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"Chromatographic Characterisation of Poly(vinyl Alcohol)"

by

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A Master's Thesis

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KEYWORDS

Poly(vinyl alcohol)
Coupled Column Chromatography
Size Exclusion Chromatography
Reverse Phase Chromatography
Copolymer Characterisation

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ABSTRACT

Poly(vinyl alcohol) (PVOH), the partially hydrolysed form of poly(vinyl acetate) (PVAc), is a complex multicomponent polymer exhibiting a number of broad molecular property distributions and is therefore somewhat difficult to characterise by analytical techniques. Coupled column chromatography (CCC) is a technique whereby such a complex polymer may be characterised by cross-fractionation from one separation method to another and may be performed using size exclusion chromatography (SEC) and reversed phase chromatography (RPC) to produce a molecular size distribution superimposed onto a compositional vinyl acetate (VAc) distribution.

Aqueous SEC has been employed using a number of eluents, including standard electrolytes and ionic surfactants, but the most favourable molecular size separation was obtained with 0.25%(w/v) sodium lauryl sulphate as eluent. RPC was examined using gradient elution with water/tetrahydrofuran (THF), and was found to separate PVOH according to composition. Fast gradients (>10%THF/minute) indicated a broad distribution of composition, which proved to be narrower for random polymer compared to blocky polymer. Slow gradients (<1%THF/minute) suggested that this was not a gradual compositional change but rather discrete fractions of similarly hydrophobic material.

A coupled system incorporating aqueous SEC followed by fast gradient RPC showed that within the molecular size distribution there existed a compositional distribution such that hydrophobicity decreased slightly (i.e. the degree of hydrolysis or sequence length

increased) with decreasing molecular weight. It was found that a coupled technique proved only to be applicable to PVOH with an average degree of hydrolysis less than 80% hydrolysed.

CHAPTER 1 - INTRODUCTION

Partially hydrolysed poly(vinyl alcohol) (PVOH), produced from poly(vinyl acetate) (PVAc), represents an important class of water soluble polymers used extensively in textile and paper treatments, adhesive technology and as emulsion stabilisers [1]. Material selection for these applications requires an understanding of the molecular properties of the polymer, such as molecular weight, degree of hydrolysis and sequence length. However, quantification of these parameters by analytical techniques proves difficult due to the polymer's complex nature. Existing methods have required an initial preparatory step such as reacetylation prior to analysis in order to minimise any compositional inhomogeneities, but these do not give a true reflection of the polymer properties, and their inter-relation. Separations by liquid chromatography techniques appear to be useful in this type of characterisation since separation mechanisms based on molecular size and composition may be achieved. Furthermore, the combination of different separation techniques in a coupled column chromatography (CCC) system has a great deal of potential for a fuller characterisation.

In this study size exclusion chromatography (SEC) has been used to investigate the molecular weight distribution of PVOH samples. In such work the polymer is separated according to molecular size in solution. This molecular size depends greatly upon the eluent being used and the method development involved the study of different eluent systems, to be applicable to a wide range of degrees of hydrolysis.Compositional separation

based upon hydrophobicity may be achieved by reversed phase chromatography (RPC) and literature suggests that gradient elution can enhance the resolution of chemically similar species.

Whilst both SEC and RPC techniques yielded useful information in their own right, a far more detailed analysis of PVOH required the developement of a CCC system, to produce information relating molecular size and compositional distributions.

CHAPTER 2 - LITERATURE SURVEY

2.1 POLY(VINYL ALCOHOL)

Unlike other vinyl polymers, PVOH cannot be made by the polymerisation of its monomer; attempts to hydrolyse vinyl acetate into vinyl alcohol result in its rearrangement product acetaldehyde. Alternative methods have therefore been required, and by far the most successful is the hydrolysis of poly(vinyl acetate) (PVAc) with acid or alkali catalysts [1].

Completion of this reaction results in fully hydrolysed PVOH. However, careful control of the reaction can lead to the production of PVOH with residual acetate groups. Far from being impure, these grades are highly useful polymers in their own right. Referred to as partially hydrolysed PVOH, they are in reality a copolymer system of vinyl alcohol and vinyl acetate. There are fundamental differences between partially and fully hydrolysed grades, affecting their usage. Partially hydrolysed PVOH is preferred to fully hydrolysed as a protective colloid in emulsion polymerisation since it produces finer emulsions of high viscosity (because of its greater interfacial activity) and improves the compatibility of the product with pigments and inorganic salts. However, for fibre sizing applications, although partially hydrolysed grades are easier to wash out, fully hydrolysed PVOH is preferred as it does not have a tendency to foam [1].

Vinyl acetate monomer may be prepared by the oxidation of ethylene in the presence of acetic acid, and is polymerised by a free-radical initiator in methanol in the temperature range 40-70°C [2].

The molecular weight of the PVAc (and hence the PVOH after hydrolysis) is dependent upon temperature, feed rate, solvent concentration and reactor residence time. Careful control of these parameters, coupled with precision hydrolysis, results in a product perfectly suited to a particular application; however, it is very difficult to control these properties within the desired range [3].

Partially hydrolysed PVOH is not unlike other copolymer systems, in that it is a complex polymer with broad molecular property distributions dependent upon the production history. Whilst the molecular weight and the degree of hydrolysis may be controlled during polymerisation and hydrolysis respectively, other properties, such as sequence length (blockiness), tacticity and stereoregularity are dependent upon reaction mechanisms. The latter two physical properties are established during polymerisation and affect the polymers resistance to water and the swellability of its film in water respectively.

The sequence length, or blockiness, is dependent upon the method of hydrolysis, the most important aspect being the nature of the catalyst. PVOH can be prepared by the alcoholysis of PVAc in methanol or hydrolysis in water; with both, the use of an alkaline catalyst such as sodium hydroxide or sodium methoxide results in a blocky polymer (long sequences of similar monomer units). Random PVOH (monomer units arranged statistically, i.e. shorter sequence lengths) is produced using mineral acid as a catalyst [4]. The blockiness can be investigated using nuclear magnetic resonance spectroscopy (NMR).

In order to ascertain the blockiness of a PVOH polymer, the methylene carbon atom must be considered. The resulting spectrum consists of three peaks corresponding to the three possible chain sequences (i.e. whether the two neighbouring methine carbons have hydroxyl groups, acetate groups or a combination of the two attached) which can be used to calculate relative block lengths [5].

Blockiness of PVOH samples is an important consideration; in its application as an emulsion stabiliser it is found that the efficiency of the PVOH increases with blockiness [6]. However, it is also found that the more blocky the PVOH is, the less compatible it is with hydroxypropyl methylcellulose, a dispersant often used in conjunction with PVOH for poly(vinyl chloride) polymerisation [7].

