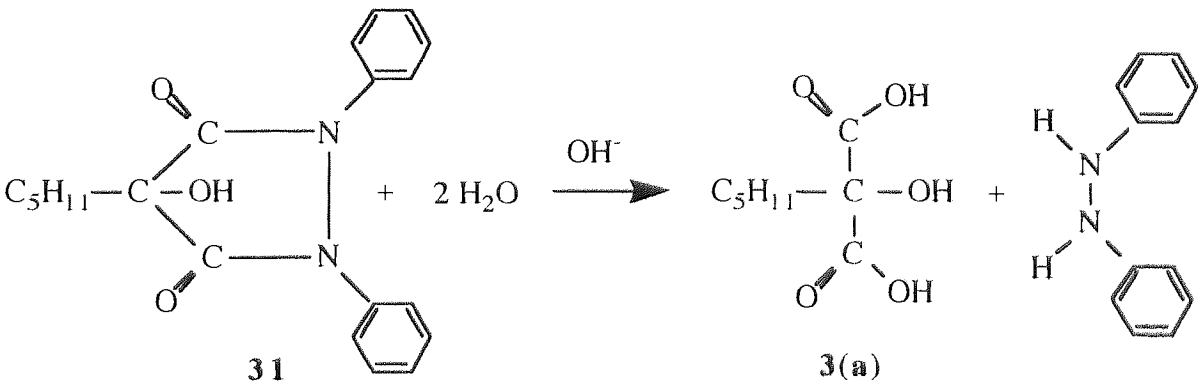


Grandjean[59] subsequently adopted Jadot's synthesis as a general route to 1,2-diphenyl-3,5-dioxo-4-hydroxy-4-alkyl pyrazolidines.

An attempt was made to produce the pentyl adduct **31** using the above procedure. A little 1,2-diphenyl-3,5-dioxo-4-pentyl pyrazolidine **26** was placed in a large round-bottomed flask. By removing the condenser from a rotary evaporator, and by spinning the contents of the flask using an oil bath as a heater, an ideal system was created not only allowing a thin molten film of the starting material to be formed, but also an adequate air supply.

1,2-diphenyl-3,5-dioxo-4-hydroxy-4-pentyl pyrazolidine **31** was obtained in quantitative yield. However, the major limitation of this technique is that in order to create a thin film, only small amounts of starting material can be used. This strongly suggested that Grandjean's synthetic scheme would not be suitable for the bulk synthesis of alkyltartronic acids.

5.2.5 Synthesis of Pentyltartronic Acid **3(a)**.



31 **3(a)**

Pentyltartronic acid **3(a)** was successfully obtained via the hydrolysis of **31** in 12M potassium hydroxide solution.

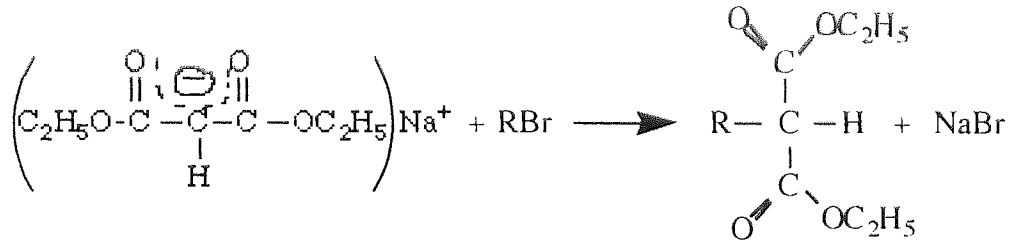
After work up, and recrystallisation from acetone/hexane, the desired product **3(a)** was obtained in 62% yield and shown to be spectroscopically pure.

It was noted that Grandjean[59] had suggested the recrystallisation of alkyltartronic acids from chloroform. It was found that pentyltartronic acid was in fact insoluble in both hot and cold chloroform.

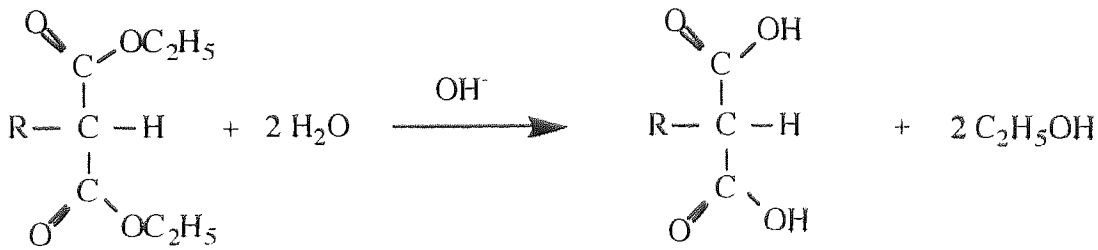
5.3 A NOVEL SYNTHESIS OF ALKYLTARTRONIC ACIDS.

5.3.1 Introduction.

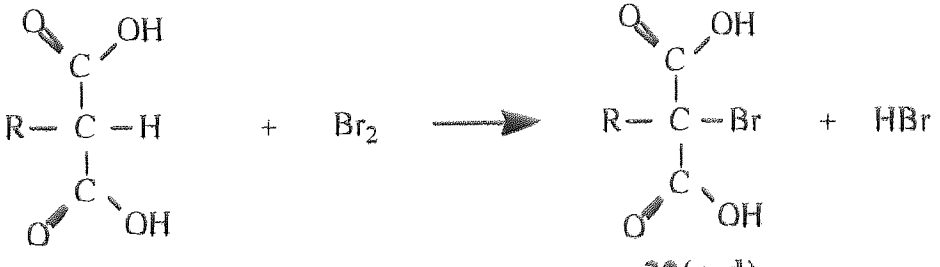
It was clear that Grandjean's method was not suitable for the large scale synthesis of alkyltartronic acids. A new method had to be devised. A novel, straightforward synthesis of alkyltartronic acids **3** for R= pentyl, octyl, stearyl and isopropyl is described schematically as follows.



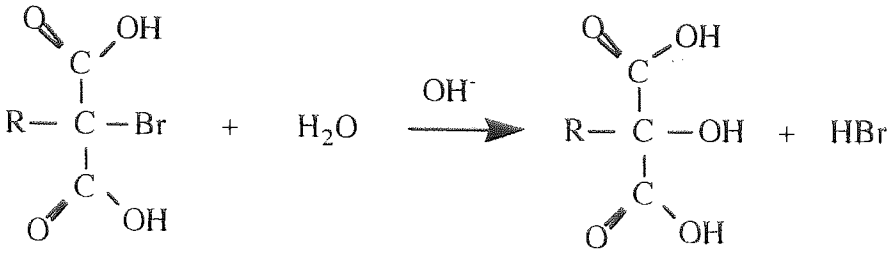
24 **25(a-d)**



25(a-d) **28(a-d)**



28(a-d) **32(a-d)**



32(a-d) **3(a-d)**

Where:-

(**a**) = R = Pentyl

(**b**) = R = Octyl

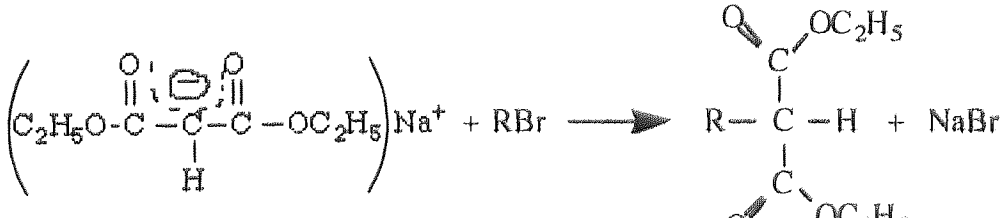
(**c**) = R = Stearyl

(**d**) = R = Isopropyl

The method relies on the initial formation of the relevant α-bromo-alkylmalonic acid **32(a-d)** and its subsequent hydrolysis to the desired alkyltartronic acid. This method enabled the bulk synthesis of various alkyltartronic acids **3(a-d)**, and is considered to be an excellent general synthetic route to any alkyltartronic acid.

A discussion of the individual steps in this four-stage synthesis now follows.

5.3.2 The Synthesis of Diethyl Alkylmalonates **25(a-d)**.



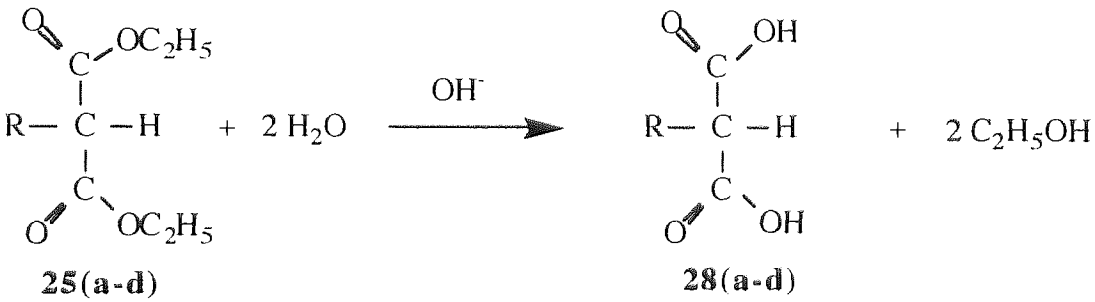
24 **25(a-d)**

The derivatives were all obtained in good yield by the above route. The history, theory and an outline of the practical application of this general synthesis has already been discussed in Section 5.2.2.

The yields of the diethyl alkylmalonates, after purification by distillation, were as follows:-

Diethyl Pentylmalonate **25(a)**	81%
Diethyl Octylmalonate **25(b)**	75%
Diethyl Stearylmalonate **25(c)**	75%
Diethyl Isopropylmalonate **25(d)**	75%

5.3.3 The Synthesis of Alkylmalonic Acids **28(a-d)**.



25(a-d) **28(a-d)**

Alkylmalonic acids, with R= pentyl **28(a)**, octyl **28(b)**, stearyl **28(c)** and isopropyl **28(d)**, were obtained after saponification of their respective diethyl alkylmalonates **25(a-d)**.

The saponification was carried out using a modified version of a method, previously reported by Marvel[129], for the synthesis of *sec* -butylmalonic acid from diethyl *sec* -butylmalonate. Diethyl *sec* -butylmalonate was added in a steady stream to a hot 22M aqueous solution of potassium hydroxide with vigorous stirring. The

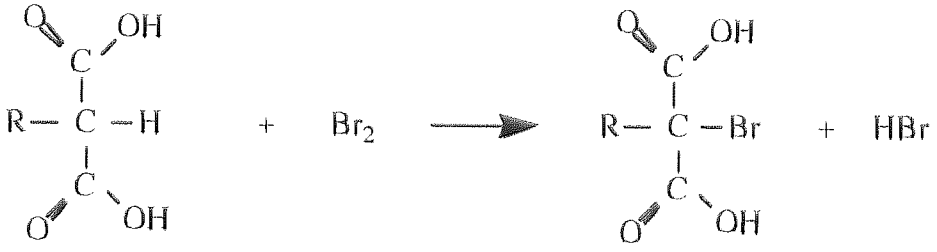
mixture was then heated for five hours, the ethanol removed under vacuum to assess reaction completeness. After cooling the mixture in ice, the mixture was acidified to pH 3 with concentrated hydrochloric acid, whilst maintaining the temperature of the solution below 20°C. It was noted by Marvel that, after addition of approximately half of the required quantity of acid, the monopotassium salt of *sec* -butylmalonic acid precipitated out of solution. On addition of further acid, however, the salt was converted into the soluble *sec* -butylmalonic acid. The acidified solution was extracted with diethyl ether, the ether layer dried and finally the solvent removed under vacuum to give the desired product.

This synthetic procedure worked perfectly for the synthesis of both **28(a)** and **28(d)**. In both cases, the monopotassium salt was seen to precipitate and then to go back into solution on further addition of acid. However, for both **28(b)** and **28(c)**, Marvel's procedure worked smoothly until the acidification step. Here again the monopotassium salt appeared but in both cases did not go back into solution on further addition of acid. The procedure had to be modified to allow for the insolubility of both **28(b)** and **28(c)** in water. As before the mixture was acidified to pH 3 but for both octyl- and stearyl- derivatives the insoluble solid was filtered and dried in a vacuum oven for 48 hours at 50°C and then in a vacuum dessicator over P_2O_5 for a further week. The crude malonic acids **28(b,c)** were taken up in some diethyl ether, and then filtered. The solid matter, insoluble in ether, had a melting point greater than 300°C suggesting it to be ionic. An IR spectrum of the compound, run as a KBr disc, showed no absorption at all until complete absorption occured at approximately 600 cm^{-1}. These two observations suggested that the solid was probably sodium chloride, a small amount of which may have precipitated out of solution with the organic solid at the acidification stage. Removal of the ether under vacuum gave the octyl- **28(b)** or stearyl- **28(c)** malonic acid.

The yields of the adducts obtained were as follows

Pentylmalonic Acid **28(a)**	85%
Octylmalonic Acid **28(b)**	84%
Stearylmalonic Acid **28(c)**	84%
Isopropylmalonic Acid **28(d)**	90%

5.3.4 The Synthesis of α-Bromo-alkylmalonic Acids **32(a-d)**.



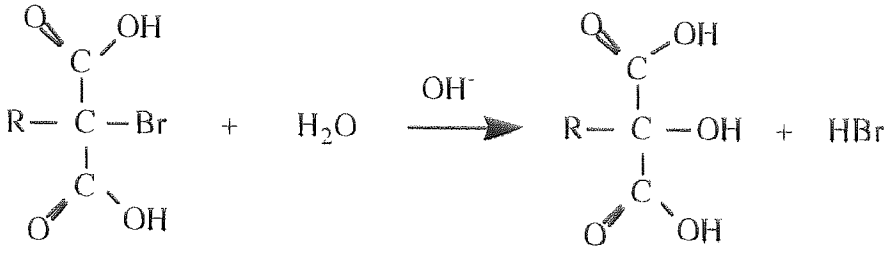
28(a-d) **32(a-d)**

The bromination of pentyl- **28(a)**, octyl- **28(b)**, stearyl- **28(c)** and isopropyl- **28(d)** malonic acids was carried out by the following technique.

A quantity of the acid was dissolved in ether, bromine was then added, cautiously at first. The reaction generated some heat causing the ether to reflux gently. Reaction was also indicated by decolourisation of the mixture following each bromine addition. After complete addition of bromine, water was added to remove any hydrogen bromide and excess bromine from the ether layer. After drying the ether layer, the ether was removed to give either an oil or a solid which was used without further purification in the fourth and final stage of the synthesis.

Conversion of the alkylmalonic acid **28(a-d)** to the α-bromo-alkylmalonic acid **32(a-d)**, was achieved in quantitative yield for pentyl **32(a)**, octyl **32(b)**, stearyl **32(c)** and isopropyl **32(d)** derivatives.

5.3.5 The Synthesis of Alkyltartronic Acids **3(a-d)**.



32(a-d) **3(a-d)**

Due to the differences in aqueous solubility of the pentyl **3(a)**, octyl **3(b)**, stearyl **3(c)** and isopropyl **3(d)** derivatives, three different procedures were adopted for this final hydrolysis stage.

The synthetic procedure for both pentyltartronic acid **3(a)** and isopropyltartronic acid **3(d)** can be outlined as follows. The α-bromo-alkylmalonic acids **32(a,d)** were dissolved in water and heated to 50°C. Enough 2M aqueous sodium hydroxide was added slowly with stirring to produce a final 1M solution. The mixture was then heated for two hours. After cooling in ice, the solution was acidified to pH 3 with 2M hydrochloric acid. The resultant solution was concentrated under vacuum, and then extracted with ether. This concentration was necessary because of the high solubility of both **3(a)** and **3(d)** in water. The concentration was performed at a temperature of no greater than 50°C to avoid any decarboxylation of the product. The ether extract was dried and the solvent removed under vacuum to give a crude product.

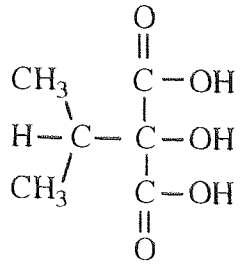
In the case of **3(b)**, the bromo-acid **32(b)** was dispersed in water and heated to 50°C. On addition of 2M aqueous sodium hydroxide, the dispersion became a solution as the acid dissolved as its sodium salt. As above, the mixture was stirred and then acidified. On acidification, octyltartronic acid **3(b)** precipitated out of aqueous solution. Transferring the 'milky' suspension to a separating funnel allowed the acid to be taken up in ether. Drying the ether layer, and subsequent removal of the solvent under vacuum, gave a crude product.

The procedure for **3(c)** was different again. The bromo-acid **32(c)** was found to be insoluble in both water and aqueous base. THF was therefore employed as a co-solvent (water:THF / 1:1(volume)). The procedure was then the same as for **3(a)** and **3(d)** until after the final reaction mixture had been acidified. At this point, the mixture was concentrated under vacuum to remove the THF. Stearyltartronic acid **3(c)** then precipitated out of solution and the remainder of this procedure was then identical to that used for octyltartronic acid **3(b)**.

Analysis of the crude pentyl **3(a)**, octyl **3(b)** and stearyl derivatives **3(c)**, using IR and NMR, showed all to be slightly impure. Pure samples were obtained by recrystallisation from acetone/hexane.

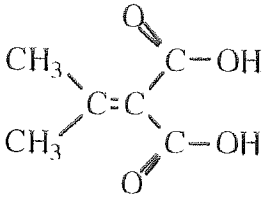
Analysis of the crude isopropyl derivative **3(d)** showed a mixture of products containing a degree of unsaturation. By fractionally recrystallising this mixture from acetone/hexane, it was possible to isolate firstly a crop of needle-like crystals and secondly a crop of leaf-like crystals.

The leaf-like crystals were shown by analysis to be pure isopropyltartronic acid **3(d)**.



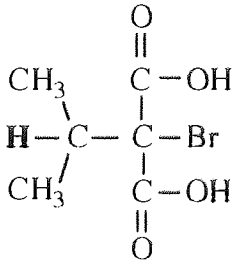
3(d)

The needle-like crystals were shown, by analysis, to have a structure consistant with that of **33**



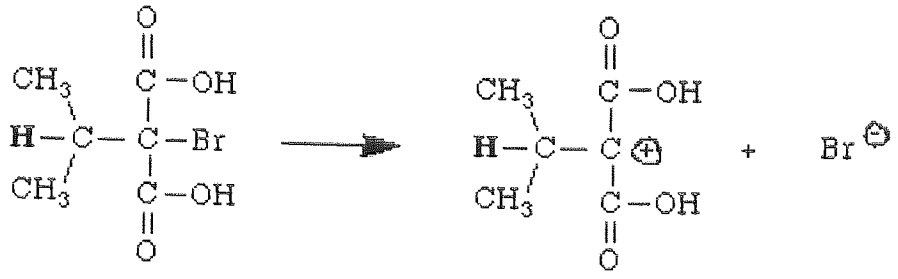
33

suggesting that the attack of the hydroxide ion had not only created the substitution product, **3(d)**, but also some of the elimination product **33**. This is feasible if the structure of α-bromo-isopropylmalonic acid **32(d)** is considered:-



32(d)

It is extremely probable that substitution reactions undergone by α-bromo-isopropylmalonic acid will follow an S_N2 mechanism. For the mechanism to follow an S_N1 route, the first step in the reaction would be as follows:-



32(d) **34**

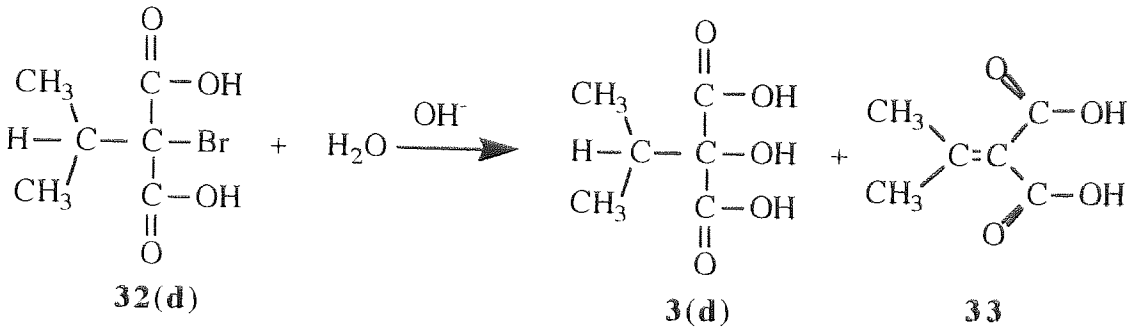
Although the subsequent carbonium ion **34** would gain a degree of inductive stabilisation from the isopropyl group, the destabilizing electron withdrawing effect of attached carboxyl groups is likely to be the dominant factor. This will inhibit the formation of **34**.

A side-reaction associated with S_N2 substitution reactions, is the E2 elimination of a hydrogen halide to give an unsaturated product.

Since it is attack at the highlighted ß-proton that causes elimination, it is the basicity rather than the nucleophilicity of the attacking species which is important. In this case, the attacking species is the hydroxide ion which, as well as being a good nucleophile, is also a strong base which will encourage elimination.

The structure of the substrate is also important in determining the substitution/elimination ratio. For steric reasons, the greater the branching around the potential leaving group, the slower the substitution reaction. Attack at the ß-proton hence becomes more favourable. α-Bromo-isopropylmalonic acid **32(d)** has branching at both α– and ß-carbons making substitution slower and elimination more favourable.

A more accurate representation of the hydrolysis of α-bromo-isopropylmalonic acid is therefore:-



32(d) **3(d)** **33**

The reported method is highly successful in producing pentyl- **3(a)**, octyl- **3(b)**, stearyl- **3(c)** and isopropyl- **3(d)** tartronic acids with the following yields:-

Pentyltartronic Acid **3(a)**	82%
Octyltartronic Acid **3(b)**	87%
Stearyltartronic Acid **3(c)**	88%
Isopropyltartronic Acid **3(d)**	37%

5.4 CONCLUSIONS.

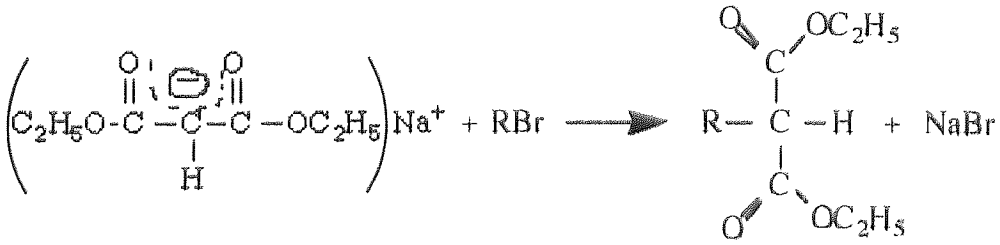
Although the synthesis of tartronic acid is well documented, the synthesis of alkyltartronic acids is not. The only general synthetic pathway to alkyltartronic acids was published by Grandjean[59] in 1969. He described a four stage synthesis from which he was able to obtain alkyltartronic acids for R= ethyl, propyl, hexyl, heptyl, isopentyl, cyclohexyl and benzyl. This report was illustrated by a pictorial reaction scheme but very little experimental detail.

Pentyltartronic acid **3(a)** was successfully synthesised, using Grandjean's scheme, applying either existing experimental knowledge or invention. In completing the synthesis, two modifications had to be made to Grandjean's approach. Firstly, it was pointed out in Section 5.2.3 that the direct condensation of diethyl pentylmalonate **25(a)** with hydrazobenzene at 150-200°C, to form 1,2-diphenyl-3,5-dioxo-4-pentyl pyrazolidine **26**, gave a very poor yield. Extensive decomposition of the reaction

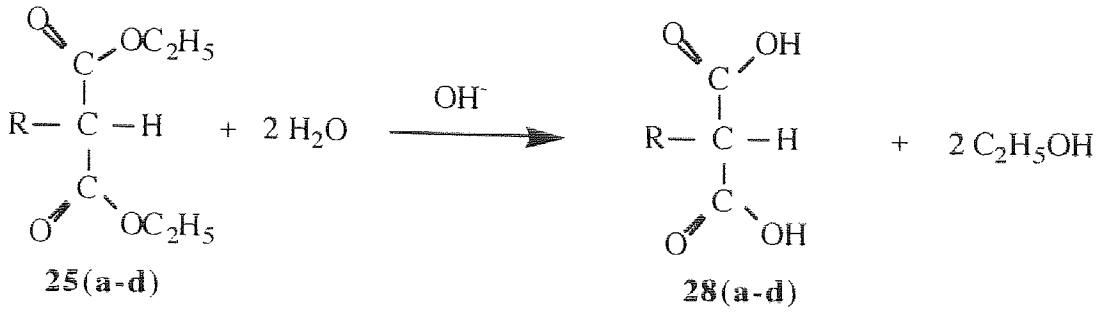
mixture was seen to occur. The synthesis of **26** was successfully achieved by the conversion of **25(a)** firstly to pentylmalonic acid **28(a)**, and then to pentylmalonyl chloride **29**. The acid chloride was then condensed with hydrazobenzene, in pyridine, to give **19**. Secondly, it was noted that Grandjean advocated the use of chloroform as a solvent for recrystallizing pentyltartronic acid. Observation showed that the acid was in fact insoluble in both hot and cold chloroform which meant that a different solvent had to be found. Acetone/hexane was found to be the most successful solvent system for this purpose.