Fractionation experiments have shown that the intrinsic viscosity in water of PVOH increases with increasing molecular weight and also with degree of hydrolysis [8], and that a relationship between intrinsic viscosity, degree of hydrolysis and viscosity-average molecular weight can be calculated from quantitative analysis of the Mark-Houwink equation:

$$[n] = K.M_v^a$$

where

[n] is intrinsic viscosity

M_v is viscosity-average molecular weight

K and a are constants for a given PVOH in a defined aqueous solvent at a fixed temperature

In the majority of its applications, PVOH is used in aqueous solutions; for partially hydrolysed polymer, its

solubility depends upon the degree of hydrolysis and also the temperature. Although its hydroxyl groups are hydrophilic, and any residual acetate groups are hydrophobic, solubility increases, at ambient temperature, with decreasing degree of hydrolysis [9]. This is because residual acetate groups reduce the extent of hydrogen bonding between hydroxyl groups, allowing the polymer to dissolve. However, at elevated temperatures the solubility increases with increasing degree of hydrolysis. This is because the heat breaks down the weak hydrogen bonds, freeing hydrophilic hydroxyl groups to help dissolve the polymer; the presence of hydrophobic acetate groups will impede solubility.

Many papers concerning PVOH have suggested association in solution[10][11][12][13]. This has recently been explained as the interaction of a minor content of hydrophobic monomer units, namely acetate groups [14][15]. The addition of a surfactant to such solutions has been shown to lead to dissociation of these micellar-like associates due to polymer-surfactant binding. The tendency to bind was shown to increase with decreasing degree of hydrolysis, suggesting that dissociation was due to interaction between the surfactant and acetate groups [13]. Further studies indicated the nature of this interaction to be a complex formation rather than micellization and that binding increased with increasing surfactant concentration, up to the critical micelle concentration (cmc); the tendency to bind was dependent upon the surfactant used [16].

2.2 SIZE EXCLUSION CHROMATOGRAPHY

SEC is governed by the equilibrium of solute molecules between a mobile phase and a porous column packing; separation of these molecules is according to their size in solution and hence their ability to diffuse through the porous matrix [17][18][19][20][21]. The column is packed with material of narrow particle size distribution and controlled pore size, referred to as the stationary phase. A mobile phase, or eluent, is pumped through the column at a constant rate and a dilute solution of the sample is injected into the eluent flow via an injection valve.

For a given stationary phase pore size there will be a molecular size, above which solute molecules are unable to enter the porous structure, known as the exclusion limit. Molecules larger than this limiting size are said to be excluded and flow straight through the column. There will also be a molecular size, below which it is almost impossible for the column to resolve different sizes, known as total permeation. Between these two limits, the time taken for a molecule to flow through the column, known as retention time, is a function of its size in solution and is found to increase with decreasing molecular size.

The concentration of species eluting from the column is continuously monitored by means of an on-line detector. There are various types of detector available, but the most commonly used are differential refractometers (RI detector) and ultra-violet photometers (UV detector). An RI detector measures the difference between the refractive indices of the eluting solution and the pure solvent. Since many solutes have a

different RI to the solvent being used it is a widely used detector for SEC. A UV detector, on the other hand, is limited in its applications since it monitors a fixed wavelength of UV energy and requires the sample to have a chromophore which absorbs at this wavelength, and a non-interfering solvent. Hence despite the UV detector being less sensitive to temperature and pressure variations, the wider application of the RI detector leads to it being more often used [22].

Whichever type of concentration detector is used, the resulting chromatogram shows the distribution of solute concentration with retention time. In order to obtain a molecular weight distribution from the resulting peak, various calculations are necessary [19][21][23][24], including an all important calibration technique which converts retention times into molecular weights. This is done by recording the elution times of a series of narrow molecular weight distribution samples. These have a known molecular weight at their peak retention time, and so a relationship between retention time and molecular weight may be calculated, in the form of a calibration curve. However, there are only a limited number of polymer types for which such narrow molecular weight distribution samples are available; for other polymer types, an available "standard" of similar molecular properties must be used, and results expressed as "equivalent" molecular weights, unless, that is, Mark-Houwink coefficients for the polymer-solvent system are known in which case the universal calibration technique may be used [19][21][23][24].

However, also now available are techniques whereby no calibration is required in order to obtain good

results. On-line molecular weight-sensitive detectors, such as low angle laser light scattering (LALLS) and viscometry detectors, measure a parameter from which a molecular weight value can be calculated [22].

For a given sample, before a molecular weight distribution (MWD) can be produced, a number of operating parameters need to be considered. Of paramount importance is the choice of solvent, but also of great interest are flow rate, sample volume and sample concentration.

As the flow rate is decreased, so the resolution between consecutive peaks is improved, resulting in longer run times. Optimum flow rate must balance out the need for good resolution with quick analysis, taking into account that high molecular weight samples require low eluent velocity to maintain resolving power, due to reduced mass-transfer through the porous structure. Sample volume must be kept to a minimum to reduce band-broadening which affects resolution. Sample concentration is limited due to its effect on the viscosity of a polymer solution; a high viscosity in the column affects mass-transfer and imparts band-broadening effects. These reduce resolution and can lead to peak splitting or even shear degradation of the polymer. Since viscosity increases with molecular weight, this loss of resolution is less important for low molecular weight samples, allowing increased concentration [19].

In SEC, retention and resolution are generally determined by the stationary phase, and so choice of mobile phase is usually determined by sample solubility, or in some cases solvent viscosity. There are two types of SEC, distinguished by the nature of the eluent, namely aqueous or organic.

The selection of mobile phase is much more important in aqueous SEC, since interaction between the sample and stationary phase is more likely [25]. Ideally, the stationary phase must be highly hydrophilic but also charge-free since many water soluble polymers have an associated charge. Ionic interaction, either adsorption or exclusion, between the sample and matrix leads to erroneous results. It is, however, very difficult to produce a charge-free matrix; in addition, the matrix may contain non-polar sites which encourage adsorption of hydrophobic polymer. Ionic interaction can be suppressed by the addition of a salt/buffer system to the water or by its pH adjustment, whereas hydrophobic interaction can be eliminated by the addition of an organic modifier (e.g. methanol).

PVOH is regarded as a non-ionic water soluble polymer, but other results have shown that commercially available products do contain charged components [26]. As the degree of hydrolysis is decreased, so the hydrophobicity of the sample increases, due to increased acetate content. These two factors may lead to sample-matrix interaction, depending on the stationary phase, with pure water as eluent; suppression of this requires solvent modification. Since PVOH also has a tendency to form associations in aqueous solutions, characterisation of its MWD has usually been by organic SEC of a reacetylated sample using THF as eluent [27]. However, PVOH has been successfully characterised using unmodified porous silica with 50/50 v/v 0.025M tetramethylammonium nitrate/methanol as eluent [28]. Ion exchange was inhibited by maintaining the pH at 3.0 thus reducing SiOH ionisation (although this increases the number of available hydrogen bonding sites).

Aqueous SEC of PVOH has also been performed using a polymer based support matrix with 0.1M sodium nitrate as eluent [29], and a method using low-angle laser light scattering (LALLS) in association with aqueous SEC to characterise PVOH has been proposed [26]. Modifications to this method, incorporating multi-angle laser light scattering (MALLS) and differential viscometry have been reported [27].

In SEC, separation is achieved according to molecular size, and hence accurate MWD characterisation relies on their being little variation in molecular size at constant molecular weight. For linear homopolymers this assumption is true but for copolymers, samples of similar molecular weight may have a different molecular size due to a variation in a compositional distribution. The elution characteristics of a copolymer, such as PVOH, will therefore vary depending upon both its MWD and chemical composition distribution (CCD). Methods of characterisation of both MWD and CCD include the use of a UV-RI dual-detector system [30]. This requires one of the copolymer constituents to have a UV chromophore, and also calibration of response factors using the respective homopolymers. In the case of PVOH, although the residual acetate groups have a UV chromophore, PVAc is insoluble in aqueous solution and so this method is unworkable.

2.3 LIQUID CHROMATOGRAPHY

LC is governed by the ability of the support matrix to retain solute molecules to a varying extent dependent upon the physical properties of the sample. This adsorption may be due to hydrogen bonding, coulombic or

solvophobic interaction, and in some cases a combination of these [20][21].

In normal phase mode, a polar stationary phase is used with gradient elution from a non-polar to polar solvent.

Hydrophobic interaction chromatography (HIC) is a method used to separate solute molecules due to their polarity. This is usually achieved by applying gradient elution, whereby the composition of the mobile phase flowing through the system is gradually altered.

In reversed phase mode the gradient elution is from a polar to non-polar solvent with a non-polar stationary phase [31].

For proteins it has been shown that a strong interaction occurs with a stationary phase based on polystyrene/divinyl benzene (PS/DVB) and that desorption occurs for a specific solvent composition dependent upon sample hydrophobicity [32]. For PVOH, adsorption to a polystyrene latex has been shown to increase with a decrease in the solvency of the medium [33], water being a better solvent as the degree of hydrolysis increases; adsorption was also found to increase with a decrease in the polarity of the latex [34], suggesting that it was due to hydrophobic interaction between the latex and any acetate groups. This all suggests that liquid chromatography of PVOH may lead to separation according to hydrophobicity, and hence degree of hydrolysis.

In the field of polymer characterisation, LC has been used to analyse poly(methyl methacrylate) in both normal and reversed phase modes, separation being found to depend upon tacticity, although there also appeared to be some size exclusion [35].

The mechanisms involved in the separation of large molecules by gradient elution remains the subject of some debate [36][37]. Solute molecules may have a greater affinity to the column packing than the solvent, in which case they are adsorbed by the matrix, desorption only occurring as and when the composition of the solvent is "good" for the sample in question. However, sample molecules may have no affinity to the matrix, but since they travel through the column at greater velocity than the solvent, they will flow into regions of "poor" solvent and be precipitated onto the matrix. Redissolution of this requires time for the "good" solvent to catch up. It has been shown that adsorption usually occurs for small, soluble molecules whereas large, less-soluble molecules undergo a precipitation-redissolution process. However, there is no reason to suspect a single model applies for any given sample, but rather a combination of the two.

Gradient elution is the altering of mobile phase with time. This may be either a gradual or step-wise change in any solvent property (e.g. polarity or acidity) and is achieved by mixing two or more solvents prior to sample introduction, at either high or low pressure [31][38].

The need for gradient elution in the separation of large molecules has been a major factor in the development of an alternative detector for liquid chromatography. An RI detector cannot be used with a gradient unless a dual-column system is set up to constantly change the reference, but this still suffers from poor temperature stability; hence there has been no suitable detection method available for polymers without a usable chromophore for ultra-violet detection. Methods

in which the solvent is removed before detection are a possible alternative and such a technique is the basis of the evaporative light-scattering detector (often referred to as a mass detector) [22]. With this detection the eluent is atomised in a nebuliser and the resulting vapour stream enters a heated chamber where volatile solvent is evaporated. Solute molecules, less volatile than the solvent, exist as a cloud of fine particles. The light scattered by this cloud is measured by a photomultiplier, and is proportional to solute concentration. Because of its evaporative mechanism, this detector is limited to non-volatile samples (relative to the eluent) but is insensitive to ambient temperature variations and can be used with gradient elution. Polymer adsorption chromatography of poly(alkylacrylate) and poly(alkylmethacrylate) homopolymers and copolymers has been carried out using a mass detector, other detection methods being non-applicable [39].

An extreme case of adsorption chromatography has been reported for the separation of functional group-containing polymers. Polymer characterisation at the critical point of adsorption operates at an eluent composition between exclusion and adsorption and is independent of molecular weight. It requires the use of a precise binary eluent which will interact with functional groups but not the rest of the polymer chain. In effect the polymer chain becomes invisible, and this method has been used in the analysis of block copolymers where selective interaction with just one of the constituents occurs [40].

2.4 COUPLED COLUMN CHROMATOGRAPHY

Coupled column chromatography (CCC), sometimes referred to as orthogonal chromatography, is a method of characterising complex polymers using two separation techniques in sequence and may be used in cases where SEC or adsorption chromatography on their own prove problematic [41][42].

Since SEC separates solutes according to their size in solution, it relies upon a relationship between molecular size and molecular weight in order to characterise a polymer. However, many polymer systems exhibit a variation in molecular weight for a given molecular size due to a superimposed chemical composition distribution, hence for such polymers SEC does not give adequate information or accurate results.

Adsorption chromatography is used extensively for the separation of small molecules according to composition. However, separation of large molecules by such a method is more difficult; to avoid any separation due to size a very small pore size must be chosen such that no molecule may enter; however, a small pore size may lead to poor resolution. To improve resolution the pore size may be increased, but this leads to size exclusion effects. The use of a very large pore size such that all molecules may enter is a possibility for improved resolution, and minimal size exclusion, whilst maintaining a high surface area for interaction.