A further observation was made whilst using Grandjean's synthesis. Section 5.2.4 illustrates the conversion of **26** to 1,2-diphenyl-3,5-dioxo-4-hydroxy-4-pentyl pyrazolidine **31**. This step, in the synthesis of **3(a)**, occurs via an oxygen insertion reaction when a thin molten film of the **26** is exposed to air over a time. It was found experimentally that it was impractical to make thin films of anything except small amounts of **26**. This prohibited the use of the Grandjean synthesis in the production of large quantities of alkyltartronic acids.

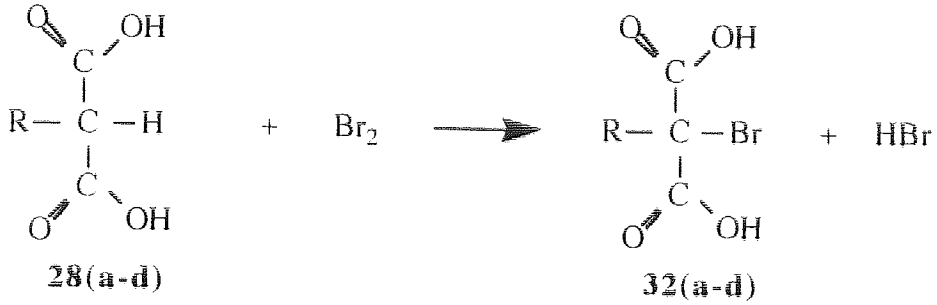
In response to the above observations, this chapter continues in Section 5.3 with a description of our novel four stage synthesis of alkyltartronic acids for R= pentyl **3(a)**, octyl **3(b)**, stearyl **3(c)** and isopropyl **3(d)**. The synthesis can be represented as:-



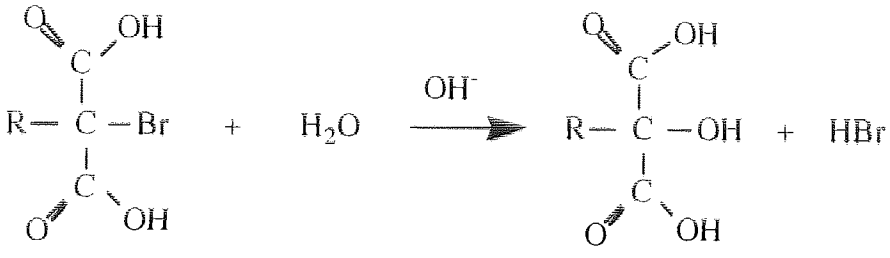
24 **25(a-d)**



25(a-d) **28(a-d)**



28(a-d) **32(a-d)**



32(a-d) **3(a-d)**

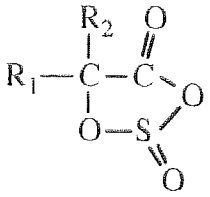
The synthesis is not only straight forward but is also considered to be the first general synthetic route to all alkyltartronic acids that gives products in bulk quantities. For these reasons this synthetic route is regarded as a highly valuable contribution to preparative chemistry.

CHAPTER 6

THE SYNTHESIS OF DERIVATIVES OF ALKYLTARTRONIC ACIDS.

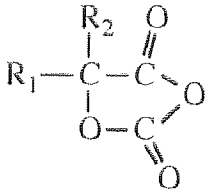
6.1 GENERAL INTRODUCTION.

The various methods that have been used to polymerise hydroxy acids into polyesters were introduced in Chapter 1. It was pointed out that some of the older methods employed were not in fact suitable for the polymerisation of α-hydroxy acids. The potential biomedical uses of poly(α-hydroxy acid)s and how they can be successfully synthesised by the ring-opening of cyclic anhydride monomers, namely the α-hydroxy acid anhydrosulphite **7** and α-hydroxy acid anhydrocarboxylate **8**:-



7

Anhydrosulphate

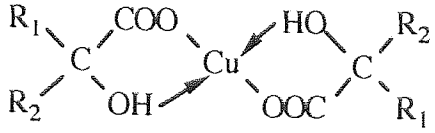


8

Anhydrocarboxylate

were also discussed in the opening chapter.

These monomers **7** and **8** can be produced from the relevant parent acids by direct combination of the parent acid with thionyl chloride in the case of the anhydrosulphite **7**, or phosgene in the case of the anhydrocarboxylate **8**. However, it has been found that these direct combination syntheses can lead to products highly contaminated with unwanted chlorinated impurities. Although the crude products can be purified by stirring in diethyl ether with baked silver oxide, and then further by either vacuum distillation or vacuum sublimation, methods have been developed to effect a cleaner reaction where products are obtained in higher yields. Conversion of the hydroxy acid to the metal carboxylate, commonly the copper(II) salt **13**:-



13

before combination with thionyl chloride or phosgene, has made for a cleaner reaction system with fewer impurities. This is believed to be because of a difference in reaction mechanism. When **13** is used the initial intermediate formed, before cyclisation occurs, is believed to be either the acyl chlorosulphinate or the acyl chloroformate. When the parent acid is used the intermediates are believed to be the alkyl chlorosulphinate or alkyl chloroformate. As the acyl intermediates are generally more stable than their alkyl analogues, less of the detrimental intramolecular reactions or rearrangements occur and this leads to a much reduced concentration of unwanted chlorinated by-products. These observations are illustrated in more detail in Chapter 1.

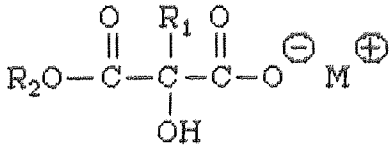
Al Mesfer and Tighe[77], polymerised tartronic acid via its anhydrosulphite (**7**; R_1=COOH, R_2=H) and anhydrocarboxylate (**7**; R_1=COOH, R_2=H). This was reasonably successful in that some low molecular weight polymers were obtained. However, many problems were encountered by them when using tartronic acid as a precursor. Synthesis of the cyclic anhydride monomers was achieved by direct combination of the parent acid with either thionyl chloride or phosgene. As discussed earlier, the concentrations of chloride containing impurities are minimised by using the metal salt of the hydroxy acid **13**. This was not possible in the case of tartronic acid due to the presence of two equivalent carboxyl groups in the parent acid. In other words it was impossible to react one of the carboxyl functions without first protecting the other. The crude products obtained were purified somewhat by stirring with baked silver oxide. Anhydrosulphites **7** are normally further purified by vacuum distillation and anhydrocarboxylates **8** by vacuum sublimation. Unfortunately, however, it was found that the high boiling point of tartronic acid anhydrosulphite (TAAS), coupled

with its low thermal stability, precluded the use of vacuum distillation as a purification technique. The requirement to distil at relatively high temperatures lead to the complete decomposition of the anhydrosulphite. Similar difficulties were encountered in the attempted purification of tartronic acid anhydrocarboxylate (TAAC) by vacuum sublimation. Polymerisations of these cyclic anhydrides were therefore made without further sample purification and this gave impure polymer products.

This thesis is concerned with the development of alkyltartronic acids as precursors to poly(alkyltartronic acids). This particular chapter is devoted to the synthetic modification of the alkyltartronic acids **3(a-d)**, to create more suitable precursors to poly(alkyltartronic acids) **4** than the parent acids themselves. The precursors described in this chapter were synthesised with the future synthesis and polymerisation of cyclic anhydride monomers in mind. Because of the similarity between the alkyltartronic acids described in this thesis and tartronic acid itself, particular attention was paid to the work and observations of Al Mesfer and Tighe[77].

It is clear that the major problem encountered in their work was that they were unable to obtain pure cyclic monomers. This problem was largely due to the presence of two equivalent carboxyl groups in the molecule.

Our objective was to synthesise metal alkyl alkyltartronates **16**:-

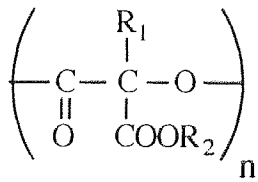


16

Such derivatives of alkyltartronic acids **3**, and tartronic acid, would theoretically have significant advantages over the parent acid in cyclic anhydride

synthesis. The protection of one carboxyl function, as an alkyl ester, would allow the activation of the other carboxyl group by conversion to the metal carboxylate **16**. This would encourage the formation of acyl intermediates during a prospective cyclic anhydride synthesis and could therefore reduce the concentration of unwanted chlorinated impurities in the final product. In addition to this, the presence of an ester, rather than a carboxyl-function, on the cyclic anhydride ring should significantly reduce the boiling points of the respective anhydrosulphites and melting points of anhydrocarboxylates. This might allow the purification of cyclic anhydride samples by either vacuum distillation or vacuum sublimation. These techniques were precluded in the work of Al Mesfer and Tighe.

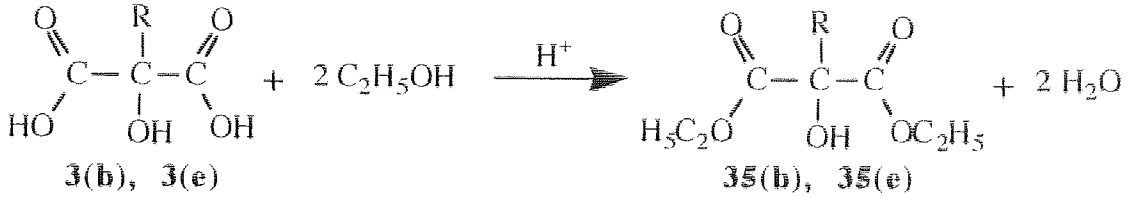
It was anticipated that the ring-opening of cyclic anhydrides formed from the proposed metal alkyl alkyltartronates would produce a series of polymers with the following repeat unit:-



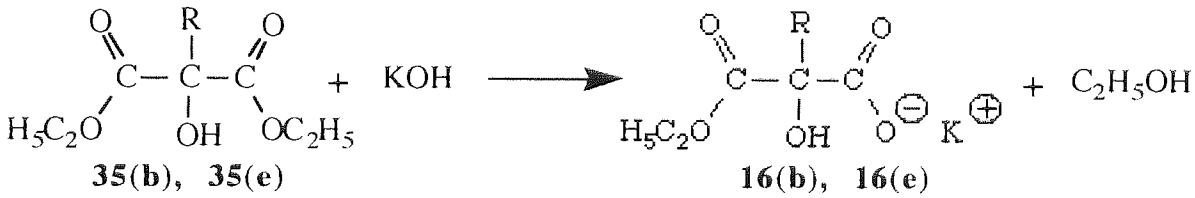
Such a series of polymers would therefore have pendant ester groups. As the ultimate purpose of this exercise was to produce polymers with pendant carboxyl functions these ester groups would have to be cleaved. This poses a problem as cleavage of the pendant ester groups is likely to be in competition with hydrolysis of the ester linkages that constitute the polymer backbone. Ultimately therefore the development of metal alkyl alkyltartronates **16** with extremely labile ester functions would be desirable so that cleavage of the pendant ester groups will occur without significant hydrolysis of the polymer chain. Esters that form stable alkoxide leaving groups that are also poor nucleophiles would be ideal for this purpose. This requires further research but alcohols such as 2,2,2-trichloroethanol and benzyl alcohol are

known to form labile esters. However, for the purposes of the studies in this chapter ethanol has been used in the esterifications described. This was largely due to reactant availability but it is considered that the syntheses described would be applicable to systems using other alcohols.

In the remainder of this chapter the successful synthesis and isolation of both potassium ethyl tartronate **16(e)** and potassium ethyl octyltartronate **16(b)** as examples of metal alkyl alkyltartronates are described. Synthesis was achieved using the following general reaction scheme:-



3(b), 3(e) **35(b), 35(e)**



35(b), 35(e) **16(b), 16(e)**

Where:-

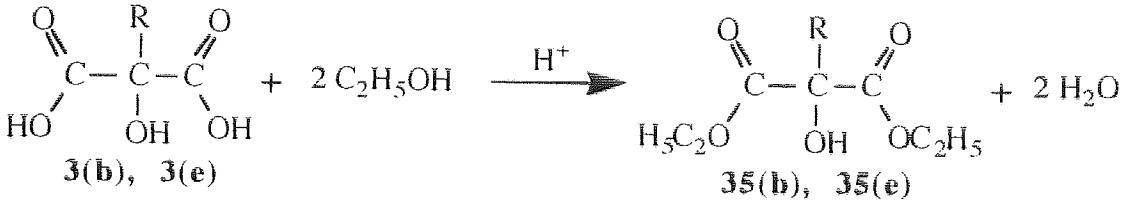
(b) = R = Octyl

(e) = R = H

This scheme is considered to offer a general synthetic procedure to any potassium alkyl alkyltartronate.

6.2 A NOVEL SYNTHESIS OF POTASSIUM ETHYL ALKYLTARTRONATES.

6.2.1 Synthesis of Diethyl Alkyltartronates 35(b,e).



3(b), 3(e) **35(b), 35(e)**

Diethyl tartronate **35(e)** and diethyl octyltartronate **35(b)** were both synthesised successfully by direct combination of the relevant tartronic acid, **3(e,b)** with ethanol using a mineral acid catalyst. To avoid any self-esterification of the hydroxy acid occuring the reaction was carried out in a dilute ethanolic solution. The presence of a large excess of ethanol also had the effect of driving the esterification equilibrium towards the desired ester product. Using a large excess of one reactant to achieve this purpose is necessary because a modest reaction temperature has to be employed, to avoid decarboxylation of the hydroxy acid. The removal of product water during the progress of the esterification is, therefore, impossible.

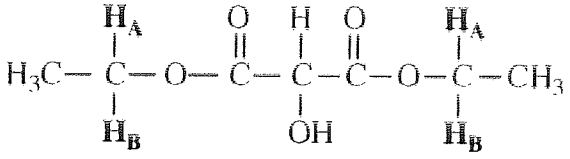
When the reaction was complete, triethylamine was added to the reaction mixture to neutralise the mineral acid catalyst. Excess ethanol was then removed, under vacuum, to leave a residue which was redissolved in dichloromethane. Washing with 5% HCl(aq) removed any excess triethylamine and subsequent washing with water removed any triethylamine hydrosulphate salts and any excess hydroxy acid that might be present in the organic layer. The remaining organic phase was then dried over anhydrous magnesium sulphate. Removing the solvent under vacuum gave the relevant crude diethyl alkyltartronate **35(b,e)** .

Pure diethyl alkyltartronates **35(b,e)** were obtained by distilling the crude products under vacuum. Both diethyl tartronate **35(e)** and diethyl octyltartronate **35(b)** were obtained as colourless liquids. The product yields were as follows:-

Diethyl Tartronate **35(e)** 90%

Diethyl Octyltartronate **35(b)** 91%

N.M.R. analysis of the diester products yielded an interesting observation. Although the observation was made for both diesters formed, the phenomenon is most clearly seen for the chemically simpler of the two. The general structure of a diethyl tartronate **35(e)** is as follows:-

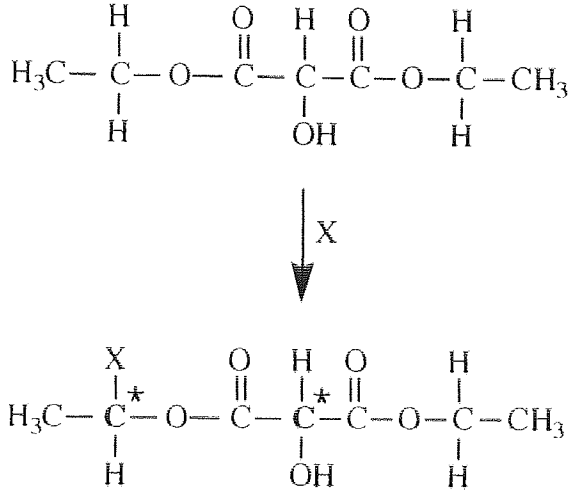


35(e)

The molecule is symmetrical and thus contains two identical methyl groups and two identical methylene groups. On first inspection, one might expect to see a triplet, corresponding to the methyl groups, a singlet corresponding to the methine proton, a singlet corresponding to the hydroxyl group, and a quartet corresponding to the methylene groups. The expected splitting patterns are all observed except for the pattern attributable to the methylene protons. Instead of the anticipated quartet, a sixteen line pattern is observed. This can be attributed to the actual chemical and magnetic inequivalence of the methylene protons H_A and H_B. H_A and H_B are diastereotopic.

If the reaction of one of two seemingly identical groups as opposed to the other leads to diastereomeric products, then the groups are said to be diastereotopic.

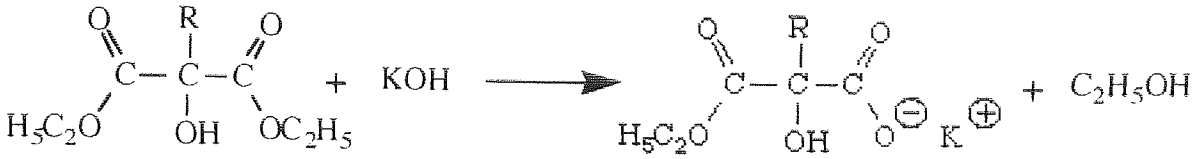
In the case of diethyl tartronate a group X substituting for one of the methylene protons will simultaneously create two chiral centres within the molecule and give rise to diastereomeric products:-



Different products would be obtained, depending on which of the two inequivalent methylene protons was substituted by X. As a consequence of their nonequivalence, these methylene protons should experience different shielding effects and consequently have different chemical shifts in the N.M.R. spectrum. In addition further splitting will be observed due to the interaction of the methylene protons.

It is proposed, therefore, that the observed spectral splitting pattern of sixteen lines is due to the methylene protons splitting each other to form four peaks. Each of these four peaks is then split into a further four peaks on coupling with the three neighbouring methyl protons to give sixteen observed peaks in total. Several observations of this phenomenon are recorded in the literature[130-133].

6.2.2 Synthesis of Potassium Ethyl Alkyltartronates **16(b,e)**.



35(b), 35(e) **16(b), 16(e)**

The conversion of diethyl malonate to potassium ethyl malonate is described in the literature[134]. Reaction is carried out by combining equimolar quantities of the diester and potassium hydroxide in ethanolic solution. The preferential cleavage of only a single ester function is apparently achieved because after the monocarboxylate is formed the product immediately precipitates out of solution. As diethyl malonate and diethyl tartronate **35(e)** are very similar in chemical structure it was considered that this procedure offered an excellent route to potassium ethyl alkyltartronates **16(b,e)**. **16(b)** and **16(e)** were both synthesised successfully using a modified version of this technique.

To an ethanolic solution of the relevant diester **35(b,e)** was added an equimolar quantity of potassium hydroxide, also in ethanolic solution. Addition was carried out slowly over a one hour period with continuous stirring. When the addition was complete, the solution was stirred for a further hour.

When the reaction was complete, the solvent was removed to yield a white solid product. Due to the affinity of the product for ethanol, referred to below, the product obtained using this procedure was a little 'sticky'. It was therefore dried in a vacuum oven before analysis.

In an analogous experiment using diethyl malonate a precipitate was seen to appear both during and after addition. However, with both diethyl tartronate **35(e)** and diethyl octyltartronate **35(b)** no such precipitate appeared. An attempt was made

to encourage precipitation by completing the reaction in a smaller amount of solvent. It was also considered that product precipitation may be promoted by using a combined solvent of ethanol and hexane. It was hoped that a lower solvent polarity would allow the product to drop out of solution. All attempts to promote precipitation were unsuccessful. The absence of precipitation was attributed to the increased polarity of diethyl alkyltartronate derivatives over diethyl malonate derivatives induced by the presence of the hydroxyl group on the α-carbon. This created a greater affinity for ethanol, inhibiting precipitation.

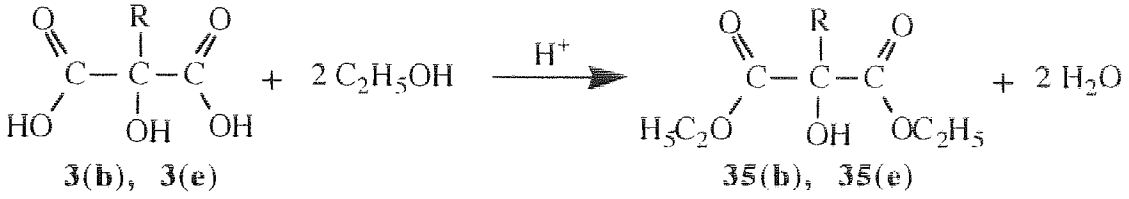
It was feared that product precipitation was crucial to ensuring the cleavage of only one ester group and that without its occurence the product would be a mixture of potassium ethyl alkyltartronate, potassium alkyltartronate and starting material. However, I.R., N.M.R. and elemental analytical studies showed that the products obtained using the above procedure were practically pure potassium ethyl alkyltartronates **16(b,e)** obtained in almost quantitative yield.

The observation that malonate diesters yield malonate monoesters, when treated with a single molar equivalent of potassium or sodium hydroxide, has been made before in the literature[135-139]. Patai[140] suggests that the preferential cleavage of only a single ester function is due to electronic factors but he does not enlarge on this observation. Further literature research failed to yield an adequate explanation for this observation.

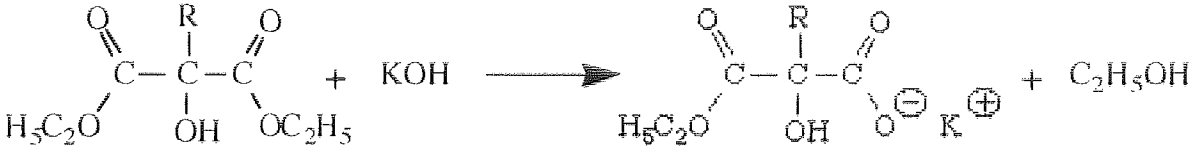
Finally, it should be noted that a complex splitting pattern was again observed in the proton N.M.R., for the methylene protons present in both **16(b)** and **16(e)**. As for diethyl tartronate **35(e)** and diethyl octyltartronate **35(b)** this is due to the methylene protons being diastereotopic and therefore magnetically nonequivalent.

6.3 CONCLUSIONS.

In this chapter the successful novel synthesis of potassium ethyl alkyltartronates is outlined. The synthetic route used can be shown schematically as follows:-



3(b), 3(e) **35(b), 35(e)**



35(b), 35(e) **16(b), 16(e)**

Potassium ethyl tartronate **16(e)** and potassium ethyl octyltartronate **16(b)** were prepared using this method. It is, however, considered that this general scheme could be used as a general route to any potassium alkyl alkyltartronate.

As explained in the introduction, it is thought that these novel derivatives may be potentially more useful as precursors to anhydrosulphite and anhydrocarboxylate type cyclic anhydrides than their respective parent acids. The resulting cyclic anhydride monomers should contain less unwanted chlorinated by-products and feasibily be easier to purify than analogous anhydrides synthesised using the α-hydroxy acids themselves. Subsequent ring-opening of the anhydrides should yield novel poly(α-hydroxy acid)s which, as discussed thoroughly in Chapter 1, would be of considerable interest in biomedical applications.

CHAPTER 7

CONCLUSIONS

7.1 CONCLUSIONS

The aims of this project were:-

1) the synthesis of a range of new polyether-based vinylic monomers and their incorporation into poly(2-hydroxyethyl methacrylate) (poly(HEMA)) based hydrogel networks, of interest to the contact lens industry.

2) the synthesis of a range alkyltartronic acids, and their derivatives. These molecules may ultimately be used to produce functionalised poly(α-hydroxy acids) of potential interest in either drug delivery or surgical suture applications.