Complex polymers such as copolymers or blends may be separated by SEC or adsorption chromatography and fractions collected for re-analysis. However, this will usually only yield an average composition for a given molecular size from SEC, or an average molecular weight

for a given functionality from adsorption chromatography. For an accurate characterisation of a polymer the re-analysis method must yield a distribution of the relative property. By combining SEC and adsorption chromatography in a coupled column system this is possible.

Coupling the two techniques can be off-line where fractions from one are re-injected onto the other, or can be on-line via a switching valve which selectively re-directs the eluent of one onto the other. On-line coupling is preferred although in some cases it is not possible due to either poor solvent compatibility or low solute concentration. In such cases an off-line technique must be employed with some method of intermediate treatment.

A coupled system has been used to identify additives in compounded rubber which proved difficult to analyse using a single separation. This involved SEC followed by RPC and comparison of the samples with known standards. This method was also used to monitor pesticide content in vegetable matter and limonin content in grapefruit peel [43].

A coupled system consisting of three interconnected separations has been used for the determination of polycyclic aromatic hydrocarbons in coal liquids and oils. Low-resolution RPC was followed by SEC to provide fractions for the final high-resolution RPC step. In this method the preliminary low-resolution RPC step was simply required as a clean-up step to limit the number of compounds and hence improve resolution in the final analysis [44].

Styrene-methyl methacrylate random copolymer has been characterised using a coupled system employing

adsorption chromatography followed by SEC. This resulted in separation according to chemical composition followed by molecular weight analysis of individual fractions [45].

Styrene-n-butyl methacrylate copolymer has been characterised using a coupled system comprised of SEC followed by adsorption chromatography, in both normal and reversed phase modes; separation in the secondary stage was achieved according to copolymer composition, although size exclusion effects were apparent [46].

The size exclusion effects present in adsorption chromatography have been assumed to be disadvantageous to complex polymer characterisation by CCC. However, in some cases it has been shown that the change in a molecule's size with a change in solvent can actually improve secondary stage resolution [47].

A coupled system incorporating SEC and adsorption chromatography at the critical point of adsorption has been used to characterise 1,3,6-trioxocane polymers synthesised in the presence of benzyl alcohol (which have various functional end-groups). An estimate of molar mass and functionality distributions was obtained with analytical SEC following a preparative "critical" adsorption step [48].

CHAPTER 3 - EXPERIMENTAL

PVOH samples were derived from a single source of PVAc by alcoholysis in a methanol/methyl acetate medium, with an alkaline catalyst to produce blocky polymer and an acid catalyst to produce random polymer; the degree of hydrolysis was determined by a titration method involving hydrochloric acid and sodium hydroxide [49] (all performed by Harlow Chemical Company, UK). The blockiness factor of these polymers was determined by ^{13}C NMR spectroscopy [5] (performed by European Vinyl Corporation, UK).

Solutions for both SEC and RPC analysis were prepared by stirring an accurately weighed sample of the polymer in eluent and heating to 90°C for dissolution.

SEC analysis was carried out using a system comprising a model 64 pump, a model 98 refractive index detector (both Knauer, Germany) and a model 7125 injection valve (Rheodyne, USA). The first columns used (2 in series) were polymeric based with a particle size of 8µm and an exclusion limit of 200,000 relative to polyethylene oxide (PL aquagel-OH 40 8µm 300 x 7.5mm, Polymer Laboratories, UK). Further analysis used columns with the same particle size but a higher exclusion limit of 1,000,000 (PL aquagel-OH 50 8µm 300 x 7.5mm). An eluent flow rate of 1.0 ml/min was used and samples were analysed in various eluent systems, with an injection volume of 200µl and sample concentration of 0.5%(w/v). The eluent modifiers $NaNO_3$, NaH_2PO_4, methanol, ammonium formate, sodium lauryl sulphate (all Fisons, UK), Aerosol OT and Aerosol IB45 (Cyanamid, UK) required no special treatments and were used as supplied.

RPC analysis was carried out using a gradient system comprising two model 64 pumps controlled by a model 50 HPLC programmer, a dynamic mixing chamber (all Knauer, Germany), a model 7125 injection valve (Rheodyne, USA), and a model PL-EMD 950/14 evaporative mass detector (Polymer Laboratories, UK). The column used was a polymeric based reversed phase packing of polystyrene/divinylbenzene with a particle size of 8µm and a pore size of 4000Å (PLRP-S 8µ 4000Å 50 x 4.6mm, Polymer Laboratories, UK). An eluent flow rate of 1.0 ml/min was used throughout and samples were analysed at room temperature using various linear gradients of water/THF, with an injection volume of 50µl and sample concentration of 0.2%(w/v) (fast gradient) or 1ml and 0.5%(w/v) (slow gradient). UHP water and HPLC grade unstabilised THF (Fisons, UK) were used throughout. The mass detector was operated at an evaporation temperature of 90°C using compressed air as nebuliser gas at a flow rate of 16 l/min.

A CCC system was produced by linking the SEC and RPC techniques together by a model 7010 switching valve (Rheodyne, USA) which allowed a fraction from the primary separation to be loaded on to the secondary separation.

The two techniques had to be slightly modified to allow coupling. With RPC as the primary separation, the mass detector was replaced by the SEC system and only one SEC column used to reduce cross-fraction analysis time (FIGURE 1). An RPC gradient of 99/1 to 40/60 (v/v) water/THF in 150 minutes was used and cross-fractionation switching times were estimated from a previous RPC analysis, the fraction volume being 500µl.

With SEC as the primary separation the RPC system was put in series after the RI detector (FIGURE 2). The