The synthesis of a range of MPEGA products **1**(**a**-**e**) was successfully achieved in very good yields, using a one step method, by combination of the desired MPEG with acryloyl chloride. The synthesis of a range of PEGA products **2**(**a**-**e**) was also successfully achieved in good yields. A three step synthesis was used that employed the *tert* - butyldimethylsilyl function as a protecting group for one of the two terminal hydroxyl groups on the PEG chain. Both MPEGA and PEGA derivatives were studied using ESMS. Using this technique the addition of the acryloyl group, with a molecular weight of 55 Daltons, to the relevant polyether starting material could be accurately assessed and was seen to be successful for all adducts synthesised.

All MPEGA and PEGA derivatives synthesised were copolymerised with HEMA to give two ranges of novel hydrogel materials. Results showed that gels containing either methoxy- or hydroxy-terminated derivatives had higher EWC values than occurs for a gel containing only poly(HEMA). Studies of the surface properties of these gels showed that the total surface free energy of the gels increased with ether repeat unit concentration. Further more this increase was manifestly due to a specific increase in the polar surface energy component.

MPEGA and PEGA derivatives were also copolymerised with a mixture of HEMA and MMA to give two further ranges of hydrogel materials. The inclusion of MMA as a third comonomer led to their being more hydrophobic than those described above. EWC values of 16.9 - 33.7% were measured.

An interesting overview of the cell adhesion behaviour of hydrogels is available[116]. Within this report it was emphasised that hydrogels containing between 5% and 35% water were responsive to cell adhesion studies. At water contents >35%, the cell response decreased suddenly producing very slow adhesion that was impossible to study over a short time span. Cell adhesion studies, carried out *in vitro* using 3T3 cells, on the two ranges of HEMA/MMA based hydrogels yielded very interesting results. Inclusion of MPEG derivatives caused a decrease in the observed cell count relative to that found for a gel consisting purely of HEMA and MMA. This decrease also appeared to be directly related to the length of the polyether side-chain. However, inclusion of the hydroxy terminated PEG derivatives caused either little difference or even an increase in the protein deposition rate compared to that found for a gel consisting purely of HEMA and MMA.

A combination of these conclusions, made on analysis of both bulk and surface properties, suggests that methoxy-terminated polyether acrylates may be suitable biomaterials when copolymerised into HEMA based systems . The observed increase in EWC suggests that polyether modified materials should have superior oxygen permeability, which may make them useful in soft contact lens applications. Additionally it would appear that the surface properties of these polyether-modified materials are largely controlled by the polar polyether side-chains. This suggestion is apparently confirmed by cell adhesion studies, which show that inclusion of polyether chains reduces protein deposition on the hydrogel surface. This observation is presumably due to the chains providing an excluded volume about the surface of the material.

When copolymerised with HEMA:MMA, hydroxy-terminated polyether acrylates were seen to have excellent EWC and surface energy properties. However these hydrogels have a fairly poor resistance to protein deposition. This may bring into question their use in HEMA-based biomaterials. This seemingly opposing effect to that observed for their methoxy-terminated analogues could be due to the fact that any excluded volume to protein deposition, created by the hydroxy-terminated polyether chains expressing themselves at the hydrogel surface, is counteracted by hydrogen bonding interactions between protein molecules and the hydroxy-terminus of the polyether chain.

The synthesis of a range of alkyltartronic acids **3(a-d)**, as potential 'building blocks' for a novel range of poly(α-hydroxy acid)s, was successfully achieved using a four-step synthesis. Products were obtained in very good yields. This novel synthesis was found to be superior to the only previously existing general route to alkyltartronic acids published by Grandjean, as the new synthesis enabled bulk quantities of the acids to be produced.

Alkyltartronic acids were converted, highly successfully, into potassium ethyl alkyltartronates **16(b,e)** using a novel two-step synthesis. Although no polyesterification is described in this thesis, it is thought that these novel derivatives may be more useful as precursors to anhydrosulphite **7** and anhydrocarboxylate **8** type cyclic anhydrides than their respective parent acids. The resulting cyclic anhydride monomers should contain less unwanted chlorinated by-products and feasibily be easier to purify than analogous anhydrides synthesised using the α-hydroxy acids themselves. Subsequent ring-opening of the anhydrides should yield novel poly(α-hydroxy acid)s, that could be of considerable interest in biomedical applications.

CHAPTER 8

MATERIALS AND METHODS.

8.1 REAGENTS.

All reagents were used as supplied unless otherwise stated.

Triethylamine and pyridine were dried, and distilled, over KOH prior to use.

HPLC grade toluene, ethyl acetate and chloroform were used where the dry solvent was required, i.e. in attempts at trityl cleavage using a silica gel column.

Optically pure 2-hydroxyethyl methacrylate was used as supplied by Kelvin Lenses.

8.1.1 Synthesis of Polyether Acrylates.

Compound	R.M.M.	Supplier
Acryloyl chloride	90.51	Aldrich
tert -Butyldimethylsilyl chloride	150.73	Aldrich
Chloroform	119.38	Fisons
Dichloromethane	84.93	Fisons
Davisil silica gel	-	Aldrich
Ethyl acetate	88.11	Fisons
Hydrochloric acid	36.46	Fisons
Magnesium sulphate, anhydrous	120.37	Aldrich
Methoxy poly(ethylene glycol)-350	350	Aldrich
Methoxy poly(ethylene glycol)-550	550	Aldrich
Methoxy poly(ethylene glycol)-750	750	Aldrich
Methoxy poly(ethylene glycol)-2000	2000	Aldrich
Methoxy poly(ethylene glycol)-5000	5000	Aldrich
Petroleum ether, 40-60°C	-	Fisons
Phosphate buffer solution (pH 7.2)	-	Aldrich
Poly(ethylene glycol)-200	200	Aldrich
Poly(ethylene glycol)-400	400	Aldrich
Poly(ethylene glycol)-1000	1000	Fluka
Sodium hydrogen carbonate	84.01	Aldrich
Sodium hydroxide	40.00	Fisons

Tetra-butylammonium fluoride, 1.0M solution in THF	261.47	Aldrich
Toluene	92.14	Fisons
Triethylamine	101.19	Aldrich
Trityl chloride	278.78	Aldrich

Table 8.1 : Materials for the Synthesis of Polyether Acrylates.

8.1.2 Synthesis of Hydrogels.

Compound	R.M.M.	Supplier
Azobisisobutyronitrile	136.20	Fluka
Ethylene glycol dimethacrylate	198.22	BDH
2-Hydroxyethyl methacrylate	130.14	KelvinLenses
Methyl methacrylate	100.12	Aldrich
Nitrogen	28.02	BOC

Table 8.2 : Additional Materials for the Synthesis of Hydrogels.

8.1.3 Synthesis of Alkyltartronic Acids and their Derivatives.

Compound	R.M.M.	Supplier
Acetone	58.08	Fisons
Bromine	159.82	Aldrich
Diethyl ether	74.12	Fisons
Diethyl malonate	160.17	Aldrich
Ethanol	46.07	BDH
Hexane	86.18	Fisons
Hydrazobenzene	184.24	Aldrich
Isopropyl bromide	123.00	Aldrich
Octyl bromide	193.13	Aldrich
Pentyl bromide	151.05	Aldrich
Phosphorus pentoxide	141.95	Aldrich

Potassium hydroxide	56.11	Fisons
Pyridine	79.10	Fisons
Sodium ethoxide	68.05	Aldrich
Stearyl bromide	333.41	Aldrich
Sulphuric acid	98.08	Fisons
Tartronic acid	120.06	Heraeus
Tetrahydrofuran	72.11	Fisons
Thionyl chloride	118.97	Aldrich

Table 8.3 : Additional Materials for the Synthesis of Alkyltartronic Acids and their Derivatives.

8.2 EXPERIMENTAL METHODS

8.2.1 Synthesis of Polyether Acrylates.

8.2.1.1 Synthesis of Methoxy Poly(ethylene glycol)-350 Acrylate 1(a).

25cm^3 (0.0781 moles) of methoxy poly(ethylene glycol)-350 **17(a)** and 13.1cm^3 (0.0937 moles) of triethylamine were dissolved in 100cm^3 of dichloromethane. The resulting solution was then stirred continuously and cooled to -78°C using a dry ice/ acetone bath. 7.7cm^3 (0.0937 moles) of acryloyl chloride was then added cautiously in a dropwise manner.

On completion of the addition the mixture was stirred for a further twenty four hours during which time the bath was allowed to warm naturally and the temperature of the reaction mixture thus allowed to increase to room temperature. The mixture was then allowed to settle after which it was filtered to remove any triethylamine hydrochloride. The filtrate was washed consecutively with 3x50cm^3 of 1M HCl(aq), 3x50cm^3 of 1M NaOH(aq) and finally with 3x50cm^3 of 10% $NaHCO_3$(aq). The remaining organic phase was then dried over anhydrous magnesium sulphate.

Removal of the solvent under vacuum gave **1(a)** in 85% yield. The product was a pale yellow liquid.

I.R.(cm^{-1}; Neat): 3060 (m; ν(C-H vinylic)); 2870 (s; ν(C-H)); 1720 (s; ν(C=O)); 1620 (m; ν(C=C)); 1120 (m; ν(C-O)).

^1H N.M.R. (δ; $CDCl_3$): 3.20 (s; $\mathbf{CH_3}$) ; 3.34, 3.48, 3.55, 4.25 (m's; $\mathbf{CH_2}$); 5.72 (d), 6.03 (q), 6.26 (d) ($\mathbf{H_2C{=}CH}$).

^{13}C N.M.R. (δ; $CDCl_3$): 58.3 (+ve; $\mathbf{CH_3}$); 63.0, 66.4, 69.8, 69.9, 71.3 (-ve; $\mathbf{CH_2}$); 127.7 (+ve; =C-H); 130.3 (-ve; H_2C=); 165.3 (-ve; C=O).

8.2.1.2 Synthesis of Methoxy Poly(ethylene glycol)-550 Acrylate 1(b).

$40cm^3$ (0.0796 moles) of methoxy poly(ethylene glycol)-550 **17(b)** and $13.3cm^3$ (0.0955 moles) of triethylamine were dissolved in $100cm^3$ of dichloromethane. The resulting solution was then stirred continuously and cooled to -78°C using a dry ice/ acetone bath. $7.8cm^3$ (0.0955 moles) of acryloyl chloride was then added cautiously in a dropwise manner.

On completion of the addition the mixture was stirred for a further twenty four hours during which time the bath was allowed to warm naturally and the temperature of the reaction mixture thus allowed to increase to room temperature. The mixture was then allowed to settle after which it was filtered to remove any triethylamine hydrochloride. The filtrate was washed consecutively with $3x50cm^3$ of 1M HCl(aq), $3x50cm^3$ of 1M NaOH(aq) and finally with $3x50cm^3$ of 10% $NaHCO_3$(aq). The remaining organic phase was then dried over anhydrous magnesium sulphate.

1M HCl(aq), 3x50cm^3 of 1M NaOH(aq) and finally with 3x50cm^3 of 10% $NaHCO_3$(aq). The remaining organic phase was then dried over anhydrous magnesium sulphate.

Removal of the solvent under vacuum gave **1(b)** in 87% yield. The product was a pale yellow liquid.

I.R.(cm^{-1}; Neat): 3050 (m; ν(C-H vinylic)); 2870 (s; ν(C-H)); 1720 (s; ν(C=O)); 1620 (m; ν(C=C)); 1120 (m; ν(C-O)).

^{1}H N.M.R. (δ; $CDCl_3$): 3.11 (s; CH_3) ; 3.68, 4.10 (m^{ts}; CH_2); 5.60 (d), 5.80 (q), 6.09 (d) (H_2C=CH).

^{13}C N.M.R. (δ; $CDCl_3$): 58.3 (+ve; CH_3); 60.9, 63.1, 66.5, 69.8, 69.9, 70.0, 71.4, 72.2 (-ve; CH_2); 127.8 (+ve; =C-H); 130.4 (-ve; H_2C=); 165.3 (-ve; C=O).

8.2.1.3 Synthesis of Methoxy Poly(ethylene glycol)-750 Acrylate 1(c).

39.3g (0.0524 moles) of methoxy poly(ethylene glycol)-750 **17(c)** and 8.8cm^3 (0.0629 moles) of triethylamine were dissolved in 100cm^3 of dichloromethane. The resulting solution was then stirred continuously and cooled to -78°C using a dry ice/ acetone bath. 5.1cm^3 (0.0629 moles) of acryloyl chloride was then added cautiously in a dropwise manner.

On completion of the addition the mixture was stirred for a further twenty four hours during which time the bath was allowed to warm naturally and the temperature of the reaction mixture thus allowed to increase to room temperature. The mixture was then allowed to settle after which it was filtered to remove any triethylamine

hydrochloride. The filtrate was washed consecutively with 3x50cm^3 of 1M HCl(aq), 3x50cm^3 of 1M NaOH(aq) and finally with 3x50cm^3 of 10% $NaHCO_3$(aq). The remaining organic phase was then dried over anhydrous magnesium sulphate.

Removal of the solvent under vacuum gave **1(c)** in 86% yield. The product was a pale yellow viscous liquid.

I.R.(cm^{-1}; Neat): 3060 (m; ν(C-H vinylic)); 2860 (s; ν(C-H)); 1715 (s; ν(C=O)); 1620 (m; ν(C=C)); 1120 (m; ν(C-O)).

^{1}H N.M.R. (δ; $CDCl_3$): 3.18 (s; C**H**$_3$) ; 3.76, 4.21 (m's; C**H**$_2$); 5.71 (d), 6.02 (q), 6.29 (d) (**H**$_2$C=C**H**).

^{13}C N.M.R. (δ; $CDCl_3$): 58.2 (+ve; **C**H$_3$); 62.9, 66.3, 69.8, 71.2 (-ve; **C**H$_2$); 127.6 (+ve; =**C**-H); 130.2 (-ve; H$_2$**C**=); 165.2 (-ve; **C**=O).

8.2.1.4 Synthesis of Methoxy Poly(ethylene glycol)-2000 Acrylate 1(d).

100.0g (0.0500 moles) of methoxy poly(ethylene glycol)-2000 **17(d)** and 7.3cm^3 (0.0600 moles) of triethylamine were dissolved in 300cm^3 of dichloromethane. The resulting solution was then stirred continuously and cooled to -78°C using a dry ice/ acetone bath. 4.9cm^3 (0.0600 moles) of acryloyl chloride was then added cautiously in a dropwise manner.

On completion of the addition the mixture was stirred for a further twenty four hours during which time the bath was allowed to warm naturally and the temperature of the reaction mixture thus allowed to increase to room temperature. The mixture was

then allowed to settle after which it was filtered to remove any triethylamine hydrochloride. The filtrate was washed consecutively with $3x50cm^3$ of 1M HCl(aq), $3x50cm^3$ of 1M NaOH(aq) and finally with $3x50cm^3$ of 10% $NaHCO_3$(aq). It was noted that when washing with base that emulsions had a tendency to form. Leaving the mixture for thirty minutes allowed the emulsion to separate. The remaining organic phase was then dried over anhydrous magnesium sulphate.

Removal of the solvent under vacuum gave **1(d)** in 86% yield. The product was a cream coloured waxy solid.

The tendency of the system to form emulsions was due to the increased water solubility of the methoxy poly(ethylene glycol) acrylate products, with increased polyether chain length.

I.R.(cm^{-1}; Nujol): 2860 (s; ν(C-H)); 1730 (s; ν(C=O)); 1120 (m; ν(C-O)).

^1H N.M.R. (δ; $CDCl_3$): 3.20 (s; $\mathbf{CH_3}$) ; 3.85, 4.28 (m's; $\mathbf{CH_2}$); 5.81 (d), 6.10 (q), 6.42 (d) ($\mathbf{H_2}C=C\mathbf{H}$).

^{13}C N.M.R. (δ; $CDCl_3$): 58.4 (+ve; $\mathbf{CH_3}$); 61.0, 63.1, 66.5, 70.0, 71.3, 72.0 (-ve; $\mathbf{CH_2}$); 127.7 (+ve; =C-H); 130.4 (-ve; $H_2\mathbf{C}$=); 165.2 (-ve; C=O).

8.2.1.5 Synthesis of Methoxy Poly(ethylene glycol)-5000 Acrylate 1(e).

100.0g (0.0200 moles) of methoxy poly(ethylene glycol)-5000 **17(e)** and $2.8cm^3$ (0.0240 moles) of triethylamine were dissolved in $500cm^3$ of dichloromethane. The resulting solution was then stirred continuously and cooled to

-78°C using a dry ice/ acetone bath. 2.0cm^3 (0.0240 moles) of acryloyl chloride was then added cautiously in a dropwise manner.

On completion of the addition the mixture was stirred for a further twenty four hours during which time the bath was allowed to warm naturally and the temperature of the reaction mixture thus allowed to increase to room temperature. The mixture was then allowed to settle after which it was filtered to remove any triethylamine hydrochloride. The filtrate was washed consecutively with 3x100cm^3 of 0.5M HCl(aq), 3x100cm^3 of 0.5M NaOH(aq) and finally with 3x50cm^3 of 10% $NaHCO_3$(aq). It was noted that when washing with base that emulsions had a tendency to form. Leaving the mixture to separate naturally took a considerable amount of time. It was found, however, that addition of a small amount of NaCl to the system facilitated two-phase separation almost immediately. The remaining organic phase was then dried over anhydrous magnesium sulphate.

Removal of the solvent under vacuum gave **1(e)** in 93% yield. The product was a cream coloured waxy solid.

The tendency of the system to form emulsions was due to the increased water solubility of the methoxy poly(ethylene glycol) acrylate products, with increased polyether chain length.

I.R.(cm^{-1}; Nujol): 2880 (s; ν(C-H)); 1730 (s; ν(C=O)); 1120 (m; ν(C-O)).

^{1}H N.M.R. (δ; $CDCl_3$): 3.34 (s; CH_3) ; 3.68, 3.84, 4.23 (m$^{'s}$; CH_2); 5.82 (d), 6.16 (q), 6.39 (d) (H_2C=CH).

^{13}C N.M.R. (δ; $CDCl_3$): 63.4, 66.8, 70.3, 71.6 (-ve; CH_2); 128.0 (+ve; =C-H); 130.7 (-ve; H_2C=); 176.5 (-ve; C=O).

8.2.1.6 Attempted Synthesis of Poly(ethylene glycol)-200 Acrylate Via Tritylation.

8.2.1.6.1 Synthesis of Poly(ethylene glycol)-200 Trityl Ether **19(a)**.

240.0g (1.20 moles) of poly(ethylene glycol)-200 **18(a)** and 5.6cm^3 (0.04 moles) of triethylamine were dissolved in 700cm^3 of dichloromethane. The resulting solution was then stirred continuously and cooled to -78°C using a dry ice/ acetone bath. 11.1g (0.04 moles) of trityl chloride, dissolved in 50cm^3 of dichloromethane, was then added cautiously in a dropwise manner.

On completion of the addition the mixture was stirred for a further twenty four hours during which time the bath was allowed to warm naturally and the temperature of the reaction mixture thus allowed to increase to room temperature. The mixture was then allowed to settle after which it was filtered to remove any triethylamine hydrochloride. The filtrate was washed with consecutively with 1x1400cm^3 and 5x700cm^3 of phosphate buffer solution (pH 7.2). The remaining organic phase was then dried over anhydrous magnesium sulphate.

Removal of the solvent under vacuum gave **19(a)** in 71% yield. The product was a colourless viscous liquid.

I.R.(cm^{-1}; Neat): 3450 (s; ν(O-H)); 3090, 3065, 3035 (m; ν(C-H aromatic)); 2880 (s; ν(C-H)); 1600, 1495 (m; ν(C=C) aromatic); 1090 (m; ν(C-O)).

8.2.1.6.2 Synthesis of Poly(ethylene glycol)-200 Tritylether Acrylate **20(a)**.

10.0g (0.023 moles) of poly(ethylene glycol)-200 trityl ether **19(a)** and 3.78cm^3 (0.027 moles) of triethylamine were dissolved in 200cm^3 of dichloromethane. The resulting solution was then stirred continuously and cooled to -78°C using a dry ice/ acetone bath. 2.21cm3 (0.027 moles) of acryloyl chloride was then added cautiously in a dropwise manner.

On completion of the addition the mixture was stirred for a further twenty four hours during which time the bath was allowed to warm naturally and the temperature of the reaction mixture thus allowed to increase to room temperature. The mixture was then allowed to settle after which it was filtered to remove any triethylamine hydrochloride. The filtrate was then washed consecutively with 3x50cm^3 0.1M HCl(aq) and 3x50cm^3 1M NaOH(aq). The remaining organic phase was then dried over anhydrous magnesium sulphate.

Removal of the solvent under vacuum gave **20(a)** in 84% yield. The product was a pale yellow viscous liquid.

I.R.(cm^{-1}; Neat): 3080, 3060, 3010 (m; ν(C-H aromatic)); 2860 (s; ν(C-H)); 1720 (s; ν(C=O)); 1595, 1490 (m; ν(C=C) aromatic); 1445 (m; ν(C=C) vinylic); 1090 (m; ν(C-O)).

8.2.1.6.3 Attempted Synthesis of Poly(ethylene glycol)-200 Acrylate **2(a)** By Suspending Poly(ethylene glycol)-200 Tritylether Acrylate **20(a)** in 0.5M HCl(aq).

5g (0.010 moles) of **20(a)** were suspended in 200cm^3 of 0.5M HCl(aq). Suspension was attained by rapid stirring of the mixture.

After stirring for one hour, the suspension was extracted consecutively with 1x200cm^3 and 3x100cm^3 of dichloromethane. The organic layers were combined and dried over anhydrous magnesium sulphate. The solvent was then removed under vacuum.

An IR spectrum of the product obtained showed that cleavage of the trityl ether, to give the desired product, had been unsuccessful. Infact the product obtained was poly(ethylene glycol)-200 tritylether acrylate starting material. Lack of success was attributed to the insolubility of the starting material in the aqueous acid, resulting in no reactive interaction.

I.R.(cm^{-1}; Neat): 3080, 3060, 3010 (m; ν(C-H aromatic)); 2860 (s; ν(C-H)); 1720 (s; ν(C=O)); 1595, 1490 (m; ν(C=C) aromatic); 1445 (m; ν(C=C) vinylic); 1090 (m; ν(C-O)).

8.2.1.6.4 Attempted Synthesis of Poly(ethylene glycol)-200 Acrylate **2(a)** By Solution of Poly(ethylene glycol)-200 Tritylether Acrylate **20(a)** in 0.5M HCl(aq)/THF.

5g (0.010 moles) of **20(a)** were suspended in 200cm^3 of 0.5M HCl(aq). Enough THF was then added to the mixture to create a homogeneous system. The solution was stirred continuously.

After stirring for one hour, the suspension was extracted consecutively with 1x200cm^3 and 3x100cm^3 of dichloromethane. The organic layers were combined and dried over anhydrous magnesium sulphate. The solvent was then removed under vacuum.

Infrared analysis of the product obtained showed that a carboxylic acid hydroxyl function was present. It was concluded that, under the acidic reaction conditions employed, undesirable cleavage of the acrylate ester had occured. This had occured preferentially to the cleavage of the trityl ether.

I.R.(cm^{-1}; Neat): 3045 (s; ν(O-H)); 2880 (s; ν(C-H)); 1700 (s; ν(C=O)) 1600, 1495 (m; ν(C=C) aromatic); 1090 (m; ν(C-O)).

8.2.1.6.5 Attempted Synthesis of Poly(ethylene glycol)-200 Acrylate **2(a)** By Trityl Ether Cleavage of Poly(ethylene glycol)-200 Tritylether Acrylate **20(a)** on a Silica Gel Column.

100g of Davisil silica gel (grade 634, 100-200 mesh, 60Å) was calcined at 300°C for three hours and left to cool in the furnace. Removing surface water from the gel ensured maximum adsorptive surface area. The gel was slurried in toluene and then poured slowly into a glass column, of 5cm diameter, to establish the silica gel column. The glass column was equipped with a tap and had previously been plugged with glass wool. The column was then allowed to settle. 'Tapping' the column gently allowed any air bubbles to escape. It is necessary that a layer of solvent, not less than 2cm, was maintained above the column at all times. This was to prevent the column from drying out, which would lead to cracks and channels reducing both the potential path length, of any substrate passing through the column, and the absorptive surface area of the column.

1g of **20(a)** was dissolved in $10cm^3$ of toluene. This solution was then poured carefully onto the top of the column. Using a further $200cm^3$ of toluene, the substrate was then developed through the column at a flow rate of approximately $2cm^3$/minute. When this procedure was complete, the column was left for sixteen hours to develop fully.

The column was eluted firstly with 500cm^3 of chloroform, in an attempt to elute the less polar triphenylcarbinol. Elution was carried out at a rate of 3cm^3/minute. Secondly, the column was eluted with 500cm^3 of ethyl acetate in an attempt to elute **2(a)** as the desired product. Elution was again completed at a rate of 3cm^3/minute.

The chloroform extract was a colourless viscous liquid. Infra-red analysis of the product showed the presence of the hydroxyl group of a carboxylic acid, ether linkages and aromatic character. The ethyl acetate extract was a viscous yellow liquid. Infra-red analysis showed this product to be poly(ethylene glycol)-200 tritylether acrylate starting material.

From the observations made in this study, it was concluded that the silica gel column had caused the cleavage of the acrylate ester instead of the desired cleavage of the trityl ether.

Chloroform Extract:-

I.R.(cm^{-1}; Neat): 3050 (s; ν(O-H)); 2880 (s; ν(C-H)); 1700 (s; ν(C=O)) 1600, 1500 (m; ν(C=C) aromatic); 1095 (m; ν(C-O)).

Ethyl Acetate Extract:-

I.R.(cm^{-1}; Neat): 3085, 3055, 3030 (m; ν(C-H aromatic)); 2865 (s; ν(C-H)); 1720 (s; ν(C=O)); 1595, 1490 (m; ν(C=C) aromatic); 1455 (m; ν(C=C) vinylic); 1090 (m; ν(C-O)).

8.2.1.7 Synthesis of Poly(ethylene glycol)-400 Tritylether Acrylate As a Potential Precursor to Poly(ethylene glycol)-400 Acrylate.

8.2.1.7.1 Synthesis of Poly(ethylene glycol)-400 Trityl Ether **19(b)**.

250.0g (0.625 moles) of poly(ethylene glycol)-400 **18(b)** and 2.9cm^3 (0.021 moles) of triethylamine were dissolved in 700cm^3 of dichloromethane. The resulting solution was then stirred continuously and cooled to -78°C using a dry ice/ acetone bath. 5.79g (0.021 moles) of trityl chloride, dissolved in 50cm^3 of dichloromethane, was then added cautiously in a dropwise manner.

On completion of the addition the mixture was stirred for a further twenty four hours during which time the bath was allowed to warm naturally and the temperature of the reaction mixture thus allowed to increase to room temperature. The mixture was then allowed to settle after which it was filtered to remove any triethylamine hydrochloride. The filtrate was washed consecutively with 1x1400cm^3 and 5x700cm^3 of phosphate buffer solution (pH 7.2). The remaining organic phase was then dried over anhydrous magnesium sulphate.

Removal of the solvent under vacuum gave **19(b)** in 65% yield. The product was a colourless viscous liquid.

I.R.(cm^{-1}; Neat): 3480 (s; ν(O-H)); 3100, 3070, 3040 (m; ν(C-H aromatic)); 2880 (s; ν(C-H)); 1610, 1500 (m; ν(C=C) aromatic); 1120 (m; ν(C-O)).

8.2.1.7.2 Synthesis of Poly(ethylene glycol)-400 Tritylether Acrylate **20(b)**.

8.5g (0.013 moles) of poly(ethylene glycol)-400 trityl ether **19(b)** and 2.2cm^3 (0.016 moles) of triethylamine were dissolved in 100cm^3 of dichloromethane. The resulting solution was then stirred continuously and cooled to -78°C using a dry ice/ acetone bath. 1.3cm3 (0.016 moles) of acryloyl chloride was then added cautiously in a dropwise manner.

On completion of the addition the mixture was stirred for a further twenty four hours during which time the bath was allowed to warm naturally and the temperature of the reaction mixture thus allowed to increase to room temperature. The mixture was then allowed to settle after which it was filtered to remove any triethylamine hydrochloride. The filtrate was then washed consecutively with 3x50cm^3 of 0.1M HCl(aq) and 3x50cm^3 1M NaOH(aq). The remaining organic phase was then dried over anhydrous magnesium sulphate.

Removal of the solvent under vacuum gave **20(b)** in 80% yield. The product was a pale yellow viscous liquid.

I.R.(cm^{-1}; Neat): 3085, 3055, 3020 (m; ν(C-H aromatic)); 2865 (s; ν(C-H)); 1720 (s; ν(C=O)); 1600, 1490 (m; ν(C=C) aromatic); 1090 (m; ν(C-O)).

8.2.1.8 Synthesis of Poly(ethylene glycol)-1000 Tritylether Acrylate As a Potential Precursor to Poly(ethylene glycol)-1000 Acrylate.

8.2.1.8.1 Synthesis of Poly(ethylene glycol)-1000 Trityl Ether **19(c)**.

420.0g (0.42 moles) of poly(ethylene glycol)-1000 **18(c)** and 1.95cm^3 (0.014 moles) of triethylamine were dissolved in 1000cm^3 of dichloromethane. The resulting solution was then stirred continuously and cooled to -78°C using a dry ice/ acetone bath. 3.9g (0.014 moles) of trityl chloride, dissolved in 50cm^3 of dichloromethane, was then added cautiously in a dropwise manner.

On completion of the addition the mixture was stirred for a further twenty four hours during which time the bath was allowed to warm naturally and the temperature of the reaction mixture thus allowed to increase to room temperature. The mixture was then allowed to settle after which it was filtered to remove any triethylamine hydrochloride. The filtrate was washed consecutively with 1x1400cm^3 and 5x700cm^3 of phosphate buffer solution (pH 7.2). The remaining organic phase was then dried over anhydrous magnesium sulphate.

Removal of the solvent under vacuum gave **19(c)** in 77% yield. The product was a colourless waxy solid.

I.R.(cm^{-1}; Neat): 3450 (m; ν(O-H)); 3085, 3060, 3040 (m; ν(C-H aromatic)); 2880 (s; ν(C-H)); 1600, 1495 (m; ν(C=C) aromatic); 1075 (m; ν(C-O)).

8.2.1.8.2 Synthesis of Poly(ethylene glycol)-1000 Tritylether Acrylate **20(c)**.

12.4g (0.010 moles) of poly(ethylene glycol)-1000 trityl ether **19(c)** and 1.67cm^3 (0.012 moles) of triethylamine were dissolved in 200cm^3 of dichloromethane. The resulting solution was then stirred continuously and cooled to -78°C using a dry ice/ acetone bath. 0.97cm^3 (0.012 moles) of acryloyl chloride was then added cautiously in a dropwise manner.

On completion of the addition the mixture was stirred for a further twenty four hours during which time the bath was allowed to warm naturally and the temperature of the reaction mixture thus allowed to increase to room temperature. The mixture was then allowed to settle after which it was filtered to remove any triethylamine hydrochloride. The filtrate was then washed consecutively with 3x50cm^3 0.1M HCl(aq) and 3x50cm^3 1M NaOH(aq). The remaining organic phase was then dried over anhydrous magnesium sulphate.

Removal of the solvent under vacuum gave **20(c)** in 75% yield. The product was a pale yellow waxy solid.

I.R.(cm^{-1}; Nujol): 3060, 3020 (m; ν(C-H aromatic)); 2860 (s; ν(C-H)); 1720 (s; ν(C=O)); 1595, 1490 (m; ν(C=C) aromatic); 1455 (m; ν(C=C) vinylic); 1100 (m; ν(C-O)).

8.2.1.9 Novel Synthesis of Poly(ethylene glycol)-200 Acrylate.

8.2.1.9.1 Synthesis of Poly(ethylene glycol)-200*tert* -Butyldimethylsilyl Ether **22(a)**.

400.0g (2.000 moles) of poly(ethylene glycol)-200 **18(a)** and 9.3cm^3 (0.067 moles) of triethylamine were dissolved in 700cm^3 of dichloromethane. The resulting solution was then stirred continuously and cooled to -78°C using a dry ice/ acetone bath. 10.0g (0.067 moles) of *tert* -butyldimethylsilyl chloride was dissolved in a further 50cm^3 of dichloromethane and then added cautiously to the reaction mixture in a dropwise manner.

On completion of the addition the mixture was stirred for a further twenty four hours during which time the bath was allowed to warm naturally and the temperature of the reaction mixture thus allowed to increase to room temperature. The mixture was then allowed to settle after which it was filtered to remove any triethylamine hydrochloride. The filtrate was washed consecutively with 1x1400cm^3 and 5x700cm^3 of phosphate buffer solution (pH 7.2). The remaining organic phase was then dried over anhydrous magnesium sulphate.

Removal of the solvent under vacuum gave **22(a)** in 92% yield. The product was a colourless liquid.

I.R.(cm^{-1}; Neat): 3454 (s; ν(O-H)); 2929, 2859 (s; ν(C-H)); 1255 (s; ν(C-Si)); 1108 (s; ν(C-O)).

^{1}H N.M.R. (δ; $CDCl_3$): -0.11 (s; $(CH_3)_2Si$); 0.80 (s; $(CH_3)_3C$); 3.29 (s; OH); 3.56 (m; CH_2).

^{13}C N.M.R. (δ; $CDCl_3$): -5.3 (+ve; $(CH_3)_2Si$); 18.3 (-ve; C); 25.9 (+ve; $(CH_3)_3C$); 61.4, 61.5, 62.6, 70.5, 72.6 (-ve; CH_2).

8.2.1.9.2 Synthesis of Poly(ethylene glycol)-200*tert* -Butyldimethylsilylether Acrylate **23(a)**.

19.0g (0.061 moles) of poly(ethylene glycol)-200*tert* - butyldimethylsilyl ether **22(a)** and 8.4cm^3 (0.073 moles) of triethylamine were dissolved in 100cm^3 of dichloromethane. The resulting solution was then stirred continuously and cooled to -78°C using a dry ice/ acetone bath. 5.9cm^3 (0.073 moles) of acryloyl chloride was then added cautiously to the reaction mixture in a dropwise manner.

On completion of the addition the mixture was stirred for a further twenty four hours during which time the bath was allowed to warm naturally and the temperature of the reaction mixture thus allowed to increase to room temperature. The mixture was then allowed to settle after which it was filtered to remove any triethylamine hydrochloride. The filtrate was washed with consecutively with 3x25cm^3 of 1M NaOH(aq). The remaining organic phase was then dried over anhydrous magnesium sulphate.

Removal of the solvent under vacuum gave **23(a)** in 90% yield. The product was a colourless liquid.

I.R.(cm^{-1}; Neat): 3038 (m; ν(C-H vinylic)); 2930, 2859 (s; ν(C-H)); 1729 (s; ν(C=O)); 1638, 1408 (m; ν(C-H vinylic)); 1256 (s; ν(C-Si)); 1109 (s; ν(C-O)).

^{1}H N.M.R. (δ; $CDCl_3$): -0.04 (s; $(CH_3)_2Si$); 0.78 (s; $(CH_3)_3C$); 3.45, 3.56, 3.67, 4.22 (m's; CH_2); 5.74 (d), 6.08 (q), 6.34 (d) ($H_2C=CH$).

^{13}C N.M.R. (δ; $CDCl_3$): -5.3 (+ve; $(CH_3)_2Si$); 18.3 (-ve; C); 25.9 (+ve; $(CH_3)_3C$); 62.6, 63.6, 69.0, 70.5, 72.6 (-ve; CH_2); 128.2 (+ve; =C-H); 130.9 (-ve; $H_2C=$); 166.0 (-ve; C=O).

8.2.1.9.3 Synthesis of Poly(ethylene glycol)-200 Acrylate **2(a)** By Cleavage of Poly(ethylene glycol)-200*tert* -Butyldimethylsilylether Acrylate **23(a)** Using Tetra-butylammonium Fluoride.

2g ($5.43x10^{-3}$ moles) of **23(a)** was dissolved in $10.3cm^3$ of THF. The mixture was stirred continuously and cooled to 0°C with an ice bath. $10.9cm^3$ of a 1.0M solution of $(C_4H_9)_4NF$ in THF (0.011 moles) was then added dropwise, over a ten minute period, to give a total solvent volume of $21.2cm^3$ ($10.6cm^3$ THF / g Starting material).

The mixture was stirred for five minutes at 0°C. The ice bath was then removed and stirring continued at room temperature for a further hour.

After removing the THF under vacuum, the residue was redissolved in $100cm^3$ of dichloromethane. The solution was washed with $1x10cm^3$ of water, with vigorous shaking, for three minutes. The remaining organic layer was then dried over anhydrous magnesium sulphate.

After removing the dichloromethane under vacuum, the residue was then shaken in petroleum spirit 40-60°C for one hour. The product was separated from the petroleum ether and then dried under vacuum.

2(a) was obtained in 88% yield. The product obtained was analysed using conventional techniques, and also using Electrospray Mass Spectrometry (ESMS). A general discussion of this technique, and also the results obtained on analysis of poly(ethylene glycol)-200 acrylate, can be found in Chapter 4 Section 4.4.

I.R.(cm^{-1}; Neat): 3261 (s; ν(O-H)); 2961, 2875 (s; ν(C-H)); 1724 (s; ν(C=O)); 1634, 1408 (m; ν(C-H vinylic)); 1127 (s; ν(C-O)).

^1H N.M.R. (δ; $CDCl_3$): 3.21, 3.51, 3.66 (m's; CH_2); 4.42 (s; OH); 5.71 (d), 6.17 (q), 6.39 (d) (H_2C=CH).

^{13}C N.M.R. (δ; $CDCl_3$): 58.8, 61.4, 63.6, 66.7, 70.4 (-ve; CH_2); 128.2 (+ve; =C-H); 131.0 (-ve; H_2C=); 166.2 (-ve; C=O).

8.2.1.10 Novel Synthesis of Poly(ethylene glycol)-400 Acrylate.

8.2.1.10.1 Synthesis of Poly(ethylene glycol)-400*tert* -Butyldimethylsilyl Ether **22(b)**.

400.0g (1.000 moles) of poly(ethylene glycol)-400 **18(b)** and 4.7cm^3 (0.033 moles) of triethylamine were dissolved in 700cm^3 of dichloromethane. The resulting solution was then stirred continuously and cooled to -78°C using a dry ice/ acetone bath. 5.0g (0.033 moles) of *tert* -butyldimethylsilyl chloride was dissolved in a

further 50cm^3 of dichloromethane and then added cautiously to the reaction mixture in a dropwise manner.

On completion of the addition the mixture was stirred for a further twenty four hours during which time the bath was allowed to warm naturally and the temperature of the reaction mixture thus allowed to increase to room temperature. The mixture was then allowed to settle after which it was filtered to remove any triethylamine hydrochloride. The filtrate was washed consecutively with 1x1400cm^3 and 5x700cm^3 of phosphate buffer solution (pH 7.2). The remaining organic phase was then dried over anhydrous magnesium sulphate.

Removal of the solvent under vacuum gave **22(b)** in 78% yield. The product was a colourless viscous liquid.

I.R.(cm^{-1}; Neat): 3456 (s; ν(O-H)); 2933, 2861 (s; ν(C-H)); 1256 (s; ν(C-Si)); 1110 (s; ν(C-O)).

^{1}H N.M.R. (δ; $CDCl_3$): -0.10 (s; $(CH_3)_2Si$); 0.82 (s; $(CH_3)_3C$); 3.30 (s; OH); 3.57 (m; CH_2).

^{13}C N.M.R. (δ; $CDCl_3$): -5.5 (+ve; $(CH_3)_2Si$); 18.2 (-ve; C); 25.8 (+ve; $(CH_3)_3C$); 61.6, 61.8, 62.6, 70.6, 72.6 (-ve; CH_2).

8.2.1.10.2 Synthesis of Poly(ethylene glycol)-400*tert* -Butyldimethylsilylether Acrylate **23(b)**.

13.0g (0.025 moles) of poly(ethylene glycol)-400*tert* -butyldimethylsilyl ether **22(b)** and 4.2cm^3 (0.030 moles) of triethylamine were dissolved in 100cm^3 of dichloromethane. The resulting solution was then stirred continuously and cooled to

-78°C using a dry ice/ acetone bath. 2.5cm^3 (0.030 moles) of acryloyl chloride was then added cautiously to the reaction mixture in a dropwise manner.

On completion of the addition the mixture was stirred for a further twenty four hours during which time the bath was allowed to warm naturally and the temperature of the reaction mixture thus allowed to increase to room temperature. The mixture was then allowed to settle after which it was filtered to remove any triethylamine hydrochloride. The filtrate was washed consecutively with 3x25cm^3 of 1M NaOH(aq). The remaining organic phase was then dried over anhydrous magnesium sulphate.

Removal of the solvent under vacuum gave **23(b)** in 73% yield. The product was a colourless viscous liquid.

I.R.(cm^{-1}; Neat): 3039 (m; ν(C-H vinylic)); 2928, 2863 (s; ν(C-H)); 1727 (s; ν(C=O)); 1638, 1408 (m; ν(C-H vinylic)); 1255 (s; ν(C-Si)); 1109 (s; ν(C-O)).

^{1}H N.M.R. (δ; $CDCl_3$): -0.04 (s; $(CH_3)_2Si$); 0.80 (s; $(CH_3)_3C$); 3.43, 3.57, 3.66, 4.24 (m's; CH_2); 5.72 (d), 6.10 (q), 6.34 (d) ($H_2C=CH$).

^{13}C N.M.R. (δ; $CDCl_3$): -5.5 (+ve; $(CH_3)_2Si$); 18.2 (-ve; C); 25.8 (+ve; $(CH_3)_3C$); 62.6, 63.5, 69.2, 70.4, 72.5 (-ve; CH_2); 128.0 (+ve; =C-H); 130.9 (-ve; $H_2C=$); 166.1 (-ve; C=O).

8.2.1.10.3 Synthesis of Poly(ethylene glycol)-400 Acrylate **2(b)** By Cleavage of Poly(ethylene glycol)-400*tert* -Butyldimethylsilylether Acrylate **23(b)** Using Tetra-butylammonium Fluoride.

2g ($3.52x10^{-3}$ moles) of **23(b)** was dissolved in $14.2cm^3$ of THF. The mixture was stirred continuously and cooled to 0°C with an ice bath. $7.0cm^3$ of a 1.0M solution of $(C_4H_9)_4NF$ in THF ($7.04x10^{-3}$ moles) was then added dropwise, over a ten minute period, to give a total solvent volume of $21.2cm^3$ ($10.6cm^3$ THF / g Starting material).

The mixture was stirred for five minutes at 0°C. The ice bath was then removed and stirring continued at room temperature for a further hour.

After removing the THF under vacuum, the residue was redissolved in $100cm^3$ of dichloromethane. The solution was washed with $1x10cm^3$ of water, with vigorous shaking, for three minutes. The remaining organic layer was then dried over anhydrous magnesium sulphate.

After removing the dichloromethane under vacuum, the residue was then shaken in petroleum spirit 40-60°C for one hour. The product was separated from the petroleum ether and then dried under vacuum.

2(b) was obtained in 85% yield. The product obtained was analysed using conventional techniques, and also using Electrospray Mass Spectrometry (ESMS). A general discussion of this technique, and also the results obtained on analysis of **2(b)**, can be found in Chapter 4 Section 4.4.

I.R.(cm^{-1}; Neat): 3259 (s; ν(O-H)); 2959, 2877 (s; ν(C-H)); 1723 (s; ν(C=O)); 1634, 1409 (m; ν(C-H vinylic)); 1109 (s; ν(C-O)).

^1H N.M.R. (δ; $CDCl_3$): 3.02, 3.36 (m^{ts}; CH_2); 4.22 (s; OH); 5.54 (d), 6.05 (q), 6.27 (d) ($H_2C=CH$).

^{13}C N.M.R. (δ; $CDCl_3$): 58.4, 61.0, 63.4, 68.7, 70.2 (-ve; CH_2); 128.0 (+ve; =C-H); 130.9 (-ve; $H_2C=$); 167.3 (-ve; C=O).

8.2.1.11 Novel Synthesis of Poly(ethylene glycol)-1000 Acrylate.

8.2.1.11.1 Synthesis of Poly(ethyleneglycol)-1000*tert* -Butyldimethylsilyl Ether **22(c)**.

500.0g (0.500 moles) of poly(ethylene glycol)-1000 **18(c)** and 2.3cm^3 (0.017 moles) of triethylamine were dissolved in 1000cm^3 of dichloromethane. The resulting solution was then stirred continuously and cooled to -78°C using a dry ice/ acetone bath. 2.5g (0.017 moles) of *tert* -butyldimethylsilyl chloride was dissolved in a further 50cm^3 of dichloromethane and then added cautiously to the reaction mixture in a dropwise manner.

On completion of the addition the mixture was stirred for a further twenty four hours during which time the bath was allowed to warm naturally and the temperature of the reaction mixture thus allowed to increase to room temperature. The mixture was then allowed to settle after which it was filtered to remove any triethylamine hydrochloride. The filtrate was washed consecutively with 1x1400cm^3 and 7x700cm^3 of phosphate buffer solution (pH 7.2). The remaining organic phase was then dried over anhydrous magnesium sulphate.

Removal of the solvent under vacuum gave **22(c)** in 75% yield. The product was a colourless waxy solid.

I.R.(cm^{-1}; Nujol): 3451 (s; ν(O-H)); 2927, 2868 (s; ν(C-H)); 1253 (s; ν(C-Si)); 1109 (s; ν(C-O)).

^1H N.M.R. (δ; $CDCl_3$): -0.11 (s; $(CH_3)_2Si$); 0.82 (s; $(CH_3)_3C$); 3.30 (s; OH); 3.57 (m; CH_2).

^{13}C N.M.R. (δ; $CDCl_3$): -5.5 (+ve; $(CH_3)_2Si$); 18.3 (-ve; C); 25.8 (+ve; $(CH_3)_3C$); 61.5, 61.7, 62.7, 70.6, 72.6 (-ve; CH_2).

8.2.1.11.2 Synthesis of Poly(ethylene glycol)-1000*tert* -Butyldimethylsilylether Acrylate **23(c)**.

17.7g (0.016 moles) of poly(ethylene glycol)-1000*tert* -butyldimethylsilyl ether **22(c)** and 2.65cm^3 (0.019 moles) of triethylamine were dissolved in 100cm^3 of dichloromethane. The resulting solution was then stirred continuously and cooled to -78°C using a dry ice/ acetone bath. 1.55cm^3 (0.019 moles) of acryloyl chloride was then added cautiously to the reaction mixture in a dropwise manner.

On completion of the addition the mixture was stirred for a further twenty four hours during which time the bath was allowed to warm naturally and the temperature of the reaction mixture thus allowed to increase to room temperature. The mixture was then allowed to settle after which it was filtered to remove any triethylamine hydrochloride. The filtrate was washed with consecutively with 3x25cm^3 of 1M NaOH(aq). The remaining organic phase was then dried over anhydrous magnesium sulphate.

Removal of the solvent under vacuum gave **23(c)** in 79% yield. The product was a colourless waxy solid.

I.R.(cm^{-1}; Nujol): 2930, 2862 (s; ν(C-H)); 1724 (s; ν(C=O)); 1633, 1411 (m; ν(C-H vinylic)); 1257 (s; ν(C-Si)); 1115 (s; ν(C-O)).

^1H N.M.R. (δ; $CDCl_3$): -0.05 (s; $(CH_3)_2Si$); 0.81 (s; $(CH_3)_3C$); 3.44, 3.57, 3.66, 4.22 (m's; CH_2); 5.72 (d), 6.10 (q), 6.33 (d) ($H_2C=CH$).

^{13}C N.M.R. (δ; $CDCl_3$): -5.5 (+ve; $(CH_3)_2Si$); 18.3 (-ve; C); 25.8 (+ve; $(CH_3)_3C$); 62.5, 63.5, 69.2, 70.5, 72.5 (-ve; CH_2); 128.1 (+ve; =C-H); 130.9 (-ve; $H_2C=$); 166.0 (-ve; C=O).

8.2.1.11.3 Synthesis of Poly(ethylene glycol)-1000 Acrylate **2(c)** By Cleavage of Poly(ethylene glycol)-1000*tert* -Butyldimethylsilylether Acrylate **23(c)** Using Tetra-butylammonium Fluoride.

17.5g (0.015 moles) of **23(c)** was dissolved in 156cm^3 of THF. The mixture was stirred continuously and cooled to 0°C with an ice bath. 30.0cm^3 of a 1.0M solution of $(C_4H_9)_4NF$ in THF (0.030 moles) was then added dropwise, over a ten minute period, to give a total solvent volume of 186cm^3 (10.6cm^3 THF / g Starting material).

The mixture was stirred for five minutes at 0°C. The ice bath was then removed and stirring continued at room temperature for a further hour.