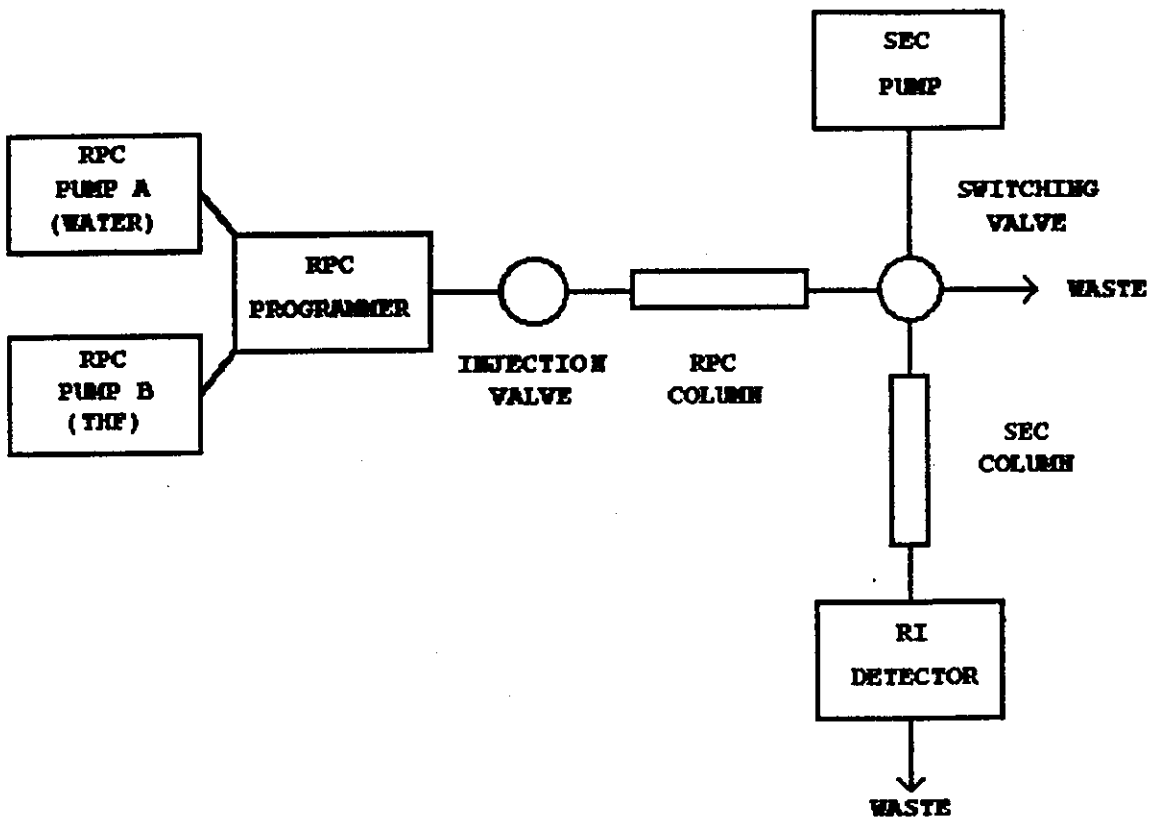


FIGURE 1 Proposed CCC system with RPC preceding SEC

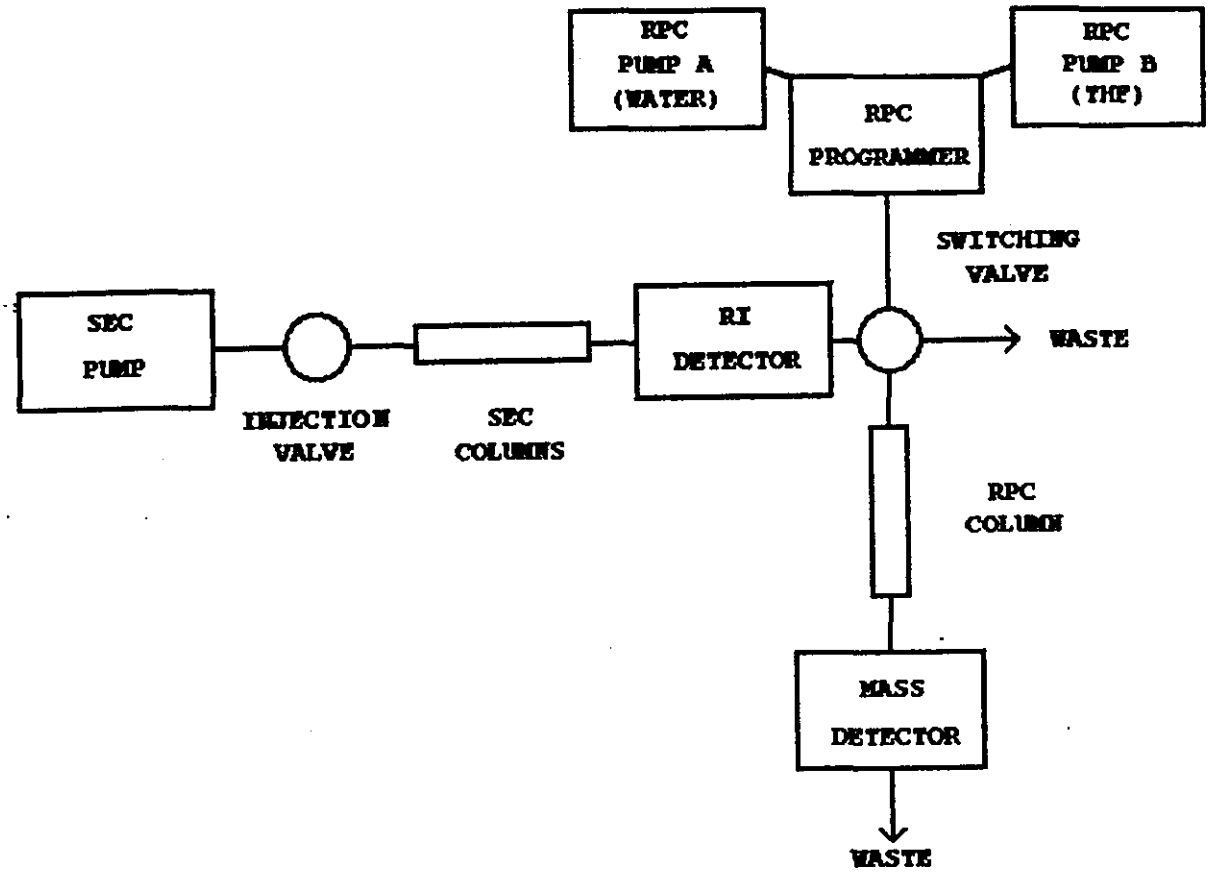


FIGURE 2 Proposed CCC system with SEC preceding RPC

two modes of analysis were discontinuous (one RPC analysis per SEC analysis, repeated injections) and continuous (many RPC analyses per SEC analysis, no repeated injections).

In discontinuous mode, an RPC gradient of 99/1 to 1/99 (v/v) water/THF in 10 minutes was used to analyse a fraction volume of 200µl switched from normal SEC.

In continuous mode the SEC flow rate was reduced to 0.2 ml/min to increase the time covered by the peak. The RPC gradient was altered so that it could be run and reconditioned several times within this time period. A step gradient was chosen such that any impurity peak eluted prior to any of the sample, so an RPC gradient of 65/35 to 30/70 (v/v) water/THF in 90 seconds was run, after holding at 65/35 for 30 seconds, to analyse a fraction volume of 200µl. The speed of this gradient proved too fast for the pumps to manage, so data was collected for 4 minutes. The eluent was allowed to recondition to 65/35 water/THF whilst the next fraction was being collected in the loop of the switching valve.

The signals from all detectors were collected and analysed using a PL Caliber Workstation (Polymer Laboratories, UK).

CHAPTER 4 - RESULTS AND DISCUSSION

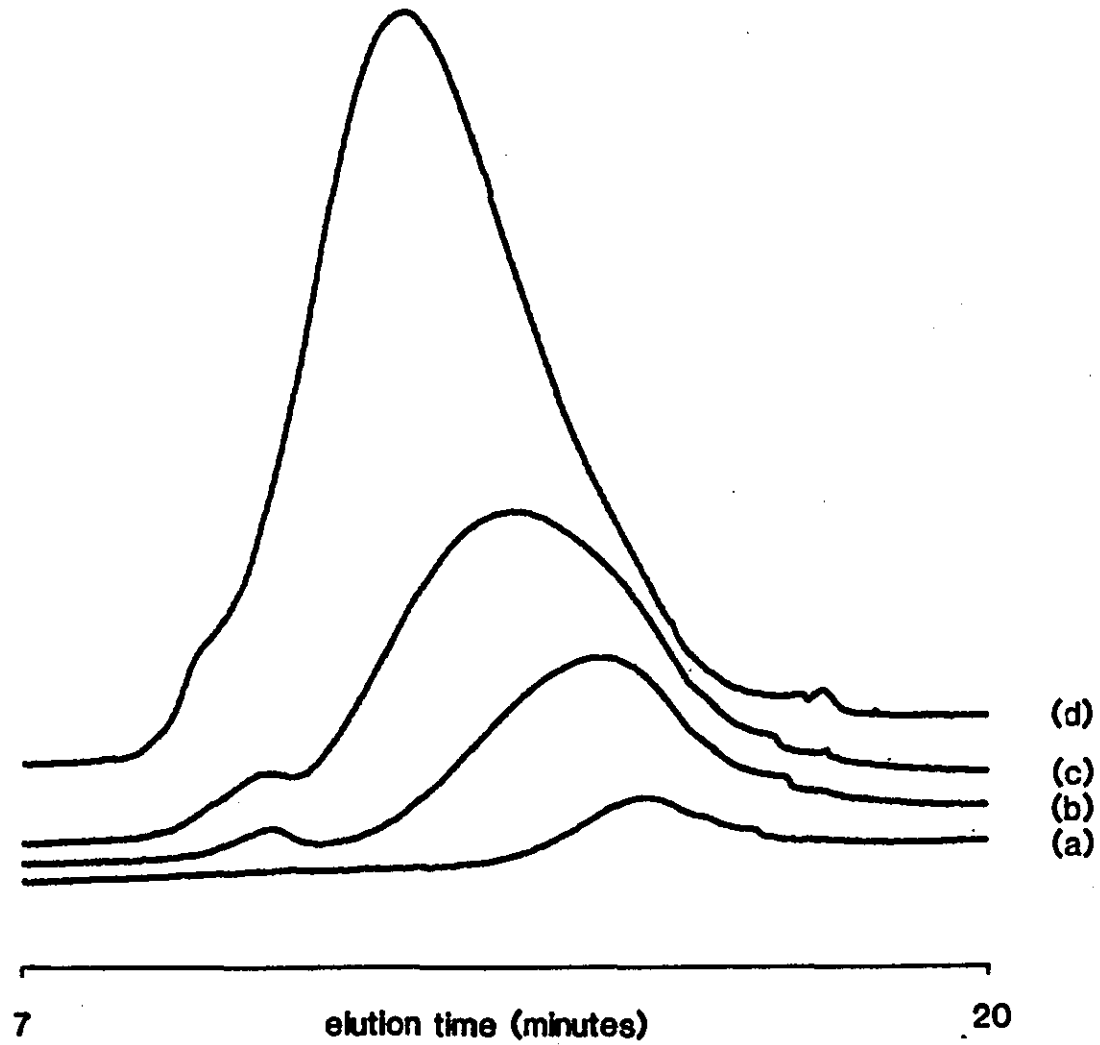
Prior to setting up a coupled system for the analysis of PVOH, the individual methods of SEC and RPC were investigated. Although the primary objective of these was to produce separations according to size and hydrophobicity respectively, an important consideration was the compatibility of the two eluent systems, as this would greatly affect any coupled sytstem.

4.1 SIZE EXCLUSION CHROMATOGRAPHY

Specially prepared samples covering a range of degree of hydrolysis (TABLE 1) were analysed by aqueous SEC with water as eluent. All of these samples were prepared from the same parent PVAc polymer and therefore had, within this range of degree of hydrolysis, similar molecular weights, and ought to produce similar SEC chromatograms. The resultant chromatograms, for partially hydrolysed grades, exhibited excluded peaks, total permeation peaks and increasing retention time with decreasing degree of hydrolysis (FIGURE 3). In true size exclusion these would be caused by molecules larger than the exclusion limit and a variation in molecular size, respectively. However some PVOH molecules may have been ionically excluded, and the variation in retention time may be caused by hydrophobic interactions. The hydrophobicity of the polymer increases with increasing acetate content (i.e. decreasing degree of hydrolysis) and hence any hydrophobic interaction would increase accordingly. The increasing acetate content may also

SAMPLE NAME	DEGREE OF HYDROLYSIS (%)	VISCOSITY (cP)	HYDROLYSIS TECHNIQUE
PLS 342	100	9.5	ALKALINE
PLS 344	67.6	(not measured)	ALKALINE
PLS 345	72.2	10.7	ALKALINE
PLS 346	77.7	8.9	ALKALINE
PLS 347	80.0	9.0	ALKALINE
PLS 348	83.4	9.5	ALKALINE
PLS 349	86.7	9.9	ALKALINE
PLS 350	90.6	9.8	ALKALINE
PLS 362	69.6	18.7	ALKALINE
PLS 363	73.7	12.6	ALKALINE
PLS 364	79.6	10.3	ALKALINE
PLS 365	83.6	10.1	ALKALINE
PLS 366	87.3	10.2	ALKALINE
PLS 367	89.8	10.7	ALKALINE
PLS 368	91.8	11.7	ALKALINE
PLS 370	82.9	4.7	ALKALINE
PLS 371	84.5	7.8	ALKALINE
PLS 379	81.2	34.7	ALKALINE
PLS 380	75.6	6.1	ACID
PLS 381	84.6	10.6	ACID
PLS 382	89.1	8.8	ACID
PLS 383	93.9	8.5	ACID
PLS 384	72.2	6.4	ACID
PLS 385	97.2	9.8	ACID

TABLE 1 Properties of the specially prepared PVOH samples



2 x PL aquagel-OH 40 columns and RI detector

FIGURE 3 SEC chromatograms for PVOH samples with different degrees of hydrolysis: (a) 77.7%; (b) 83.4%; (c) 86.7%; (d) 90.6%, using pure water as eluent.