After removing the THF under vacuum, the residue was redissolved in $100cm^3$ of dichloromethane. The solution was washed with $1x10cm^3$ of water, with vigorous shaking, for three minutes. The remaining organic layer was then dried over anhydrous magnesium sulphate.

After removing the dichloromethane under vacuum, the residue was then shaken in petroleum spirit 40-60°C for one hour. The product was separated from the petroleum ether and then dried under vacuum.

2(c) was obtained in 84% yield. The product obtained was analysed using conventional techniques, and also using Electrospray Mass Spectrometry (ESMS). A general discussion of this technique, and also the results obtained on analysis of **2(c)**, can be found in Chapter 4 Section 4.4.

I.R.(cm^{-1}; Nujol): 3260 (s; ν(O-H)); 2960, 2877 (s; ν(C-H)); 1725 (s; ν(C=O)); 1638, 1408 (m; ν(C-H vinylic)); 1109 (s; ν(C-O)).

^1H N.M.R. (δ; $CDCl_3$): 3.52 (m; CH_2); 4.21 (s; OH); 5.72 (d), 6.13 (q), 6.34 (d) (H_2C=CH).

^{13}C N.M.R. (δ; $CDCl_3$): 58.7, 61.3, 63.3, 68.7, 70.2 (-ve; CH_2); 127.9 (+ve; =C-H); 130.7 (-ve; H_2C=); 168.0 (-ve; C=O).

8.2.2 Synthesis of Hydrogels.

Hydrogels were produced using a thermally induced bulk polymerisation in a mould. The mould consisted of two glass plates each fitted with a sheet of melinex (poly(ethylene) terephthalate). The melinex ensured that the mould could be easily

separated.Between the two plates was a poly(ethylene) gasket. The mould was held together with clips as shown in Figure 8.1.

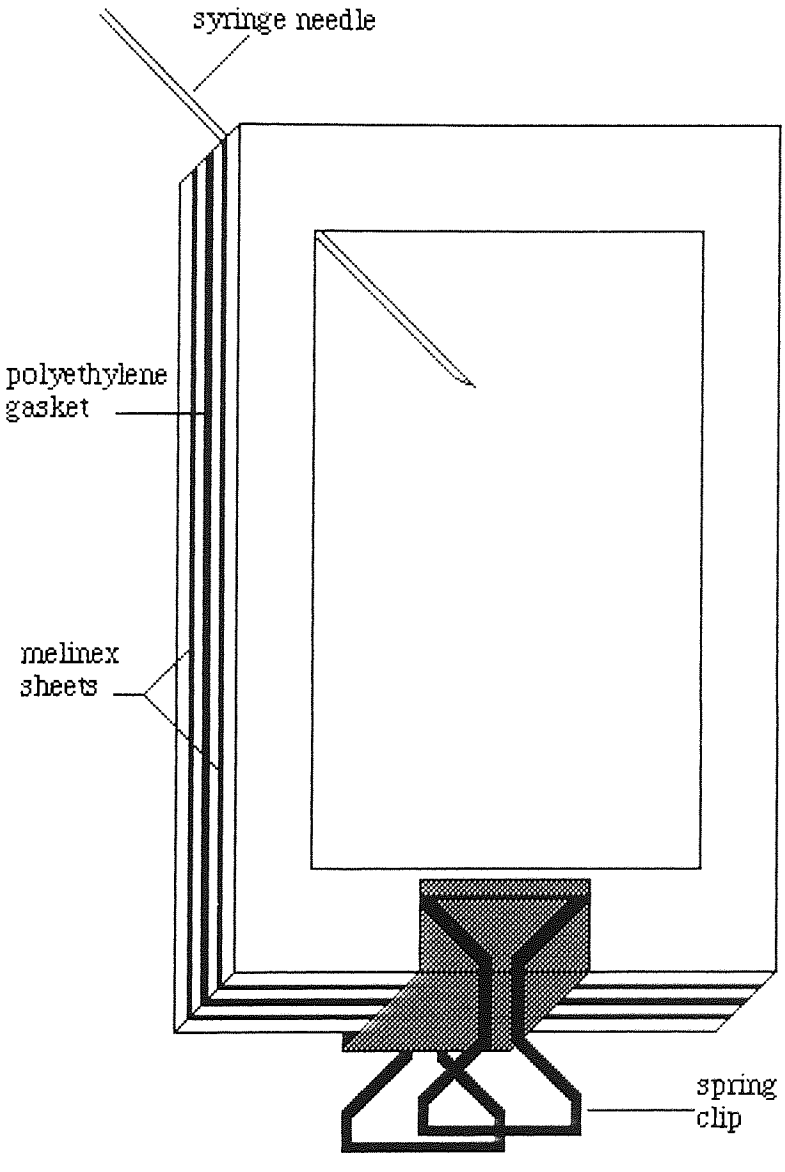


Figure 8.1 Membrane Mould.

The desired mixture of HEMA (see Chapter 3), or HEMA/MMA (see Chapter 4), and novel comonomer was prepared. To this was added ethylene dimethacrylate (EDMA) (1%wt/wt), to cross-link the polymer and azobisisobutyronitrile (AZBN) (0.5% wt/wt) to initiate polymerisation. The whole mixture was then degassed for 10

minutes with nitrogen. This removed any unwanted oxygen from the polymerisation system.

The mixture was then injected carefully into the mould, to prevent air bubbles. The mould was then placed in an oven at 60°C for 3 days and then postcured for 3 hours at 90°C.

The mould was separated whilst still warm and the xerogel peeled from the melinex sheet. The xerogel, or unhydrated gel, was then swollen in distilled water for at least 10 days, the water being changed daily, before any analysis on the gel could take place. The purpose of changing the water was to ensure that all unpolymerised low molecular weight material had been leached from the gel before gel analysis.

8.2.3 Synthesis of Alkyltartronic Acids and their Derivatives.

8.2.3.1 Grandjean[59] Synthesis of Pentyltartronic Acid.

8.2.3.1.1 Synthesis of Diethyl Pentylmalonate **25(a)**.

30.6g (0.450 moles) of sodium ethoxide was dissolved in 200cm^3 of ethanol and the solution heated to 50°C. 68.3cm^3 (0.45 moles) of diethyl malonate were then added cautiously in a dropwise manner. As the reaction was mildly exothermic a water bath was placed around the reaction vessel to help control the reaction temperature.

When a clear solution had been obtained 55.8cm^3 (0.45 moles) of pentyl bromide was added cautiously, in a dropwise manner. The water bath was retained for the duration of this second addition. Reaction was almost immediate and was signified by the appearance of a yellow precipitate, sodium bromide. After complete

addition of the pentyl bromide, the reaction mixture was refluxed for two hours or until the mixture was neutral to moist litmus paper.

After cooling, the ethanol was removed under vacuum to leave a residue. The residue was shaken in approximately 200cm^3 of water. This dissolved any sodium bromide present and allowed crude **25(a)** to separate creating a two phase system.

The crude ester was then distilled under vacuum, collecting the fraction boiling in the range 134-136°C^{14mm}, the quoted literature boiling point. Pure **25(a)** was obtained as colourless liquid in 81% yield.

I.R.(cm^{-1}; Neat): 2959 (s; ν(C-H)); 1734 (s; ν(C=O)); 1153 (m; ν(C-O)).

^{1}H N.M.R. (δ; $CDCl_3$): 0.68 (t; 3H; $CH_3(CH_2)_4$) ; 0.95 (t; 6H; CH_3CH_2O); 1.69 (m; 8H; $CH_3(CH_2)_4$); 3.09 (t; 1H; CH); 3.94 (q; 4H; CH_3CH_2O).

^{13}C N.M.R. (δ; $CDCl_3$): 13.4 (+ve; $CH_3(CH_2)_4$); 13.6 (+ve; CH_3CH_2O); 21.9, 26.5, 28.3, 31.0 (-ve; $CH_3(CH_2)_4$); 51.6 (+ve; CH); 60.6 (-ve; CH_3CH_2O); 169.0 (-ve; C=O).

Elemental Analysis:

	C	H	N
Theoretical (%)	62.58	9.63	-
Found (%)	61.83	9.47	-

8.2.3.1.2 Attempted Synthesis of 1,2-Diphenyl-3,5-dioxo-4-pentyl Pyrazolidine **26** Using Diethyl Pentylmalonate **25(a)**.

2.4g (0.035 moles) of sodium ethoxide was dissolved in $50cm^3$ of ethanol.To the resulting solution was then added $71.6cm^3$ (0.300 moles) of **25(a)** and 46.8g (0.254 moles) of hydrazobenzene. The contents of the flask were then heated to 150°C and then stirred continuously at this temperature for twelve hours.

As the reaction was heated, the contents of the reaction flask became homogeneous. Also as ethanol was formed during the reaction, it was allowed to distil off slowly over the twelve hour period.

When the reaction was complete, an attempt was made to dissolve the residue in water. Although some solution occured, it was noted that a large amount of the residue was an insoluble black tar. It was considered that a large degree of decomposition had occured under the extreme reaction conditions employed. 15% HCl(aq) was added to the aqueous extract until the pH of the extract was equal to 4. A precipitate of crude **19** formed which was filtered off.

The crude product was recrystallised from ethanol to give pure **26** as a colourless solid, of leaf-like crystals, in 11% yield.

Melting point = 102°C

I.R.(cm^{-1}; KBr disc): 3180, 3061, 3051 (s; ν(Arom C-H)); 2922 (s; ν(Aliph C-H)) 1753 (s; ν(C=O)); (s; ν(C=C)); 1299 (m; ν(C-N)).

^{1}H N.M.R. (δ; $CDCl_3$): 0.85 (t; 3H; C**H**$_3$) ; 1.30 (m; 4H; $CH_3(CH_2)_2(CH_2)_2$); 1.50 (m; 2H; $CH_3(CH_2)_2CH_2CH_2$); 2.06 (m; 2H; $CH_3(CH_2)_3CH_2$); 3.37 (t; 1H; Aliph C**H**); 7.15 (m; 2H; Arom C**H**); 7.34 (m; 8H; Arom C**H**)

^{13}C N.M.R. (δ; $CDCl_3$): 13.9 (+ve; $CH_3(CH_2)_4$); 22.3, 25.5, 28.1, 31.4 (-ve; $CH_3(CH_2)_4$); 46.2 (+ve; Aliph **C**H); 122.5, 126.8, 129.0 (+ve; Arom **C**H); 135.7 (-ve; Arom **C**); 170.4 (-ve; **C**=O).

Elemental Analysis:

	C	**H**	**N**
Theoretical (%)	74.74	6.59	8.72
Found (%)	74.55	6.95	8.57

8.2.3.1.3 Synthesis of Pentylmalonic Acid **28(a)**.

25g of KOH was dissolved in 20cm^3 of water to make a 22M solution. The solution was then heated to 50°C. 46.0g (0.200 moles) of diethyl pentylmalonate **25(a)** was then added in a dropwise manner.

The mixture was heated and stirred continuously for five hours. The progress of the reaction was estimated by removing the ethanol formed, during the saponification, under vacuum.

On completion of the reaction, the contents of the reaction vessel were cooled, using an ice bath, to approximately 15°C. Concentrated hydrochloric acid was then added at such a rate that the temperature of the mixture never rose above 20°C. It was

noted that after addition of a quantity of acid, the monopotassium salt precipitated out of solution. Stirring vigorously by hand, however, redissolved the precipitate.

When the pH of the solution was at 4, measured using indicator paper, the aqueous solution was extracted with $3x200cm^3$ of diethyl ether. The combined organic extracts were then dried over anhydrous magnesium sulphate. Removing the ether under vacuum gave crude **28(a)**.

Recrystallisation from acetone/hexane gave pure **28(a)** in 85% yield, as a colourless solid, of leaf-like crystals.

Melting Point = 84°C

I.R.(cm^{-1}; KBr disc): 2990 (s; ν(O-H)); 2932 (s; ν(C-H)); 1708 (s; ν(C=O)); 1271 (m; ν(C-O)).

^1H N.M.R. (δ; $CDCl_3$): 0.85 (t; 3H; CH_3); 1.27 (m; 6H; $CH_3(CH_2)_3CH_2$); 1.89 (q; 2H; $CH_3(CH_2)_3CH_2$); 3.40 (t; 1H; CH); 11.62 (s; 2H; COOH).

^{13}C N.M.R. (δ; $CDCl_3$): 13.8 (+ve; CH_3); 22.2, 26.8, 28.6, 31.2 (-ve; $CH_3(CH_2)_4$); 51.7 (+ve; CH); 175.4 (-ve; C=O).

Elemental Analysis:

	C	H	N
Theoretical (%)	55.16	8.10	-
Found (%)	55.96	8.43	-

8.2.3.1.4 Synthesis of Pentylmalonyl Chloride **29**.

10.0g (0.058 moles) of pentylmalonic acid **28(a)** was suspended in 14cm^3 (0.190 moles). The reaction was stirred for three days at 45-50°C and for a further six hours at 60°C. Initially the mixture was heterogeneous but, as the reaction progressed, the system became homogenised.

At the end of the reaction any excess thionyl chloride was removed under vacuum. **29** was used immediately.

8.2.3.1.5 Synthesis of 1,2-Diphenyl-3,5-dioxo-4-pentyl Pyrazolidine **26** Using Pentylmalonyl Chloride **29**.

11.2cm^3 (0.139 moles) of pyridine was dissolved in 300cm^3 of chloroform. The solution was stirred continuously and cooled to 0°C using an ice-bath.

12.2g (0.058 moles) of **29** was dissolved in a further 30cm^3 of chloroform. This was then added cautiously to the pyridine solution in a dropwise manner. A yellow saturated solution of the acylpyridinium chloride was formed.

10.2g (0.055 moles) of hydrazobenzene was dissolved in 100cm^3 of diethyl ether and added dropwise to the saturated pyridinium chloride salt solution. When the addition was complete the mixture was stirred for a further two hours at room temperature.

When the reaction was complete the reaction mixture was filtered to remove any pyridine hydrochloride formed. The filtrate was then washed with 3x100cm^3 of 1M HCl(aq) and then 3x100cm^3 of 1M NaOH(aq). The basic extracts were combined

and acidified with 1M HCl(aq) until pH4 was attained. A precipitate of crude **26** formed which was filtered.

The crude product was recrystallised from ethanol to give pure **26** as a colourless solid, of leaf-like crystals, in 87% yield.

Melting point = 102-103°C

I.R.(cm^{-1}; KBr disc): 3180, 3061, 3052 (s; ν(Arom C-H)); 2920 (s; ν(Aliph C-H)) 1752 (s; ν(C=O)); 1596, 1492 (s; ν(C=C)); 1302 (m; ν(C-N)).

^{1}H N.M.R. (δ; $CDCl_3$): 0.84 (t; 3H; CH_3) ; 1.24 (m; 4H; $CH_3(CH_2)_2(CH_2)_2$); 1.50 (m; 2H; $CH_3(CH_2)_2CH_2CH_2$); 2.08 (m; 2H; $CH_3(CH_2)_3CH_2$); 3.38 (t; 1H; Aliph CH); 7.16 (m; 2H; Arom CH); 7.32 (m; 8H; Arom CH)

^{13}C N.M.R. (δ; $CDCl_3$): 13.9 (+ve; $CH_3(CH_2)_4$); 22.2, 25.5, 28.1, 31.4 (-ve; $CH_3(CH_2)_4$); 46.2 (+ve; Aliph CH); 122.5, 126.8, 128.9 (+ve; Arom CH); 135.7 (-ve; Arom C); 170.4 (-ve; C=O).

Elemental Analysis:

	C	H	N
Theoretical (%)	74.74	6.59	8.72
Found (%)	72.13	6.78	8.91

8.2.3.1.6 Synthesis of 1,2-Diphenyl-3,5-dioxo-4-hydroxy-4-pentyl Pyrazolidine **31**.

2.0g (6.21×10^{-3} moles) of 1,2-diphenyl-3,5-dioxo-4-pentyl pyrazolidine **26** was placed in a large round-bottomed flask. The flask was then heated to 80°C, the melting point of the solid, using a liquid paraffin-bath. When the melt had been created the flask was spun, by the use of the spinner from a rotory evaporator, to create a thin film.

These conditions were maintained for four days during which a new solid, with a higher melting point, was seen to reform without the system cooling at all.

When all of the melt had been seen to resolidify at 80°C, the system was cooled to allow the light brown **31** to be collected for analysis.

Melting point = 102-103°C

I.R.(cm^{-1}; KBr disc): 3422 (s; ν(O-H)); 3185, 3062, 3050 (s; ν(Arom C-H)); 2924 (s; ν(Aliph C-H)) 1755 (s; ν(C=O)); 1597, 1492 (s; ν(C=C)); 1304 (m; ν(C-N)).

^{1}H N.M.R. (δ; $CDCl_3$): 0.82 (t; 3H; CH_3) ; 1.24 (m; 4H; $CH_3(CH_2)_2(CH_2)_2$); 1.40 (m; 2H; $CH_3(CH_2)_2CH_2CH_2$); 2.06 (m; 2H; $CH_3(CH_2)_3CH_2$); 3.86 (s; 1H; OH); 7.17 (m; 2H; CH); 7.34 (m; 8H; Arom CH)

^{13}C N.M.R. (δ; $CDCl_3$): 13.8 (+ve; $CH_3(CH_2)_4$); 22.2, 29.7, 31.4, 37.6 (-ve; $CH_3(CH_2)_4$); 122.6, 127.1, 129.0 (+ve; CH); 135.0 (-ve; Arom C); 170.4 (-ve; C=O).

Elemental Analysis:

	C	H	N
Theoretical (%)	70.99	6.55	8.28
Found (%)	71.30	6.72	8.16

8.2.3.1.7 Synthesis of Pentyltartronic Acid **3(a)**.

0.2g (2.37×10^{-3} moles) of 1,2-diphenyl-3,5-dioxo-4-hydroxy-4-pentyl pyrazolidine **31** was suspended in $20cm^3$ of 12M KOH(aq). The suspension was stirred continuously and heated at 50°C for five hours.

When the reaction was complete, the mixture was cooled in an ice-bath to approximately 15°C. Concentrated hydochloric acid was then added until the reaction mixture attained pH4. Acid was added, at such a rate, that the temperature did not rise above 20°C. The mixture was then filtered, before being concentrated under vacuum to give a saturated solution. The solution was then extracted with $3 \times 100cm^3$ of diethyl ether. The organic extracts were combined and dried over anhydrous magnesium sulphate.

Removal of the solvent under vacuum afforded an oil. Addition of a small amount of chloroform produced brown needle-like crystals of crude **3(a)**.

The crude product was recrystallised from acetone/hexane to give pure **3(a)** as a colourless solid, of leaf-like crystals, in 62% yield.

Melting Point = 123-124°C

I.R.(cm^{-1}; KBr disc): 3359 (s; ν(O-H)); 2968 (s; ν(C-H)); 1713 (s; ν(C=O)).

^{1}H N.M.R. (δ; DMSO-d_6): 0.83 (t; 3H; CH_3); 1.26 (m; 6H; $CH_3(CH_2)_3CH_2$); 1.74 (q; 2H; $CH_3(CH_2)_3CH_2$); 5.55 (s; 1H; C-OH); 12.51 (s; 2H; COOH).

^{13}C N.M.R. (δ; DMSO-d_6): 13.9 (+ve; CH_3); 22.1, 22.6, 31.4, 35.2 (-ve; $CH_3(CH_2)_4$); 78.3 (-ve; C-OH); 172.3 (-ve; C=O).

Elemental Analysis:

	C	H	N
Theoretical (%)	50.52	7.42	-
Found (%)	50.80	7.46	-

8.2.3.2 Novel Synthesis of Pentyltartronic Acid.

8.2.3.2.1 Synthesis of Diethyl Pentylmalonate **25(a)**.

30.6g (0.450 moles) of sodium ethoxide was dissolved in 200cm^3 of ethanol and the solution heated to 50°C. 68.3cm^3 (0.45 moles) of diethyl malonate was then added cautiously in a dropwise manner. As the reaction was mildly exothermic a water bath was placed around the reaction vessel to help control the reaction temperature.

When a clear solution had been obtained 55.8cm^3 (0.45 moles) of pentyl bromide was added cautiously in a dropwise manner. The water bath was retained for the duration of this second addition. Reaction was almost immediate and was signified by the appearance of a yellow precipitate, sodium bromide. After complete addition of the pentyl bromide, the reaction mixture was refluxed for two hours or until the mixture was neutral to moist litmus paper.

After cooling, the ethanol was removed under vacuum to leave a residue. The residue was shaken in approximately 200cm^3 of water. This dissolved any sodium bromide present and allowed crude **25(a)** to separate creating a two phase system.

The crude ester was then distilled under vacuum, collecting the fraction boiling in the range 134-136°C^{14mm}, the quoted literature boiling point. Pure **25(a)** was obtained as colourless liquid in 81% yield.

I.R.(cm^{-1}; Neat): 2959 (s; ν(C-H)); 1734 (s; ν(C=O)); 1153 (m; ν(C-O)).

^{1}H N.M.R. (δ; $CDCl_3$): 0.68 (t; 3H; C**H**$_3$(CH$_2$)$_4$) ; 0.95 (t; 6H; C**H**$_3$CH$_2$O); 1.69 (m; 8H; CH$_3$(C**H**$_2$)$_4$); 3.09 (t; 1H; C**H**); 3.94 (q; 4H; CH$_3$C**H**$_2$O).

^{13}C N.M.R. (δ; $CDCl_3$): 13.4 (+ve; **C**H$_3$(CH$_2$)$_4$); 13.6 (+ve; **C**H$_3$CH$_2$O); 21.9, 26.5, 28.3, 31.0 (-ve; CH$_3$(**C**H$_2$)$_4$); 51.6 (+ve; **C**H); 60.6 (-ve; CH$_3$**C**H$_2$O); 169.0 (-ve; **C**=O).

Elemental Analysis:

	C	**H**	**N**
Theoretical (%)	62.58	9.63	-
Found (%)	61.83	9.47	-

8.2.3.2.2 Synthesis of Pentylmalonic Acid **28(a)**.

25g of KOH was dissolved in 20cm^3 of water to make a 22M solution. The solution was then heated to 50°C. 46.0g (0.200 moles) of diethyl pentylmalonate **25(a)** was then added in a dropwise manner.

The mixture was heated and stirred continuously for five hours. The progress of the reaction was estimated by removing the ethanol formed, during the saponification, under vacuum.

On completion of the reaction, the contents of the reaction vessel were cooled, using an ice bath, to approximately 15°C. Concentrated hydrochloric acid was then added at such a rate that the temperature of the mixture never rose above 20°C. It was noted that after addition of a quantity of acid the monopotassium salt precipitated out of solution. Stirring vigorously by hand, however, redissolved the precipitate.

When the pH of the solution was equal to 4, when measured using indicator paper, the aqueous solution was extracted with $3x200cm^3$ of diethyl ether. The combined organic extracts were then dried over anhydrous magnesium sulphate. Removing the ether under vacuum gave crude **28(a)**.

Recrystallisation from acetone/hexane gave pure **28(a)** in 85% yield, as a colourless solid, of leaf-like crystals.

Melting Point = 84°C

I.R.(cm^{-1}; KBr disc): 2990 (s; ν(O-H)); 2932 (s; ν(C-H)); 1708 (s; ν(C=O)); 1271 (m; ν(C-O)).

^{1}H N.M.R. (δ; $CDCl_3$): 0.85 (t; 3H; CH_3); 1.27 (m; 6H; $CH_3(CH_2)_3CH_2$); 1.89 (q; 2H; $CH_3(CH_2)_3CH_2$); 3.40 (t; 1H; CH); 11.62 (s; 2H; COOH).

^{13}C N.M.R. (δ; $CDCl_3$): 13.8 (+ve; CH_3); 22.2, 26.8, 28.6, 31.2 (-ve; $CH_3(CH_2)_4$); 51.7 (+ve; CH); 175.4 (-ve; C=O).

Elemental Analysis:

	C	H	N
Theoretical (%)	55.16	8.10	-
Found (%)	55.96	8.43	-

8.2.3.2.3 Synthesis of α-Bromo-pentylmalonic Acid **32(a)**.

10.0g (0.058 moles) of pentylmalonic acid **28(a)** was dissolved in 30cm^3 of diethyl ether. The resulting solution was then stirred continuously. 3.6cm^3 (0.069 moles) of bromine was then added cautiously to this solution in a dropwise manner. During the addition a ten degree rise in temperature, and subsequent mild reflux of the solution, was observed.

After the addition of bromine was complete, 10cm^3 of water was added carefully to the mixture to avoid any violent exothermic reaction. The organic layer was then separated and dried over anhydrous magnesium sulphate.