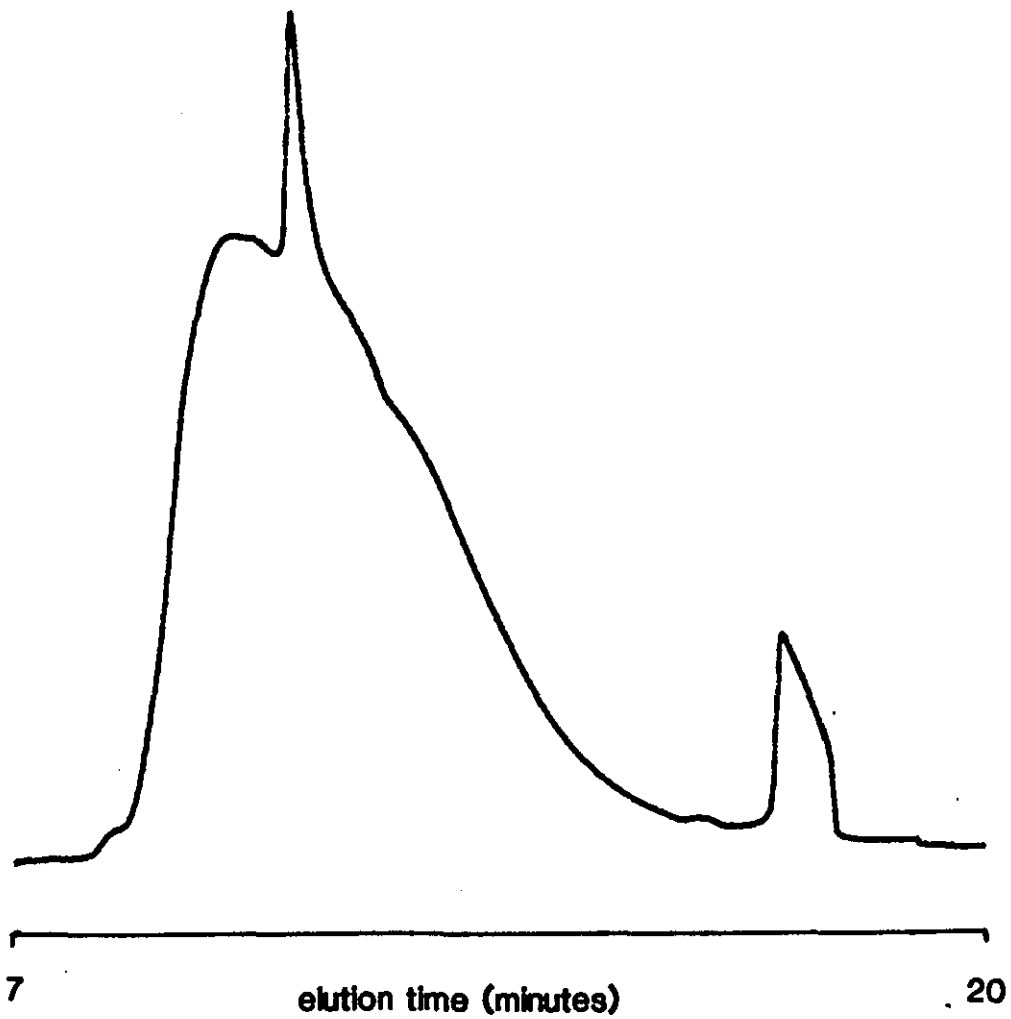
lead to a reduction in size due to intra-molecular attraction.

From these and subsequent peaks (FIGURES 3 to 14), it was evident that any peak behaviour exhibited before less than about 9 minutes was due to exclusion, and any peak behaviour exhibited after about 18 minutes was caused by total permeation.

Analysis of a fully hydrolysed sample produced a curious shaped chromatogram (FIGURE 4) which eluted at a time similar to that of the excluded peaks of the partially hydrolysed samples (in figure 3). The peak shape was found to be non-reproducible with repeated injections although retention times remained similar. The exact cause of the spiked peaks at high and low molecular size was not understood (FIGURE 4).

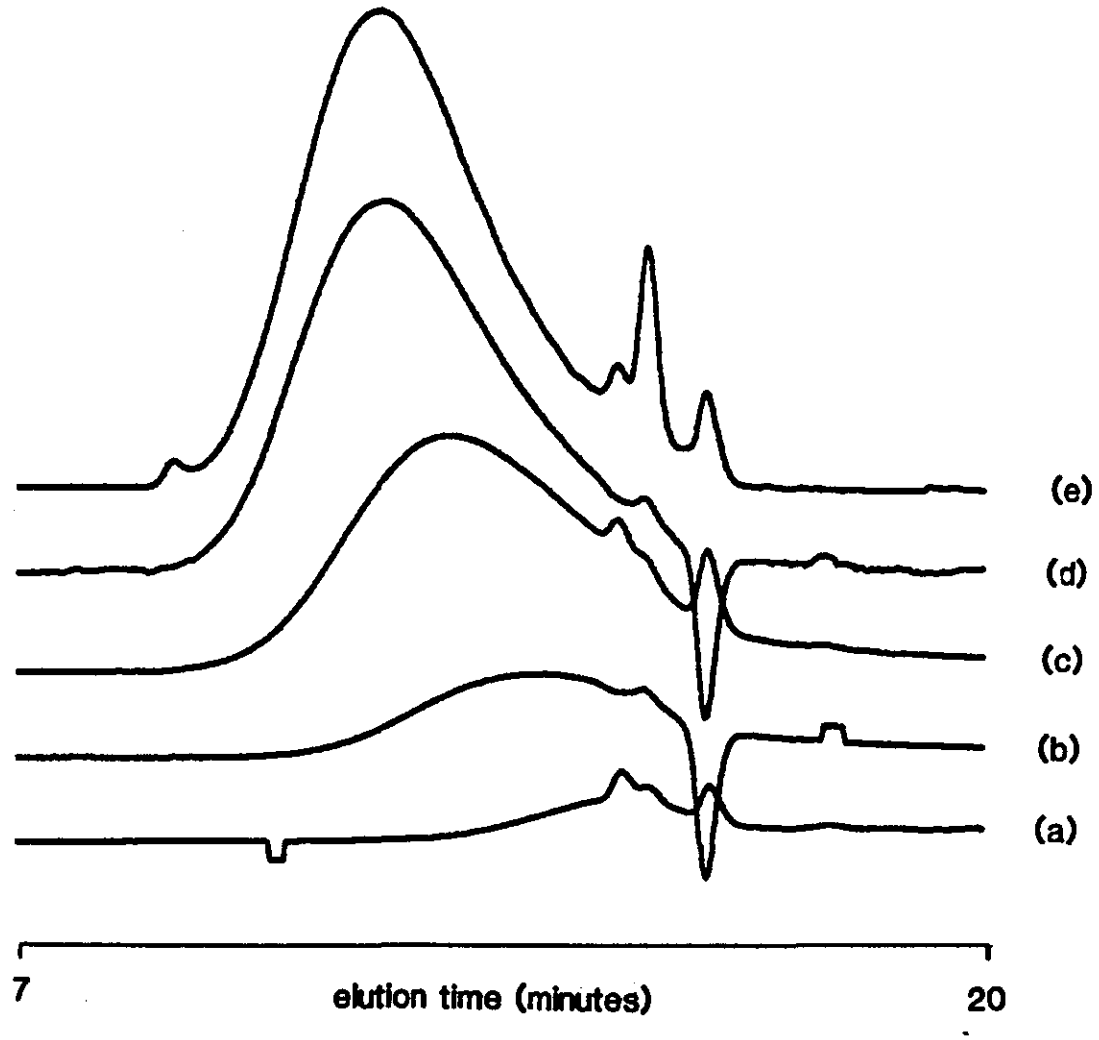
All of these observations using pure water as eluent suggested that eluent modification would be necessary to ensure separation by a true size exclusion mechanism.