Removal of the solvent under vacuum produced **32(a)** as an orange solid. Colour was presumably imparted to the product by small amounts of bromine impurity. This product was used immediately.

8.2.3.2.4 Synthesis of Pentyltartronic Acid **3(a)**.

10.0g (0.040 moles) of α-bromo-pentylmalonic acid **32(a)** was dissolved in 150cm^3 of 1M NaOH(aq) and heated to 50°C. The solution was stirred at 50°C for two hours. The mixture was cooled using an ice-bath and acidified to pH4 by cautious addition of 2M HCl(aq).

Under vacuum the acidic solution was concentrated to form a saturated solution, before being extracted with 3x200cm^3 of diethyl ether. This procedure was used only after it was found that the desired product could not be recovered in any great yield, by the continuous extraction of the dilute aqueous layer with diethyl ether.

The organic extracts were combined and dried over anhydrous magnesium sulphate. Removing the solvent under vacuum gave crude **3(a)**.

Recrystallisation from acetone/hexane gave pure **3(a)** in 82% yield, as a colourless solid, of leaf-like crystals.

Melting Point = 123-124°C

I.R.(cm^{-1}; KBr disc): 3359 (s; ν(O-H)); 2968 (s; ν(C-H)); 1713 (s; ν(C=O)).

^{1}H N.M.R. (δ; DMSO-d_6): 0.83 (t; 3H; C**H**$_3$); 1.26 (m; 6H; CH$_3$(C**H**$_2$)$_3$CH$_2$); 1.74 (q; 2H; CH$_3$(CH$_2$)$_3$C**H**$_2$); 5.55 (s; 1H; C-O**H**); 12.51 (s; 2H; COO**H**).

^{13}C N.M.R. (δ; DMSO-d_6): 13.9 (+ve; **C**H$_3$); 22.1, 22.6, 31.4, 35.2 (-ve; CH$_3$(**C**H$_2$)$_4$); 78.3 (-ve; **C**-OH); 172.3 (-ve; **C**=O).

Elemental Analysis:

	C	**H**	**N**
Theoretical (%)	50.52	7.42	-
Found (%)	50.80	7.46	-

8.2.3.3 Novel Synthesis of Octyltartronic Acid.

8.2.3.3.1 Synthesis of Diethyl Octylmalonate **25(b)**.

30.6g (0.450 moles) of sodium ethoxide was dissolved in 200cm^3 of ethanol and the solution heated to 50°C. 68.3cm^3 (0.45 moles) of diethyl malonate was then added cautiously in a dropwise manner. As the reaction was mildly exothermic a water bath was placed around the reaction vessel to help control the reaction temperature.

When a clear solution had been obtained 77.7cm^3 (0.45 moles) of octyl bromide was added cautiously in a dropwise manner. The water bath was retained for the duration of this second addition. Reaction was almost immediate and was signified by the appearance of a white precipitate, sodium bromide. After complete addition of the octyl bromide, the reaction mixture was refluxed for two hours or until the mixture was neutral to moist litmus paper.

After cooling, the ethanol was removed under vacuum to leave a residue. The residue was shaken in approximately 200cm^3 of water. This dissolved any sodium bromide present and allowed crude **25(b)** to separate creating a two phase system.

The crude ester was then distilled under vacuum, collecting the fraction boiling at 167°C^{16mm}, the quoted literature boiling point. Pure **25(b)** was obtained as colourless liquid in 75% yield.

I.R.(cm^{-1}; Neat): 2957 (s; ν(C-H)); 1736 (s; ν(C=O)); 1153 (m; ν(C-O)).

^{1}H N.M.R. (δ; $CDCl_3$): 0.77 (t; 3H; $CH_3(CH_2)_7$) ; 1.81 (t; 6H; CH_3CH_2O); 1.24 (m; 14H; $CH_3(CH_2)_7$); 3.21 (t; 1H; CH); 4.11 (q; 4H; CH_3CH_2O).

^{13}C N.M.R. (δ; $CDCl_3$): 13.4 (+ve; $CH_3(CH_2)_7$); 13.6 (+ve; CH_3CH_2O); 22.4, 27.1, 28.5, 29.0, 29.1, 29.6, 31.6 (-ve; $CH_3(CH_2)_7$); 51.6 (+ve; CH); 61.0 (-ve; CH_3CH_2O); 169.3 (-ve; C=O).

Elemental Analysis:

	C	H	N
Theoretical (%)	66.14	10.36	-
Found (%)	66.41	10.37	-

8.2.3.3.2 Synthesis of Octylmalonic Acid **28(b)**.

25g of KOH was dissolved in $20cm^3$ of water to make a 22M solution. The solution was then heated to 50°C. 54.4g (0.200 moles) of diethyl octylmalonate **25(b)** was then added in a dropwise manner.

The mixture was heated and stirred continuously for five hours. The progress of the reaction was estimated by removing the ethanol formed, during the saponification, under vacuum.

On completion of the reaction, the contents of the reaction vessel were cooled, using an ice bath, to approximately 15°C. Concentrated hydrochloric acid was then added at such a rate that the temperature of the mixture never rose above 20°C.

When the pH of the solution was equal to 4, when measured using indicator paper, the insoluble solid formed was filtered and dried in a vacuum oven for 48 hours at 50°C and then in a vacuum dessicator over phosphorus pentoxide for a further week. The crude product was then taken up in diethyl ether, and then filtered. Removal of the ether under vacuum gave crude **28(b)**.

Recrystallisation from acetone/hexane gave pure **28(b)** in 84% yield, as a colourless solid, of leaf-like crystals.

Melting Point = 113°C

I.R.(cm^{-1}; KBr disc): 2990 (s; ν(O-H)); 2919 (s; ν(C-H)); 1708 (s; ν(C=O)); 1228 (m; ν(C-O)).

^1H N.M.R. (δ; $CDCl_3$): 0.84 (t; 3H; CH_3); 1.24 (m; 12H; $CH_3(CH_2)_6CH_2$); 1.67 (q; 2H; $CH_3(CH_2)_6CH_2$); 3.07 (t; 1H; CH); 12.80 (s; 2H; COOH).

^{13}C N.M.R. (δ; $CDCl_3$): 14.0 (+ve; CH_3); 22.2, 26.9, 28.5, 28.7, 28.9, 29.4, 31.4 (-ve; $CH_3(CH_2)_7$); 51.7 (+ve; CH); 171.0 (-ve; C=O).

Elemental Analysis:

	C	H	N
Theoretical (%)	61.09	9.32	-
Found (%)	61.17	9.36	-

8.2.3.3.3 Synthesis of α-Bromo-octylmalonic Acid **32(b)**.

10.0g (0.046 moles) of octylmalonic acid **28(b)** was dissolved in 30cm^3 of diethyl ether. The resulting solution was then stirred continuously. 2.9cm^3 (0.056 moles) of bromine was then added cautiously to this solution in a dropwise manner. During the addition a ten degree rise in temperature, and subsequent mild reflux of the solution, was observed.

After the addition of bromine was complete, 10cm^3 of water was added carefully to the mixture so as to avoid any violent exothermic reaction. The organic layer was then separated and dried over anhydrous magnesium sulphate.

Removal of the solvent under vacuum produced an orange solid. This was shown by NMR and IR analysis to be practically pure **32(b)**. The colour was presumably imparted to the product by small amounts of bromine impurity.

I.R.(cm^{-1}; Nujol): 2980 (s; ν(O-H)); 2920 (s; ν(C-H)); 1715 (s; ν(C=O)); 1275 (m; ν(C-O)).

^{1}H N.M.R. (δ; $CDCl_3$): 0.81 (t; 3H; CH_3); 1.20 (m; 12H; $CH_3(CH_2)_6CH_2$); 1.66 (t; 2H; $CH_3(CH_2)_6CH_2$); 11.93 (s; 2H; COOH).

^{13}C N.M.R. (δ; $CDCl_3$): 13.9 (+ve; CH_3); 22.2, 27.0, 28.4, 28.7, 28.8, 29.5, 31.4 (-ve; $CH_3(CH_2)_7$); 65.5 (-ve; C-Br); 168.2 (-ve; C=O).

Elemental Analysis:

	C	H	N
Theoretical (%)	44.76	14.50	-
Found (%)	44.86	14.42	-

8.2.3.3.4 Synthesis of Octyltartronic Acid **3(b)**.

10.0g (0.034 moles) of α-bromo-octylmalonic acid **32(b)** was suspended in 150cm^3 of 1M NaOH(aq) and heated to 50°C at which temperature complete solution was attained. The solution was stirred at 50°C for two hours. The mixture was cooled using an ice-bath and acidified to pH4 by cautious addition of 2M HCl(aq). Acidification caused **3(b)** to precipitate out of the cold solution highlighting its solubility in only warm or hot aqueous media.

The precipitate was extracted into 3x200cm^3 of diethyl ether. The organic extracts were combined and dried over anhydrous magnesium sulphate. Removing the solvent under vacuum gave crude **3(b)**.

Recrystallisation from acetone/hexane gave pure **3(b)** in 87% yield, as a colourless solid, of leaf-like crystals.

Melting Point = 114°C

I.R.(cm^{-1}; KBr disc): 3485 (s; ν(O-H)); 2922 (s; ν(C-H)); 1698 (s; ν(C=O)); 1224 (m; ν(C-O)).

^{1}H N.M.R. (δ; DMSO-d_6): 0.84 (t; 3H; C**H**$_3$); 1.22 (m; 12H; CH$_3$(C**H**$_2$)$_6$CH$_2$); 1.76 (t; 2H; CH$_3$(CH$_2$)$_6$C**H**$_2$); 5.60 (s; 1H; C-O**H**) 12.82 (s; 2H; COO**H**).

^{13}C N.M.R. (δ; DMSO-d_6): 14.0 (+ve; CH_3); 22.2, 22.9, 28.7, 29.0, 29.2, 31.4, 35.2 (-ve; $CH_3(CH_2)_7$); 78.3 (-ve; C-OH); 172.3 (-ve; C=O).

Elemental Analysis:

	C	H	N
Theoretical (%)	56.90	8.62	-
Found (%)	56.97	8.58	-

8.2.3.4 Novel Synthesis of Stearyltartronic Acid.

8.2.3.4.1 Synthesis of Diethyl Stearylmalonate **25(c)**.

30.6g (0.450 moles) of sodium ethoxide was dissolved in 200cm^3 of ethanol and the solution heated to 50°C. 68.3cm^3 (0.45 moles) of diethyl malonate were then added cautiously in a dropwise manner. As the reaction was mildly exothermic a water bath was placed around the reaction vessel to help control the reaction temperature.

When a clear solution had been obtained 150.0g (0.45 moles) of stearyl bromide was dissolved in a further 500cm^3 of ethanol and then added cautiously in a dropwise manner. The water bath was retained for the duration of this second addition. Reaction was almost immediate and was signified by the appearance of a white precipitate, sodium bromide. After complete addition of the stearyl bromide, the reaction mixture was refluxed for two hours or until the mixture was neutral to moist litmus paper.

After cooling, the ethanol was removed under vacuum to leave a residue. The residue was shaken in approximately 200cm^3 of water. The resultant slurry was then

extracted with 3x200cm^3 of diethyl ether. The combined organic extracts were dried over anhydrous magnesium sulphate.

Removal of the ether, under vacuum, gave a white waxy solid that was shown spectroscopically to be practically pure **25(c)**. The product was obtained in 75% yield.

I.R.(cm^{-1}; Neat): 2952 (s; ν(C-H)); 1738 (s; ν(C=O)); 1154 (m; ν(C-O)).

^{1}H N.M.R. (δ; $CDCl_3$): 0.83 (t; 3H; $CH_3(CH_2)_{17}$) ; 1.83 (t; 6H; CH_3CH_2O); 1.20 (m; 34H; $CH_3(CH_2)_{17}$); 3.23 (t; 1H; CH); 4.11 (q; 4H; CH_3CH_2O).

^{13}C N.M.R. (δ; $CDCl_3$): 13.8 (+ve; $CH_3(CH_2)_{17}$); 14.0 (+ve; CH_3CH_2O); 22.6, 23.6, 27.2, 28.7, 29.1, 29.2, 29.4, 29.6, 31.8, 32.1 (-ve; $CH_3(CH_2)_{17}$); 52.0 (+ve; CH); 61.0 (-ve; CH_3CH_2O); 169.4 (-ve; C=O).

Elemental Analysis:

	C	H	N
Theoretical (%)	72.82	11.65	-
Found (%)	73.59	11.77	-

8.2.3.4.2 Synthesis of Stearylmalonic Acid **28(c)**.

250g of KOH was dissolved in 200cm^3 of water to make a 22M solution. The solution was then heated to 50°C. 82.4g (0.200 moles) of diethyl stearylmalonate **25(c)** was then stirred as a suspension in the basic solution.

The mixture was heated and stirred continuously for five hours. As saponification occured, then the suspension of **18(c)** gradually became a solution of the dipotassium salt of stearyl malonic acid. The progress of the reaction was estimated by removing the ethanol formed, during the saponification, under vacuum.

On completion of the reaction, the contents of the reaction vessel were cooled, using an ice bath, to approximately 15°C. Concentrated hydrochloric acid was then added at such a rate that the temperature of the mixture never rose above 20°C.

When the pH of the solution was equal to 4, when measured using indicator paper, the insoluble solid formed was filtered and dried in a vacuum oven for 48 hours at 50°C and then in a vacuum dessicator over phosphorus pentoxide for a further week. The crude product was then taken up in some diethyl ether, and then filtered. Removal of the ether under vacuum gave crude **28(c)**.

Recrystallisation from acetone/hexane gave pure **28(c)** in 84% yield, as a colourless solid, of leaf-like crystals.

Melting Point = 95°C

I.R.(cm^{-1}; KBr disc): 2990 (s; ν(O-H)); 2917 (s; ν(C-H)); 1705 (s; ν(C=O)); 1249 (m; ν(C-O)).

^1H N.M.R. (δ; $CDCl_3$): 0.77 (t; 3H; CH_3); 1.24 (m; 34H; $CH_3(CH_2)_{17}$); 3.15 (t; 1H; CH); 12.55 (s; 2H; COOH).

^{13}C N.M.R. (δ; $CDCl_3$): 14.0 (+ve; CH_3); 22.2, 26.9, 28.5, 28.9, 29.2, 31.4 (-ve; $CH_3(CH_2)_{17}$); 51.6 (+ve; CH); 171.0 (-ve; C=O).

Elemental Analysis:

	C	H	N
Theoretical (%)	70.74	11.31	-
Found (%)	70.91	11.40	-

8.2.3.4.3 Synthesis of α-Bromo-stearylmalonic Acid **32(c)**.

10.0g (0.0292 moles) of stearylmalonic acid **28(c)** was dissolved in 30cm^3 of diethyl ether. The resulting solution was then stirred continuously. 1.8cm^3 (0.035 moles) of bromine was then added cautiously to this solution in a dropwise manner. During the addition a ten degree rise in temperature, and subsequent mild reflux of the solution, was observed.

After the addition of bromine was complete, 10cm^3 of water was added carefully to the mixture to avoid any violent exothermic reaction. The organic layer was then separated and dried over anhydrous magnesium sulphate.

Removal of the solvent under vacuum produced an orange solid. This was shown by NMR and IR analysis to be practically pure **32(c)** α-bromo-stearylmalonic acid. The colour was presumably imparted to the product by small amounts of bromine impurity.

I.R.(cm^{-1}; Nujol): 2980 (s; ν(O-H)); 2921 (s; ν(C-H)); 1710 (s; ν(C=O)); 1263 (m; ν(C-O)).

^{1}H N.M.R. (δ; $CDCl_3$): 0.82 (t; 3H; CH_3); 1.20 (m; 32H; $CH_3(CH_2)_{16}CH_2$); 1.67 (t; 2H; $CH_3(CH_2)_{16}CH_2$); 11.53 (s; 2H; COOH).

^{13}C N.M.R. (δ; $CDCl_3$): 14.2 (+ve; CH_3); 22.6, 27.4, 28.8, 29.4, 29.7, 31.9 (-ve; $CH_3(CH_2)_{17}$); 65.8 (-ve; C-Br); 168.6 (-ve; C=O).

Elemental Analysis:

	C	H	N
Theoretical (%)	57.92	9.03	-
Found (%)	58.00	8.90	-

8.2.3.4.4 Synthesis of Stearyltartronic Acid **3(c)**.

10.0g (0.023 moles) of α-bromo-stearylmalonic acid **32(c)** was dissolved in a mixture of 100cm^3 of THF and 100cm^3 of 1M NaOH(aq) and heated to 50°C. The solution was stirred at 50°C for two hours. The mixture was cooled in using an ice-bath and acidified to pH4 by cautious addition of 2M HCl(aq).

Under vacuum the acidic solution was concentrated to remove the THF from the mixture. On removal of the THF, the **3(c)** product precipitated out highlighting its insolubility in water. The precipitate was extracted into 3x200cm^3 of diethyl ether.

The organic extracts were combined and dried over anhydrous magnesium sulphate. Removing the solvent under vacuum gave crude **3(c)**.

Recrystallisation from acetone/hexane gave pure **3(c)** in 88% yield, as a colourless solid, of leaf-like crystals.

Melting Point = 120°C

I.R.(cm^{-1}; KBr disc): 3498 (s; ν(O-H)); 2918 (s; ν(C-H)); 1699 (s; ν(C=O)).

^{1}H N.M.R. (δ; DMSO-d_6): 0.81 (t; 3H; CH_3); 1.19 (m; 32H; $CH_3(CH_2)_{16}CH_2$); 1.75 (t; 2H; $CH_3(CH_2)_{16}CH_2$); 5.47 (s; 1H; C-OH) 12.78 (s; 2H; COOH).

^{13}C N.M.R. (δ; DMSO-d_6): 13.9 (+ve; CH_3); 22.4, 23.1, 29.1, 29.5, 31.6, 35.2 (-ve; $CH_3(CH_2)_{17}$); 78.3 (-ve; C-OH); 172.4 (-ve; C=O).

Elemental Analysis:

	C	H	N
Theoretical (%)	67.74	10.75	-
Found (%)	67.95	10.87	-

8.2.3.5 Novel Synthesis of Isopropyltartronic Acid.

8.2.3.5.1 Synthesis of Diethyl Isopropylmalonate **25(d)**.

30.6g (0.450 moles) of sodium ethoxide was dissolved in 200cm^3 of ethanol and the solution heated to 50°C. 68.3cm^3 (0.45 moles) of diethyl malonate were then added cautiously in a dropwise manner. As the reaction was mildly exothermic a water bath was placed around the reaction vessel to help control the reaction temperature.

When a clear solution had been obtained 42.3cm^3 (0.45 moles) of isopropyl bromide was added cautiously in a dropwise manner. The water bath was retained for the duration of this second addition. Reaction was almost immediate and was signified by the appearance of a white precipitate, sodium bromide. After complete addition of the isopropyl bromide, the reaction mixture was refluxed for two hours or until the mixture was neutral to moist litmus paper.

After cooling, the ethanol was removed under vacuum to leave a residue. The residue was shaken in approximately 200cm^3 of water. This dissolved any sodium bromide present and allowed crude **25(d)** to separate creating a two phase system.

The crude ester was then distilled under vacuum, collecting the fraction boiling in the range 126-129°C^{44mm}, the quoted literature boiling point. Pure **25(d)** was obtained as colourless liquid in 75% yield.

I.R.(cm^{-1}; Neat): 2970 (s; ν(C-H)); 1757 (s; ν(C=O)); 1153 (m; ν(C-O)).

^{1}H N.M.R. (δ; $CDCl_3$): 0.85 (d; 6H; $(CH_3)_2CH$) ; 1.13 (t; 6H; CH_3CH_2O); 2.27 (heptet; 1H; $(CH_3)_2CH$); 2.95 (t; 1H; CH); 4.05 (q; 4H; CH_3CH_2O).

^{13}C N.M.R. (δ; $CDCl_3$): 13.8 (+ve; $(CH_3)_2CH$); 20.1 (+ve; CH_3CH_2O); 28.5 (+ve; $(CH_3)_2CH$); 58.8 (+ve; CH); 60.8 (-ve; CH_3CH_2O); 168.5 (-ve; C=O).

Elemental Analysis:

	C	H	N
Theoretical (%)	59.39	8.97	-
Found (%)	58.73	8.91	-

8.2.3.5.2 Synthesis of Isopropylmalonic Acid **28(d)**.

25g of KOH was dissolved in 20cm^3 of water to make a 22M solution. The solution was then heated to 50°C. 40.4g (0.200 moles) of diethyl isopropylmalonate **25(d)** was then added in a dropwise manner.

The mixture was heated and stirred continuously for five hours. The progress of the reaction was estimated by removing the ethanol formed, during the saponification, under vacuum.

On completion of the reaction, the contents of the reaction vessel were cooled, using an ice bath, to approximately 15°C. Concentrated hydrochloric acid was then added at such a rate that the temperature of the mixture never rose above 20°C. It was noted that after addition of a quantity of acid that the monopotassium salt precipitated out of solution. Stirring vigorously by hand, however, redissolved the precipitate.

When the pH of the solution was equal to 4, measured using indicator paper, the aqueous solution was extracted with 3x200cm^3 of diethyl ether. The combined organic extracts were dried over anhydrous magnesium sulphate. Removing the ether under vacuum gave crude **28(d)**.

Recrystallisation from acetone/hexane gave pure **28(d)** in 90% yield, as a colourless solid, of leaf-like crystals.

Melting Point = 101°C

I.R.(cm^{-1}; KBr disc): 2990 (s; ν(O-H)); 2920 (s; ν(C-H)); 1694 (s; ν(C=O)); 1239 (m; ν(C-O)).

^{1}H N.M.R. (δ; $CDCl_3$): 0.90 (d; 6H; CH_3); 2.14 (heptet; 1H; $(CH_3)_2CH$); 2.91 (t; 1H; CH); 12.53 (s; 2H; COOH).

^{13}C N.M.R. (δ; $CDCl_3$): 20.2 (+ve; CH_3); 27.8 (+ve; $(CH_3)_2CH$); 58.9 (+ve; CH); 170.3 (-ve; C=O).

Elemental Analysis:

	C	H	N
Theoretical (%)	49.31	6.90	-
Found (%)	49.22	7.05	-

8.2.3.5.3 Synthesis of α-Bromo-isopropylmalonic Acid **32(d)**.

10.0g (0.068 moles) of isopropylmalonic acid **28(d)** was dissolved in 30cm^3 of diethyl ether. The resulting solution was then stirred continuously. 4.3cm^3 (0.082 moles) of bromine was then added cautiously to this solution in a dropwise manner. During the addition a ten degree rise in temperature, and subsequent mild reflux of the solution, was observed.

After the addition of bromine was complete, 10cm3 of water was added carefully to the mixture so as to produce no violent exotherm. The organic layer was then separated and dried over anhydrous magnesium sulphate.

Removal of the solvent under vacuum produced an orange solid. This was shown by NMR and IR analysis to be practically pure **32(d)**. The colour was presumably imparted to the product by small amounts of bromine impurity.

I.R.(cm^{-1}; Nujol): 2980 (s; ν(O-H)); 2920 (s; ν(C-H)); 1738 (s; ν(C=O)); 1245 (m; ν(C-O)).

^{1}H N.M.R. (δ; $CDCl_3$): 0.97 (d; 6H; CH_3); 2.35 (heptet; 1H; $(CH_3)_2CH$); 12.10 (s; 2H; COOH).

^{13}C N.M.R. (δ; $CDCl_3$): 19.5 (+ve; CH_3); 34.7 (+ve; $(CH_3)_2CH$); 73.3 (-ve; C-Br); 168.2 (-ve; C=O).

Elemental Analysis:

	C	H	N
Theoretical (%)	32.02	4.03	-
Found (%)	31.93	3.95	-

8.2.3.5.4 Synthesis of Isopropyltartronic Acid **3(d)**.

10.0g (0.044 moles) of α-bromo-isopropylmalonic acid **28(d)** was dissolved in 150cm^3 of 1M NaOH(aq) and heated to 50°C. The solution was stirred at 50°C for two hours. The mixture was cooled using an ice-bath and acidified to pH4 by cautious addition of 2M HCl(aq).

Under vacuum the acidic solution was concentrated to form a saturated solution, before being extracted with 3x200cm^3 of diethyl ether. This procedure was used only after it was found that the desired product could not be recovered, in any great yield, by the continuous extraction of the dilute aqueous layer with diethyl ether.

The organic extracts were combined and dried over anhydrous magnesium sulphate. Removing the solvent under vacuum gave a crude mixture of two products.

Fractional recrystallisation from acetone/hexane of the product mixture obtained enabled the resolution of the product mixture into a fraction of needle like crystals identifiable as the elimination product **33** and a fraction of leaf-like crystals identifiable as pure **3(d)**. Because of the competing elimination and substitution processes in this procedure, the yield of **3(d)** was a little disappointing at 37%.

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Melting Point = 149°C

^{1}H N.M.R. (δ; DMSO-d_6): 1.95 (s; 6H; C**H**$_3$); 12.58 (s; 2H; COO**H**).

^{13}C N.M.R. (δ; DMSO-d_6): 22.6 (+ve; **C**H$_3$); 126.5 (-ve; (CH$_3$)$_2$**C**=C); 151.2 (-ve; (CH$_3$)$_2$**C**=C); 167.1 (-ve; **C**=O).

Elemental Analysis:

	C	**H**	**N**
Theoretical (%)	50.00	5.56	-
Found (%)	49.87	5.64	-

3(d)

Melting Point = 106°C

I.R.(cm^{-1}; Nujol): 3476 (s; ν(O-H)); 2927 (s; ν(C-H)); 1692 (s; ν(C=O)); 1232 (m; ν(C-O)).

^{1}H N.M.R. (δ; DMSO-d_6): 0.84 (d; 6H; C**H**$_3$); 2.40 (heptet; 1H; (CH$_3$)$_2$C**H**); 5.96 (s; 1H; C-O**H**); 12.20 (s; 2H; COO**H**).

^{13}C N.M.R. (δ; DMSO-d_6): 16.9 (+ve; **C**H$_3$); 32.6 (+ve; (CH$_3$)$_2$**C**H); 81.6 (-ve; **C**-OH); 172.1 (-ve; **C**=O).

Elemental Analysis:

	C	**H**	**N**
Theoretical (%)	44.44	6.17	-
Found (%)	44.61	6.11	-

8.2.3.6 Synthesis of Tartronic Acid- and Alkyltartronic Acid-Derivatives.

8.2.3.6.1 Synthesis of Diethyl Tartronate **35(e)**.

5.0g (0.042 moles) of tartronic acid was dissolved in 150cm^3 of ethanol. 6cm^3 of concentrated sulphuric acid was then added, carefully, to the solution. The reaction mixture was heated to 60°C and stirred continuously for seventy two hours.

When the reaction was complete the mixture was allowed to cool to room temperature under continuous stirring. Triethylamine was then added cautiously, in a dropwise manner, until the system became just alkaline. Excess ethanol was then removed, under vacuum, to leave a viscous liquid which was then redissolved in 200cm^3 of dichloromethane.

The dichloromethane solution was extracted with 1x100cm^3 of 5% HCl(aq) and 1x100cm^3 of water. The remaining organic phase was then dried over anhydrous magnesium sulphate. Removing the solvent under vacuum gave crude **35(e)**.

The crude ester was then distilled under vacuum, collecting the fraction boiling in the range 70-72°C^{2mm}. Pure **35(e)** was obtained as a colourless liquid in 90% yield.

I.R.(cm^{-1}; Neat): 3480 (s; ν(O-H)); 2986, 2941 (s; ν(C-H)) 1742 (s; ν(C=O)); 1119 (s; ν(C-O)).

^{1}H N.M.R. (δ; $CDCl_3$): 1.07 (t; 6H; C**H**$_3$) ; 4.06 (16 peaks; 4H; C**H**$_2$); 4.06 (s; 1H; O**H**); 4.51 (s; 1H; C**H**).

^{13}C N.M.R. (δ; $CDCl_3$): 13.3 (+ve; CH_3); 61.8 (-ve; CH_2); 71.1 (+ve; CH); 168.1 (-ve; C=O).

Elemental Analysis:

	C	H	N
Theoretical (%)	47.73	6.82	-
Found (%)	47.75	6.81	-

8.2.3.6.2 Synthesis of Potassium Ethyl Tartronate **16(e)**.

2.0g (0.011 moles) of diethyl tartronate **35(e)** was dissolved in $7cm^3$ of ethanol. 0.64g (0.011 moles) of potassium hydroxide was dissolved in a further mixture of $7cm^3$ of ethanol. Under continuous stirring, this solution was added dropwise to the original solution over a one hour period.

When addition was complete, the solution was stirred for a further hour. The solvent was then removed, under vacuum, to give a white solid. Due to the affinity of the product for ethanol, the solid was a little 'sticky'. It was therefore dried, in a vacuum oven at 50°C for three days, before being analysed.

Analysis showed the product to be **16(e)**, obtained in quantitative yield.

I.R.(cm^{-1}; KBr disc): 3475 (s; ν(O-H)); 2982, 2937 (s; ν(C-H)) 1737, 1598 (s; ν(C=O)); 1113 (s; ν(C-O)).

^1H N.M.R. (δ; D_2O): 1.08 (t; 3H; CH_3) ; 4.04 (16 peaks; 2H; CH_2); 4.41 (s; 1H; OH); 4.60 (s; 1H; CH).

^{13}C N.M.R. (δ; D_2O): 15.9 (+ve; CH_3); 65.3 (-ve; CH_2); 75.9 (+ve; CH); 174.9, 176.2 (-ve; C=O).

Elemental Analysis:

	C	H	N
Theoretical (%)	32.26	3.76	-
Found (%)	32.70	3.92	-

8.2.3.6.3 Synthesis of Diethyl Octyltartronate **35(b)**.

5.0g (0.022 moles) of octyltartronic acid **3(b)** was dissolved in 150cm^3 of ethanol. 6cm^3 of concentrated sulphuric acid was then added, carefully, to the solution. The reaction mixture was heated to 60°C and stirred continuously for seventy two hours.

When the reaction was complete the mixture was -78°C to cool to room temperature under continuous stirring. Triethylamine was then added cautiously, in a dropwise manner, until the system became just alkaline. Excess ethanol was then removed, under vacuum, to leave a viscous liquid which was then redissolved in 200cm^3 of dichloromethane.

The dichloromethane solution was extracted with 1x100cm^3 of 5% HCl(aq) and 1x100cm^3 of water. The remaining organic phase was then dried over anhydrous magnesium sulphate. Removing the solvent under vacuum gave crude **35(b)**.

The crude ester was then distilled under vacuum, collecting the fraction boiling in the range 84-86°C^{2mm}. Pure **35(b)** was obtained as a colourless liquid in 91% yield.

I.R.(cm^{-1}; Neat): 3500 (s; ν(O-H)); 2959, 2927 (s; ν(C-H)) 1741 (s; ν(C=O)); 1112 (s; ν(C-O)).

^1H N.M.R. (δ; $CDCl_3$): 0.74 (t; 3H; $CH_3(CH_2)_7$) ; 1.15 (m; 18H; CH_3CH_2O and $CH_3(CH_2)_6CH_2$); 1.88 (m; 2H; $CH_3(CH_2)_6CH_2$); 3.95 (s; 1H; OH); 4.11 (16 peaks; 4H; CH_3CH_2O).

^{13}C N.M.R. (δ; $CDCl_3$): 13.7 (+ve; $CH_3(CH_2)_7$); 13.8 (+ve; CH_3CH_2O); 22.4, 22.7, 28.9, 29.0, 29.2, 31.6, 34.3 (-ve; $CH_3(CH_2)_7$); 62.0 (-ve; CH_3CH_2O); 78.8 (-ve; C); 170.4 (-ve; C=O).

Elemental Analysis:

	C	H	N
Theoretical (%)	62.50	9.72	-
Found (%)	62.53	9.73	-

8.2.3.6.4 Synthesis of Potassium Ethyl Octyltartronate **16(b)**.

2.0g ($6.94x10^{-3}$ moles) of diethyl octyltartronate **35(b)** was dissolved in $7cm^3$ of ethanol. 0.39g ($6.94x10^{-3}$ moles) of potassium hydroxide was dissolved in a further $7cm^3$ of ethanol. Under continuous stirring, this solution was added dropwise to the original solution over a one hour period.

When addition was complete, the solution was stirred for a further hour. The solvent was then removed, under vacuum. to give a white solid. Due to the affinity of the product for ethanol, the solid was a little 'sticky'. It was therefore dried, in a vacuum oven at 50°C for three days, before being analysed.

Analysis showed the product to be **16(b)** obtained in quantitative yield.

I.R.(cm^{-1}; KBr disc): 3496 (s; ν(O-H)); 2963, 2924 (s; ν(C-H)) 1736, 1599 (s; ν(C=O)); 1115 (s; ν(C-O)).

^1H N.M.R. (δ; D_2O): 0.84 (t; 3H; $CH_3(CH_2)_7$) ; 1.21 (m; 13H; CH_3CH_2O and $CH_3(CH_2)_5CH_2CH_2$); 1.73 (m; 2H; $CH_3(CH_2)_5CH_2CH_2$); 1.99 (m; 2H; $CH_3(CH_2)_5CH_2CH_2$); 4.14 (s; 1H; OH); 4.14 (16 peaks; 4H; CH_3CH_2O).

^{13}C N.M.R. (δ; D_2O): 14.0 (+ve; $CH_3(CH_2)_7$); 14.1 (+ve; CH_3CH_2O); 22.7, 24.0, 29.5, 29.8, 30.0, 32.0, 36.7 (-ve; $CH_3(CH_2)_7$); 61.0 (-ve; CH_3CH_2O); 81.3 (-ve; C) 174.8, 175.6 (-ve; C=O).

Elemental Analysis:

	C	H	N
Theoretical (%)	52.35	5.37	-
Found (%)	52.61	5.44	-

8.2.4 Methods of Analysis.

8.2.4.1 Infrared Spectroscopy.

All infrared spectra were recorded on a Perkin-Elmer 1710 Fourier Transform Infrared Spectrometer. Solid samples were prepared as KBr discs, waxy solids as nujol mulls and liquids as thin films between sodium chloride plates.

8.2.4.2 Nuclear Magnetic Resonance Spectroscopy.

All nuclear magnetic resonance spectra were recorded on a Bruker AC 300 spectrometer. 13C spectra were recorded as either APT (Attached Proton Test) or DEPT (Distortionless Enhanced Polarisation Transfer) spectra.

8.2.4.3 Elemental Analysis.

Elemental microanalyses were performed by Medac Ltd., Department of Chemistry, Brunel University, Uxbridge, Middlesex.

8.2.4.4 Melting Point Determination.

Melting points were determined in capillary tubes with Gallencamp Melting Point Apparatus, Model No. ME-370, and are uncorrected.

8.2.5 Measurement of the Properties of Hydrogels.

8.2.5.1 Determination of Equilibrium Water Content.

EWC determinations were carried out on five separate pieces of gel and the average value calculated. A No.4 cork borer was used to cut out small discs of gel, which were then placed in a sample bottle of distilled water. For each determination the disc was blotted lightly with filter paper, to remove surface water, and weighed. Dehydration of the gel was achieved by placing it in a microwave oven for twelve minutes, after which the gel was reweighed. The EWC was then calculated using the equation in Chapter 1.

8.2.5.2 Determination of Surface Free Energy.

Surface energies of the hydrogels prepared were studied in the hydrated state using Hamilton's method and the captive air bubble technique. Samples were cut from the hydrogel using a No.7 cork borer. In each case the samples were cleaned using Tepol 'L' and rinsed thoroughly in distilled water. They were then left to soak in distilled water for a week before testing.

The contact angle at the three phase interface was measured using a Rame Hart goniometer. Readings were taken at each side of the drop/bubble and an average was taken. Once the contact angles had been obtained, polar, dispersive and toal surface free energy values were calculated using Macintosh Works™ which had been programmed with the relevant equations.

8.2.5.2.1 Hamilton's Method.

Surface water was removed from the sample using filter paper. The gel sample was then glued to a microscope cover slip, using super glue. The sample was inverted in an optical cell which was filled with distilled water. A small drop of octane was then placed on the surface of the sample using a G25 syringe needle and the contact angle was measured. This procedure was repeated three times for each sample.

8.2.5.2.2 Captive Air Bubble Technique.

Samples were mounted as described above. Air bubbles were generated on the gel surface using a G35 needle and the contact angle was measured. Again this procedure was repeated three times for each sample.

8.2.5.3 Cell Adhesion Studies.

For a full experimental description of the cell adhesion studies carried out in the course of this work, it is advised that the reader should consult the thesis of Dr.J.H.Fitton. The effect of polyether chain length on the adhesion of 3T3 cells was studied.

APPENDICES

APPENDIX 1 : EQUILIBRIUM WATER CONTENTS OF POLY(HEMA) HYDROGELS CONTAINING LINEAR POLYETHERS.

MPEG350-A 1(a)

wt% Comonomer	1/%	2/%	3/%	4/%	5/%	Mean EWC/%
0%	36.8	37.1	32.5	35.4	44.5	37.3
5%	40.0	40.7	39.7	40.1	40.0	40.1
10%	42.0	41.7	41.7	41.6	41.4	41.7
20%	46.9	49.0	49.1	43.4	45.9	46.9

MPEG550-A 1(b).

wt% Comonomer	1/%	2/%	3/%	4/%	5/%	Mean EWC/%
5%	41.2	40.6	40.3	40.0	39.9	40.4
10%	43.6	42.7	41.3	42.5	43.3	42.7
20%	47.0	48.8	47.6	47.8	46.7	47.6

MPEG750-A 1(c).

wt% Comonomer	1/%	2/%	3/%	4/%	5/%	Mean EWC/%
5%	40.7	40.6	40.1	40.9	40.7	40.6
10%	43.3	43.9	43.6	43.2	43.5	43.5
20%	47.6	48.2	48.3	48.3	48.6	48.2

MPEG2000-A 1(d).

wt% Comonomer	1/%	2/%	3/%	4/%	5/%	Mean EWC/%
5%	42.3	42.3	42.5	42.6	42.0	42.3
10%	47.1	45.3	45.5	44.9	43.8	45.3
20%	52.8	53.7	53.4	53.2	54.0	53.4

MPEG5000-A 1(e).

wt% Comonomer	1/%	2/%	3/%	4/%	5/%	Mean EWC/%
5%	45.6	46.1	46.0	46.0	46.3	46.0
10%	51.1	50.6	50.2	50.3	50.3	50.5
20%	58.6	58.6	58.3	58.5	58.9	58.6

PEG200-A 2(a).

wt% Comonomer	1/%	2/%	3/%	4/%	5/%	Mean EWC/%
5%	39.7	38.7	38.5	39.0	38.6	38.9
10%	40.1	42.4	39.1	39.9	40.4	40.4
20%	39.7	39.1	42.7	43.5	41.4	41.3

PEG400-A 2(b).

wt% Comonomer	1/%	2/%	3/%	4/%	5/%	Mean EWC/%
5%	39.8	41.3	38.4	43.1	43.0	41.1
10%	42.0	42.5	42.6	41.6	42.2	42.2
20%	44.2	43.3	43.4	45.5	44.2	44.1

PEG1000-A 2(c).

wt% Comonomer	1/%	2/%	3/%	4/%	5/%	Mean EWC/%
5%	41.3	40.6	41.2	41.8	41.9	41.4
10%	44.5	44.2	44.4	44.4	44.4	44.4
20%	48.6	48.0	48.3	48.2	47.8	48.2

APPENDIX 2 : EQUILIBRIUM WATER CONTENTS OF POLY(HEMA) : POLY(MMA) HYDROGELS CONTAINING LINEAR POLYETHERS.

MPEG550-A 1(b)

wt% Comonomer	1	2	3	4	5	Mean EWC/%
0%	16.4	16.4	15.3	17.1	16.7	16.4
5%	16.7	17.5	17.2	16.8	16.3	16.9
10%	17.9	17.3	16.5	16.8	17.0	17.1
20%	21.5	21.6	19.4	24.2	21.4	21.6

MPEG2000-A 1(d).

wt% Comonomer	1	2	3	4	5	Mean EWC/%
5%	18.7	20.1	19.9	21.7	20.2	20.1
10%	23.2	23.6	23.2	23.3	22.7	23.2
20%	33.6	33.4	35.8	32.1	33.7	33.7

PEG400-A 2(b).

wt% Comonomer	1	2	3	4	5	Mean EWC/%
5%	17.8	15.9	16.6	18.7	17.0	17.2
10%	21.5	21.3	21.8	20.9	21.5	21.4
20%	27.1	26.9	26.7	28.1	26.5	27.1

PEG1000-A 2(c).

wt% Comonomer	1	2	3	4	5	Mean EWC/%
5%	20.6	21.1	22.2	21.4	21.6	21.4
10%	23.1	26.5	24.8	23.8	25.1	24.7
20%	29.0	28.3	31.9	29.6	29.3	29.6

APPENDIX 3 : CONTACT ANGLE AND SURFACE FREE ENERGY MEASUREMENTS FOR POLY(HEMA) HYDROGELS CONTAINING LINEAR POLYETHERS.

MPEG350-A 1(a)

Comonomer wt%	Air/°	Octane/°	γ_s^d mN/m	γ_s^p mN/m	γ_s^t mN/m
0%	33	137	23.9	38.2	62.1
5%	32	144	20.6	41.8	62.3
10%	30	145	21.4	42.2	63.6
20%	27	146	22.7	42.7	65.4

MPEG550-A 1(b)

Comonomer wt%	Air/°	Octane/°	γ_s^d mN/m	γ_s^p mN/m	γ_s^t mN/m
20%	26	146	23.3	42.7	65.9

MPEG750-A 1(c)

Comonomer wt%	Air/°	Octane/°	γ_s^d mN/m	γ_s^p mN/m	γ_s^t mN/m
20%	26	147	22.8	43.1	65.9

MPEG2000-A 1(d)

Comonomer wt%	Air/°	Octane/°	γ_s^d mN/m	γ_s^p mN/m	γ_s^t mN/m
20%	24	152	21.6	45.2	66.8

MPEG5000-A 1(e)

Comonomer wt%	Air/°	Octane/°	γ_s^d mN/m	γ_s^p mN/m	γ_s^t mN/m
20%	22	155	21.4	46.3	67.7

PEG200-A 2(a)

Comonomer wt%	Air/°	Octane/°	γ_s^d mN/m	γ_s^p mN/m	γ_s^t mN/m
5%	30	145	21.4	42.2	63.6
10%	26	146	23.3	42.7	65.9
20%	25	147	23.3	43.1	66.4

PEG400-A 2(b)

Comonomer wt%	Air/°	Octane/°	γ_s^d mN/m	γ_s^p mN/m	γ_s^t mN/m
20%	24	149	22.9	44.0	66.9

PEG1000-A 2(c)

Comonomer wt%	Air/°	Octane/°	γ_s^d mN/m	γ_s^p mN/m	γ_s^t mN/m
20%	23	150	22.9	44.4	67.4

APPENDIX 4 : CONTACT ANGLE AND SURFACE FREE ENERGY MEASUREMENTS FOR POLY(HEMA) : POLY(MMA) HYDROGELS CONTAINING LINEAR POLYETHERS.

MPEG550-A 1(b)

Comonomer wt%	Air/°	Octane/°	γ_s^d mN/m	γ_s^p mN/m	γ_s^t mN/m
0%	36	119	36.5	28.1	64.6
5%	31	127	33.0	32.7	65.7
10%	27	133	31.0	36.1	67.1
20%	26	139	27.4	39.3	66.6

MPEG2000-A 1(d)

Comonomer wt%	Air/°	Octane/°	γ_s^d mN/m	γ_s^p mN/m	γ_s^t mN/m
5%	38	131	24.0	35.0	59.0
10%	32	133	27.4	36.1	63.5
20%	24	137	30.0	38.2	68.2

PEG400-A 2(b)

Comonomer wt%	Air/°	Octane/°	γ_s^d mN/m	γ_s^p mN/m	γ_s^t mN/m
5%	31	129	31.3	33.9	65.1
10%	27	127	36.1	32.7	68.8
20%	28	132	31.1	35.5	66.6

PEG1000-A 2(c)

Comonomer wt%	Air/°	Octane/°	γ_s^d mN/m	γ_s^p mN/m	γ_s^t mN/m
5%	31	128	32.1	33.3	65.4
10%	30	130	31.2	34.4	65.6
20%	29	134	28.9	36.6	65.5

CHAPTER 8

REFERENCES.

1) Wicherle, O. and Lim, D., Hydrophilic gels for biological use, *Nature*, 1960, **185**, 117-118.

2) Tighe, B.J., Hydrogels as contact lens materials, *Hydrogels in Medicine and Pharmacy, Vol. III*, Ed. Peppas, N.A., CRC Press, Boca-Raton, Florida, 1987, 53-82.

3) Corkhill, P.H., Hamilton, C.J. and Tighe, B.J., Synthetic Hydrogels VI. Hydrogel composites as wound dressing and implant materials, *Biomaterials*, 1989, **10**, 3-10.

4) Corkhill, P.H., Trevett, A.S. and Tighe, B.J., The potential of hydrogels as synthetic articular cartilage, Proc. Instn. Mech. Engrs., Part H, *J. Engineering in Medicine*, 1990, **204**, 147-155.

5) Corkhill, P.H., Hamilton, C.J. and Tighe, B.J., The design of hydrogels for medical applications, *Critical Reviews in Biocompatability, Vol.5 (Issue 4)*, Ed. Williams, D.F., CRC Press, Boca-Raton, Florida, 1990, 363-436.

6) Bowers, R.W.J. and Tighe, B.J., Studies of the ocular compatability of hydrogels.White spot deposits- incidence of occurence, Location and gross morphology, *Biomaterials*, 1987, **8**, 89-93.

7) Graham, N.B., Poly(ethylene oxide) and related hydrogels, *Hydrogels in Medicine and Pharmacy, Vol.II*, Ed. Peppas, N.A., CRC Press, Boca-Raton, Florida, 1987, 95-113.

8) Merrill, E.W.,Pekala, R.W. and Mahmud, N.A., Hydrogels for blood contact, *Hydrogels in Medicine and Pharmacy, Vol. III*, Ed. Peppas, N.A., CRC Press, Boca-Raton, Florida, 1987, 1-16.

9) Barnes, A., Corkhill, P.H. and Tighe, B.J., Synthetic hydrogels: 3. Hydroxyalkyl acrylate and methacrylate copolymers: Surface and mechanical properties, *Polymer*, 1988, **29**, 2191-2202.

10) Lee, H.B., Jhon, M.S. and Andrade, J.D., Nature of water in synthetic hydrogels I. Dilatometry, Specific conductivity and differential scanning calorimetry of poly hydroxyethyl methacrylate, *J. Colloid Interface Sci.*, 1975, **51**, 225-231.

11) Hatakeyama, T., Yamauchi, A. and Hatakeyama, H., Studies on water bound in poly(vinyl alcohol) hydrogel by DSC and FT-NMR, *Eur. Polym. J.*, 1984, **20**, 61-64.

12) Nagura, M., Nagura, M. and Ishikawa, H.,State of water in highly elastic poly(vinyl alcohol) hydrogels prepared by repeated freezing and melting, *Polymer Comms.*, 1984, **25**, 313-314.

13) Sung, Y.K., Pulse NMR and thermal analysis of water-swelling polymers for biomedical applications, *Polymer(Korea)*, 1986, **10**, 576-583.

14) Sung, Y.K., Gregonis, D.E., Jhon, M.S. and Andrade, J.D., Thermal and pulse NMR analysis of water in poly(2-hydroxyethyl methacrylate), *J. Appl. Polymer Sci.*, 1981, **26**, 3179-3228.

15) Kim, E.H., Jeon, S.I.,Yoon, S.C. and Jhon, M.S., The nature of water in tactic poly(2-hydroxyethyl methacrylate) hydrogels, *Bull. Korean Chem. Soc.*, 1981, **2**, 60-66

16) Jhon, M.S., Hattori, S., Ma, S.M., Gregonis, D. and Andrade, J.D., The role of water in the osmotic and viscoelastic behaviour of gel networks, *Polymer Preprints*, 1975, **16**, 281-285.

17) Pathmanathan, K. and Johari, P., Dielectric and conductivity relaxations in poly(HEMA) and of water in its hydrogels, *J. Polymer Sci.: Part B: Polymer Phys.*, 1990, **28**, 675-689.

18) Kim, E.H., Moon, B.Y., Jeon, S. and Jhon, M.S., The nature of water in copolymer hydrogels, *Bull. Korean Chem. Soc.*, 1983, **4**, 251-256.

19) Nakamura, K., Hatakeyma, T. and Hatakeyma, H., Studies on bound water of cellulose by differential scanning calorimetry, *Textile Res. J.*, 1981, **51**, 607-613.

20) Frommer, M.A. and Lancet, D., Freezing and nonfreezing water in cellulose acetate membranes, *J. Appl. Polymer Sci.*, 1972, **16**, 1295-1303.

21) Takizawa, A., Kinoshita, T., Nomura, O.and Tsujita, Y.,Characteristics of water in copoly(methyl methacrylate/ N-vinylpyrrolidone) membranes, *Polymer J.*, 1985, **17**, 747-752.

22) Pedley, D.G. and Tighe, B.J., Water binding properties of hydrogel polymers for reverse osmosis and related applications, *Br. Polym. J.*, 1979, **11**, 130-136.

23) Bouwstra, J.A., van Miltenburg, J.C., Roorda, W.E. and Junginger, H.E., Polymer-water interactions in cross-linked gels determined by calorimetric measurements, *Polym. Bull.*, 1987, **18**, 337-341.

24) Corkhill, P.H., Jolly, A.M., Ng, C.O. and Tighe, B.J., Synthetic hydrogels: 1. Hydroxyalkyl acrylate and methacrylate copolymers-water binding studies, *Polymer*, 1987, **28**, 1758-1766.

25) Roorda, W.E., Bouwstra, J.A, de Vries, M.A. and Junginger, H.E., The thermal analysis of water in p(HEMA) hydrogels, *Biomaterials*, 1988, **9**, 494-499.

26) Roorda, W.E., Bouwstra, J.A, de Vries, M.A. and Junginger, H.E., Thermal behaviour of poly hydroxyethyl methacrylate (pHEMA) hydrogels, *Pharm. Res.*, 1988, **5**, 722-725.

27) Aizawa, M. and Suzuki, S.,Properties of water in macromolecular gels III. Dilatometric studies of the properties of water in macromolecular gels, *Bull. Chem. Soc. Jpn.*, 1971, **44**, 2967-2971.

28) Carles, J.E. and Scallan, A.M., The determination of the amount of bound water within cellulosic gels by NMR spectroscopy, *J. App.Polymer Sci.*, 1973, **17**, 1855-1865

29) Nagashima, N. and Suzuki, E.I., Studies of hydration by broad line pulsed NMR, *Appl. Spec. Rev.*, 1984, **20**, 1-53.

30) Quinn, F.X., Kampff, E., Smyth, G. and McBrierty, V.J., Water in hydrogels 1. A study of water in poly(N-vinyl-2-pyrrolidone/ methylmethacrylate) copolymers, *Macromolecules*, 1988, **21**, 3191-3198.

31) Yamada-Nosaka, A., Ishikiriyama, K., Todoki, M. and Tanzawa, H., 1H-NMR studies on water in methacrylate gels I., *J. App. Polym. Sci.*, 1990, **39**, 2443-2452.

32) Roorda, W.E., de Bleyser, J., Junginger, H.E. and Leyte, J.C., Nuclear magnetic relaxation of water in hydrogels, *Biomaterials*, 1990, **11**, 17-23.

33) Tanaguchi, Y. and Horigome, S., The states of water in cellulose acetate membranes, *J. Appl. Polym. Sci.*, 1975, **19**, 2743-2748.

34) Jhon, M.S. and Andrade, J.D., Water and hydrogels, *J. Biomed. Mater. Res.*, 1973, **7**, 509-522.

35) Baier, R.E., Dutton, R.C. and Gott, V.L.,Surface chemical features of blood vessel walls and of synthetic materials exhibiting thromboresistance, *Advances in Experimental Medicine, Vol. 7, Surface chemistry of biological surfaces*, Plenum Press, New York, N.Y., 1970, 235-260.

36) Andrade, J.D., Interfacial phenomena and biomaterials, *Medical Instrumentation*, 1973, **7**, 110-120.

37) Coleman, D.L., Gregonis, D.E. and Andrade, J.D., Blood-materials interactions: The minimum interfacial free energy and the optimum polar/ apolar ratio hypothesis, *J. Biomed. Mater. Res.*, 1982, **16**, 381-393.

38) Zhu, K.J., Xiangzhou, L. and Shilin, Y., Preparation, characterisation and properties of polylactide(PLA)- poly(ethylene glycol) (PEG) copolymers:a potential drug carrier, *J. App. Polym. Sci.*, 1990, **39**, 1 - 9.

39) Hiatt, C.W., Shelokov, A., Rosenthal, E.J. and Galimore, J.M., Treatment of controlled pore glass with poly(ethylene oxide) to prevent the absorption of rabies virus, *J. Chromatogr.*, 1971, **56**, 362-364.

40) Whicher, S.J. and Brash, J.L., Platelet-foreign surface interactions: Release of granule constituents from adherent platelets, *J. Biomed. Mater. Res.*, 1978, **12**, 181-201.

41) Sa Da Costa, V., Brier-Russell, D., Trudel, G. Waugh, D.F., Saltzman, E.W. and Merrill, E.W., Polyether-polyurethane surfaces: Thrombin adsorption, Platelet adsorption and ESCA Scanning, *J. Coll. Interface Sci.*, 1980, **76**, 594-596.

42) Sa Da Costa, V., Brier-Russell, D., Saltzman, E.W. and Merrill, E.W., ESCA studies of Polyurethanes: Blood platelet activation in relation to surface composition, *J.Coll. Interface Sci.*, 1981, **80**, 445-452.

43) Okkema, A.Z., Grasel, T.G., Zdrahala, R.J., Solomon, D.D. and Cooper, S.L., Bulk, surface and blood-contacting properties of polyetherurethanes modified with polyethylene oxide, *J. Biomater. Sci. Polymer Edn.*, 1989, **1**, 43-62.

44) Liu, S.Q., Ito, Y. and Imanishi, Y., Synthesis and non-thrombogenicity of polyurethanes with poly(oxyethylene) side-chains in soft segment regions, *J.Biomater. Sci. Polymer Edn.*, 1989, **1**, 111-122.

45) Nakao, A., Nagaoka, S. and Mori, Y., Hemocompatability of hydrogel with polyethyleneoxide chains, *J. Biomater. Appl.*, 1987, **2**, 219-234.

46) Nagaoka, S. and Nakao, A., Clinical applications of antithrombogenic hydrogel with long poly(ethylene oxide) chains, *Biomaterials*, 1990, **11**, 119-121.

47) Nojiri, C., Okano, T., Jacobs, H.A., Park, K.D., Mohammad, S.F., Olsen, D.B. and Kim, S.W., Blood compatability of PEO grafted polyurethane and HEMA/ styrene block copolymer surfaces, *J. Biomed. Mater. Res.*, 1990, **24**, 1151-1171.

48) Allmer, K. Hiborn, J., Larsson, P.H., Hult, A. and Ranby, B., Surface modification of polymers V.Biomaterial applications, *J. Polymer Sci.: Part A: Polymer Chem.*, 1990, **28**, 173-183.

49) Nagaoka, S., Mori, Y., Takiuchi, H., Yokota, K., Tanzawa, H. and Nishiumi, S., *Interaction between blood components and hydrogels with poly (oxyethylene) chains, Polymers as Biomaterials*, Eds., Shalaby, S.W., Hoffman, A.S., Ratner, B.D.and Horbett, T.A., Plenum Press, New York, N.Y., 1984, 361-374.

50) Lee, J.H., Kopeckova, P., Kopecek, J. and Andrade, J.D., Surface properties of copolymers of alkyl methacrylates with methoxy (polyethylene oxide) methacrylates and their protein resistant coatings, *Biomaterials*, 1990, **11**, 455-464.

51) Schreiner, L.J., Milkovic, L. and Peemoeller, H., A determination of hydration stoichiometry in aqueous PEG400 solutions, *Polymer Commun.*, 1991, **32**, 105-107.

52) Graham, N.B., Zulfiqar, M., Nwachuku, N.E. and Rashid, A., Interaction of poly(ethylene oxide) with solvents: 4. Interaction of water with poly(ethylene oxide) crosslinked hydrogels, *Polymer*, 1990, **31**, 909-916.

53) Dessaignes.V., Ueber die nitroweinsaure und eine davon sich ableitende saure, *Ann.*, 1852, **82**, 362.

54) Osten, Nitrirung bei gegennart von phosphorsaureanhydrid, *Ann.*, 1905, **343**, 154.

55) Gruber, Ueber die einwirkung von salpetrigsaure anhydrid auf protocartechusaure, *Ber.*, 1879, **12**, 514.

56) Kekule, Ueber die carboxytartronsaure und die constitution des benzols, *Ann.*, 1883, **221**, 250.

57) Dobinson, F., Ozonisation of malonic acid in aqueous solution, *Chemistry and Industry*, 1959, 853-854.

58) Bardroff, W., Zur konstitution der bimolekularen fettsaurecyanide, *Monatsh.*, 1912, **33**, 859-871.

59) Grandjean, J., Contribution a l'etude des acides tartroniques, *Bull. Soc. Roy. des Sc. de Liege*, 1969, **38**, 288-292.

60) Fichter, F. and Beisswenger, A., Die reduction des glutarsaureanhydrids zum γ-valerolacton, *Ber.*, 1903, **36**, 1200.

61) Bischoff, C.A. and Walden, P.W., Ueber des glycolid und seine homologen, *Ber.*, 1893, **26**, 262.

62) Bischoff, C.A. and Walden, P.W., Ueber derivate der glycolsaure, *Ber.*, 1894, **27**(**4**), 633.

63) Small, P.A., Thermodynamics of polymerisation of cyclic compounds by ring opening. II. Heterocylic compounds, *Trans. Farad. Soc.*, 1955, **51**, 1717-1720.

64) Deibig, H., Geiger, J. and Sander, M., Poly(tetramethylglycolide). I. Synthesis and properties of poly(tetramethylglycolide), *Die Makromol. Chemie*, 1971, **145**, 123-131.

65) Cox, E.F. and Hostettler, F., Polymerization of cyclic esters, *U.S. Patent 3,021,313*, 1962.

66) Ballard, D.G.H. and Tighe, B.J., Studies of the reactions of the anhydrosulphites of α-hydroxy-carboxylic acids. Part I. Polymerisation of the anhydrosulphite of α-hydroxyisobutyric acid, *J. Chem. Soc.*, 1967, 702.

67) Tighe, B.J., Ring opening reactions of 5,5-dimethyl-1,3-dioxolane-2,4-dione, *Chemistry and Industry*, 1969, 1837.

68) Blackbourn, G.P. and Tighe, B.J., Studies in ring-opening polymerisation. (I). 5,5-diethyl-1,3,2-dioxathiolan-4-one-2-oxide, *J. Poly. Sci.*, 1970, **8**, 3591.

69) Blackbourn, G.P. and Tighe, B.J., Studies in Ring-Opening Polymerisation. (II). 5,5-diethyl-1,3,2-dioxathiolan-4-one-2-oxide, *J. Chem. Soc.*, 1971, **8(A-1)**, 1384.

70) Pedley, D.G. and Tighe, B.J., Studies in Ring-Opening Polymerisation. (III). 5-methyl-5-propyl and 5-methyl-5-isopropyl-1,3,2-dioxathiolan-4-one-2-oxide, *J. Poly. Sci. (Polym. Chem. Ed.)*, 1973, **11**, 779.

71) Augert, T.A., Rosensaft, N. and Perciaccante, V.A., Unsymmetrically substituted 1,4-dioxane-2,5-diones, *British Pat. 1, 494,781*, 1977.

72) Davies, W.H., Anhydrocarboxy- derivatives of hydroxy- and mercapto-acids, *J. Chem. Soc.*, 1951, 1357.

73) Tighe, B.J., Studies in ring-chain polymerisation, *Ph.D. Thesis*, University of Aston in Birmingham, 1966.

74) Thomas, M.D. and Tighe, B.J., Studies of the reactions of the anhydrosulphites of α-hydroxy-carboxylic acids. Part III. Purification and polymerisation of glycollic acid anhydrosulphite. *J. Chem. Soc.(B)*, 1970, 1039.

75) Smith,I.J. and Tighe, B.J., Studies in ring-opening polymerisation. (VI) Tertiary base initiated polymerisation of 5-phenyl-1,3-dioxalan-2,4-dione, *Makromol. Chem.* , 1981, **182**, 313.

76) Ballard, D.G.H. and Tighe, B.J., Studies of the reactions of α-hydroxy carboxylic acids. (II) Polymerisation of glycollic and lactic acid anhydrosulphites, *J. Chem. Soc.(B)*, 1967, 976.

77) Al-Mesfer, H. and Tighe, B.J., Polymers for biodegradable medical devices. (III) Polymerisation and copolymerisation of cyclic derivatives of tartronic acid, *Biomaterials*, 1987, **8**, 353-359.

78) Klibanov, A.M., Enzymes that work in organic solvents, *Chemtech*, 1986, 354-359.

79) Dordick, J.S., Enzymatic catalysis in monophasic organic solvents, *Enzyme Microb. Technol.*, 1989, **11**, 194-211.

80) Gatfield, I.L., The enzymic synthesis of esters in non-aqueous solvents, *Annals N.Y. Acad. Sci.*, 1984, **321**, 568.

81) Kirchner, G., Scollar, M.P. and Klibanov, A.M., Resolution of racemic mixtures via lipase catalysis in organic solvents, *J. Am. Chem. Soc.*, 1985, **107**, 7072-7076.

82) Bell, G., Blain, J.A., Paterson, J.D.E., Shaw, C.E.L. and Todd, R.J., Ester and glyceride synthesis by Rhizopus Arrhizus mycellia, *FEMS Microbiol. Lett.*, 1978, **3**, 223-225.

83) Marlot, C., Langrand, G., Triantaphylides, C. and Baratti, J., Ester synthesis in organic solvent catalysed by lipases immobilised on hydrophilic supports, *Biotechnol. Lett.*, 1985, **7**, 647-650.

84) Koshiro, S., Sonomoto, K., Tanaka, A. and Fukui, S.J., Stereoselective esterification of dl-menthol by polyurethane-entrapped lipase in organic solvent, *Biotechnol.*, 1985, **2**, 47-57.

85) Cesti, P., Zaks, A. and Klibanov, A.M., Preparative regioselective acylation of glycols by enzymic transesterification in organic solvents, *Appl. Biochem. Biotechnol.*, 1985, **11**, 401-407.

86) Therisod, M. and Klibanov, A.M., Regioselective acylation of secondary hydroxyl groups in sugars catalysed by lipases in organic solvents, *J. Am. Chem. Soc.*, 1987, **109**, 3977-3981.

87) Chopineau, J., McCafferty, F.D., Therisod, M. and Klibanov, A.M., Production of biosurfactants from sugar alcohols and vegetable oils catalysed by lipases in a non-aqueous medium, *Biotechnol. Bioeng.*, 1988, **31**, 208-214.

88) Riva, S. and Klibanov, A.M., Protease-catalysed regioselective esterification of sugars and related compounds in anhydrous dimethylformamide, *J. Am. Chem. Soc.*, 1988, **110**, 2

89) Makita, A., Nihira, T. and Yamada, Y., Lipase catalysed synthesis of macrocylic lactones in organic solvents, *Tetrahedron Lett.*, 1987, **28**, 805.

90) Gutman, A.L., Zuobi, K. and Boltansky, A., Lipase catalysed synthesis of macrocyclic lactones in organic solvents, Enzymatic lactonisation of γ-hydroxyesters in organic solvents. Synthesis of optically pure γ-methyl butyrolactone and γ-phenyl butyrolactone, *Tetrahedron Lett.*, 1987, **28**, 3861.

91) Oxley, H.R., Hydrogels containing linear and cyclic polyethers, *Ph.D. Thesis*, University of Aston in Birmingham, 1991.

92) Dal Pozzo, A.D., Vigo, A. and Donzelli, G., New monofunctional derivatives of polyethylene glycols via monotrityl intermediates, *Makromol. Chem.*, 1989, **190**, 2457 - 2461.

93) Helferich, B., Moog, L. and Junger, A., Replacement of reactive H atoms in sugars, hydroxy and amino acids by the triphenylmethyl residue, *Ber.*, 1925, **58**, 872.

94) Michelson, A.M. and Todd, A., Nucleotides. Part XXXVIII. An improved synthesis of uridine-diphosphate-glucose (UDPG), *J. Chem. Soc.*, 1956, 3459.

95) Lehrfeld, J., Silica gel catalysed detritylation of some carbohydrate derivatives, *J. Org. Chem.*, 1967, **32**, 2544 - 2545.

96) Corey, E.J. and Venkateswarin, A., Protection of hydroxyl groups as tert-butyl dimethylsilyl derivatives, *J. Am. Chem. Soc.*, 1972, **94**, 17.

97) Cunico, R.F. and Bedell, L., The triisopropylsilyl group as a hydroxyl-protecting function, *J. Org. Chem.*, 1980, **45**, 4797 -4798.

98) Ogilvie, K.K., Sadana, K.L., Thompson, E.A., Quilliam, M.A. and Westmore, J.B., The use of silyl groups in protecting the hydroxyl functions of ribonucleosides, *Tetrahedron Lett.*, 1974, **33**, 2861 - 2863.

99) Ogilvie, K.K., Thompson, E.A., Quilliam, M.A. and Westmore, J.B., Selective protection of hydroxyl groups in deoxnucleosides using alkylsilyl reagents, *Tetrahedron Lett.*, 1974, **33**, 2865 -2868.

100) Green, B.N., Oliver, R.W.A., Falick, A.M., Shackleton, C.H.L., Roitman, F. and Witkowska, H.E., *Biological Mass Spectrometry*, (Eds) Burlingame, A.L., McCloskey, J.A., Elsevier, 1990, 129-146.

101) Shackleton, C.H.L., Falick, A.M., Green, B.N.and Witkowska, H.E., Electrospray mass spectrometry in the clinical diagnosis of variant hemoglobins, *J. Chromatogr.*, 1991, **562**, 175 - 190.

102) Falick, A.M., Shackleton, C.H.L., Green, B.N.and Witkowska, H.E., Tandem mass spectrometry in the clinical analysis of variant haemoglobins, *Rapid Commun. Mass Spectrom.*, 1990, **4**, 396-400.

103) Hamilton, C.J., Transport phenomena in hydrogel membranes, *PhD Thesis*, The University of Aston in Birmingham, 1988.

104) Hamilton, W.C., Technique for the characterisation of hydrophilic solid surfaces, *J. Colloid Interface Sci.*, 1972, **40(2)**, 219.

105) Fowkes, F.M, Additivity of intermolecular forces at interfaces I : Determination of the contribution to surface and interfacial tensions of dispersion forces in various liquids, *J. Phys. Chem.*, 1963, **67**, 2538-2541.

106) Tamai, Y., Makuuchi, K. and Suzuki, M., Experimental analysis of interfacial forces at the plane surface of solids, *J. Phys. Chem.*, 1967, **71**, 4176-4179.

107) Young, T., On the cohesion of fluids, *Phil. Trans. Roy. Soc. (London)*, 1805, **95**, 65-87.

108) Andrade, J.D., Hydrogels for biomedical applications, *ACS Symposium Series*, 1976, **31**, Washington DC American Chemical Society.

109) Larke, J.R., Ng, C.O. and Tighe, B.J., Hydrogel polymers in contact lens applications:- A survey of existing literature- Part 1, *The Optician*, 1971, **162**, 12-16.

110) Moss, S.J., Jolly, A.J. and Tighe, B.J., Plasma oxidation of polymers, *Plasma Processing Plasma Chem.*, 1986, **6**, 401.

111) Ruckenstein, E. and Gourinsaker, S.V., A surface energy criterion of blood compatability, *J. Coll. Interface Sci.*, 1984, **101**, 436-451.

112) Lydon, M.J., Synthetic hydrogels as substrata for cell adhesion, *Br. Polym. J.*, 1986, **18**, 22-27.

113) Fitton, J.H., Cells, surfaces and adhesion, *Ph.D. Thesis*, University of Aston in Birmingham, 1993.

114) Thomas, K.D., Biological interactions with synthetic polymers, *Ph.D. Thesis*, University of Aston in Birmingham, 1988.

115) Baier, R.E., Comments on cell adhesion to biomaterial surfaces : Conflicts and concerns, *J. Biomed. Mater. Res.*, 1982, **16**, 173-175.

116) Minett, W.T., Cell adhesion to synthetic polymer substrates, *Ph.D. Thesis*, University of Aston in Birmingham, 1986.

117) Franklin, V.J., Lipoidal Species in Ocular Spoilation Processes, *PhD Thesis*, Aston University, 1990.

118) Bischoff, Studien uber verkethungen. I. Alkylining des malon- und acetessigsaureesters, *Ber.*, 1895, **28**, 2622.

119) Adams, R. and Marvel, C.S., n-Butyl malonic ester, preparation, *J. Am. Chem. Soc.*, 1920, **42**, 316.

120) Levene, P.A. and Taylor, F.A., On oxidation of tertiary hydrocarbons, *J. Biol. Chem.*, 1922, **54**, 351.

121) Bhide, B.V. and Sudborough, J.J., Esterification, *J. Indian Inst. Sci. A*, 1925, **8**, 89.

122) Adams, R. and Kamm, R.M., Ethyl n-butylmalonate (Malonic acid, butyl-, ethyl ester), *Org. Syn.*, 1941, **Coll. Vol.1**, 250-251.

123) Ruhkoph, H., Some dioxopyrazolidines, *Ber.*, 1940, **73**, 820.

124) Tsumaki, S., 3,5-Diketopyrazolidine derivatives, *Bull. Soc. Chem. Japan*, 1931, **6**, 1.

125) J.R. Geigy S.A., Improvements in or relating to the manufacture of derivatives of 3:5-dioxo-pyrazolidine, *B.P. 646,597*, 1950.

126) Budziarek, R., Drain, D.J., Macrae, F.J., McLean, J., Newbold, G.T., Seymour, D.E., Spring, F.S. and Stansfield M., 4-Alkyl-3:5-dioxo-1:2-diphenylpyrazolidine derivatives, *J. Chem Soc.*, 1955, 3158-3163.

127) McCloskey, A.L. and Fonken, G.S., Di-tert-butyl malonate (Malonic acid, di-tert-butyl ester), *Org. Syn.*, 1963, **Coll. Vol. 4**, 261-266.

128) Jadot, J., Alderweireldt, F., Thomas, G. and Cloostermans, G., Etude du produit d'oxydation par l'oxygene de l'air de la diphenyl-1-2 n. butyl-4 pyrazolidinedione, *Bull. Soc. Roy. Sci. Liege*, 1967, **36(5-6)**, 302-310.

129) Marvel, C.S., dl-isoleucine (α-amino-β-methylvaleric acid), *Org. Syn.*, 1955, **Coll. Vol. 3**, 495-498.

130) Franzen, G.R. and Binsch, G., The origin of anisochronism of geminal groups in conformationally mobile systems. I. Intrinsically diastereotopic nuclei, *J. Am. Chem. Soc.*, 1973, **95**, 175.

131) Norms, R.D. and Binsch, G., The origin of anisochronism of geminal groups in conformationally mobile systems. II. Intrinsic and conformational contributions in assymmetric fluoroethanes, *J. Am. Chem. Soc.*, 1973, **95**, 182.

132) Hill, R.K. and Chan, T-H, Magnetic non-equivalence of methylene protons in disymmetric benzylamines, *Tetrahedron*, 1965, **21**, 2015.

133) Rarban, H. and Mislow, K., The determination of optical purity by nuclear magnetic resonance spectroscopy. II. Compounds which owe their disymmetry to deuterium substitution, *Tetrahedron Lett.*, 1966, 3961.

134) Strube, R.E., Ethyl tert-butyl malonate (malonic acid, tert-butyl ethyl ester), *Org. Syn.*, 1963, **Coll. Vol. 4**, 417-419.

135) Frankel, M. and Berger, A., Synthesis of polyaspartic acid, *J. Org. Chem.*, 1951, **16**, 1513.

136) Hellmann, H., Teichmann, K. and Lingens, E., α-Acylaminoacrylic esters from acylaminomalonic esters, *Chem. Ber.*, 1958, **91**, 2487.

137) Stork, G. and Singh, G., Skatylmalonic ester, *J. Am. Chem. Soc.*, 1951, **73**, 4742.

138) Uhle, C., The synthesis of 5-keto-1,3,4,5-tetrahydrobenz[cd]indole. A synthesis of 4-substituted indoles, *J. Am. Chem. Soc.*, 1949, **71**, 761.

139) Widequist, S., The mononitriles of substituted succinic acids, *Arkiv. Kemi.*, 1948, **26A**, 16.

140) Patai, S., *The chemistry of carboxylic acids and esters*, Wiley (Interscience), 1969.