The eluent was modified to a 0.2M $NaNO_3$, 0.1M NaH_2PO_4, pH7 buffer solution, which introduced charged species into the mobile phase. These charged species inhibit ionic interactions by screening any charges in the sample or on the gel matrix. The resultant chromatograms showed no excluded peaks, except in the case of the fully hydrolysed sample (FIGURE 5); this would tend to suggest that the excluded peaks previously observed were caused by ionic exclusion [25]. Increasing retention times with decreasing degree of hydrolysis were still observed, which indicates that molecular size is not reduced by intra-molecular ionic interactions as the charged species would inhibit this, and that hydrophobic interactions either reduce molecular size or increase retention.



2 x PL aquagel-OH 40 columns and RI detector

FIGURE 4 SEC chromatograms for a fully hydrolysed PVOH sample, using pure water as eluent.



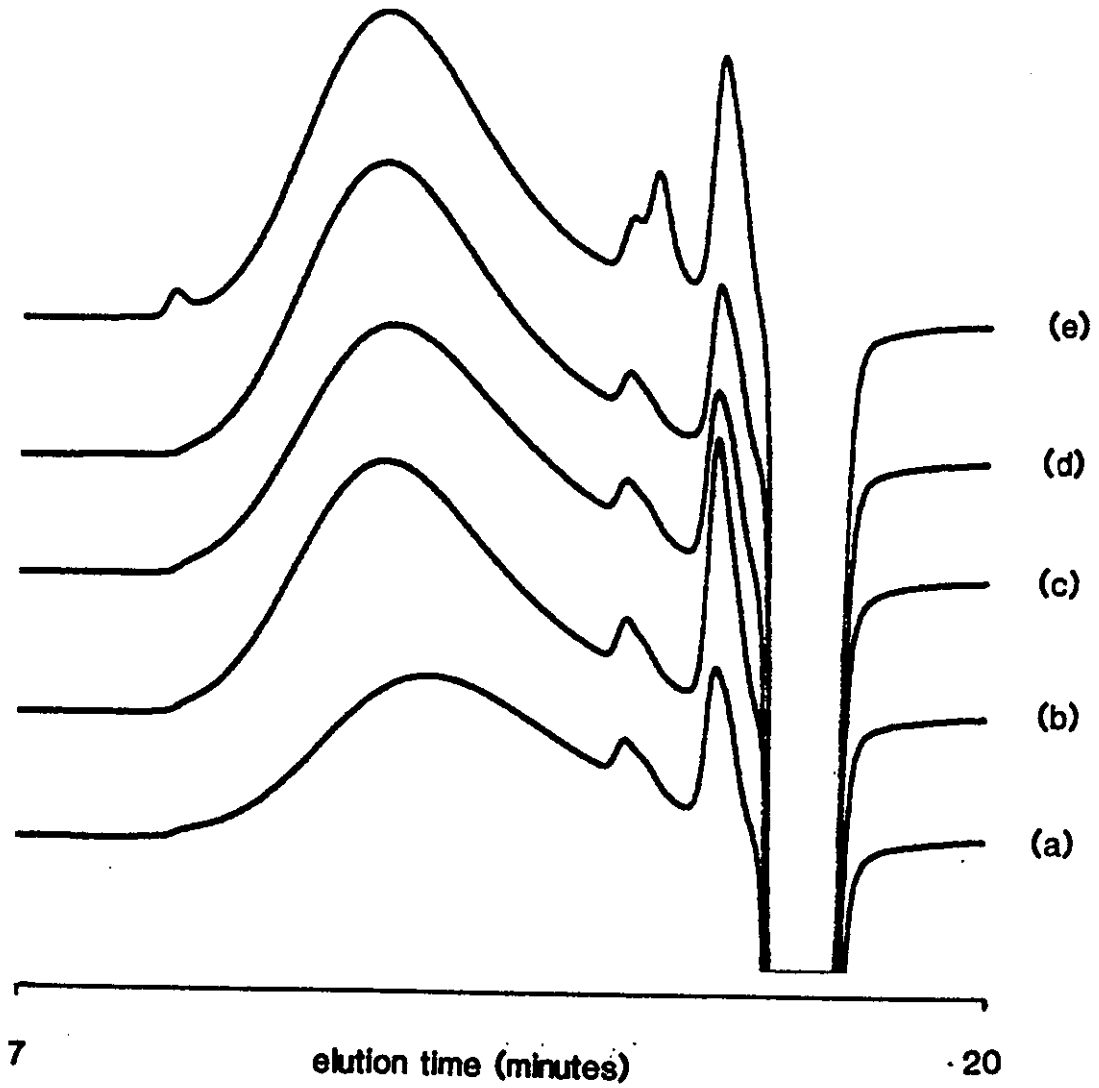
2 x PL aquagel-OH 40 columns and RI detector

FIGURE 5 SEC chromatograms for PVOH samples with different degrees of hydrolysis: (a) 77.7%; (b) 83.4%; (c) 86.7%; (d) 90.6%; (e) 100%; with 0.2M $NaNO_3$, 0.1M NaH_2PO_4, pH7 buffer as eluent.

The fully hydrolysed sample no longer exhibited strange chromatographic behaviour, which suggests that it was caused by some means of ionic interaction, or possibly poor solubility. It did still have an excluded peak, however, maybe because there were no acetate groups present to reduce molecular size.

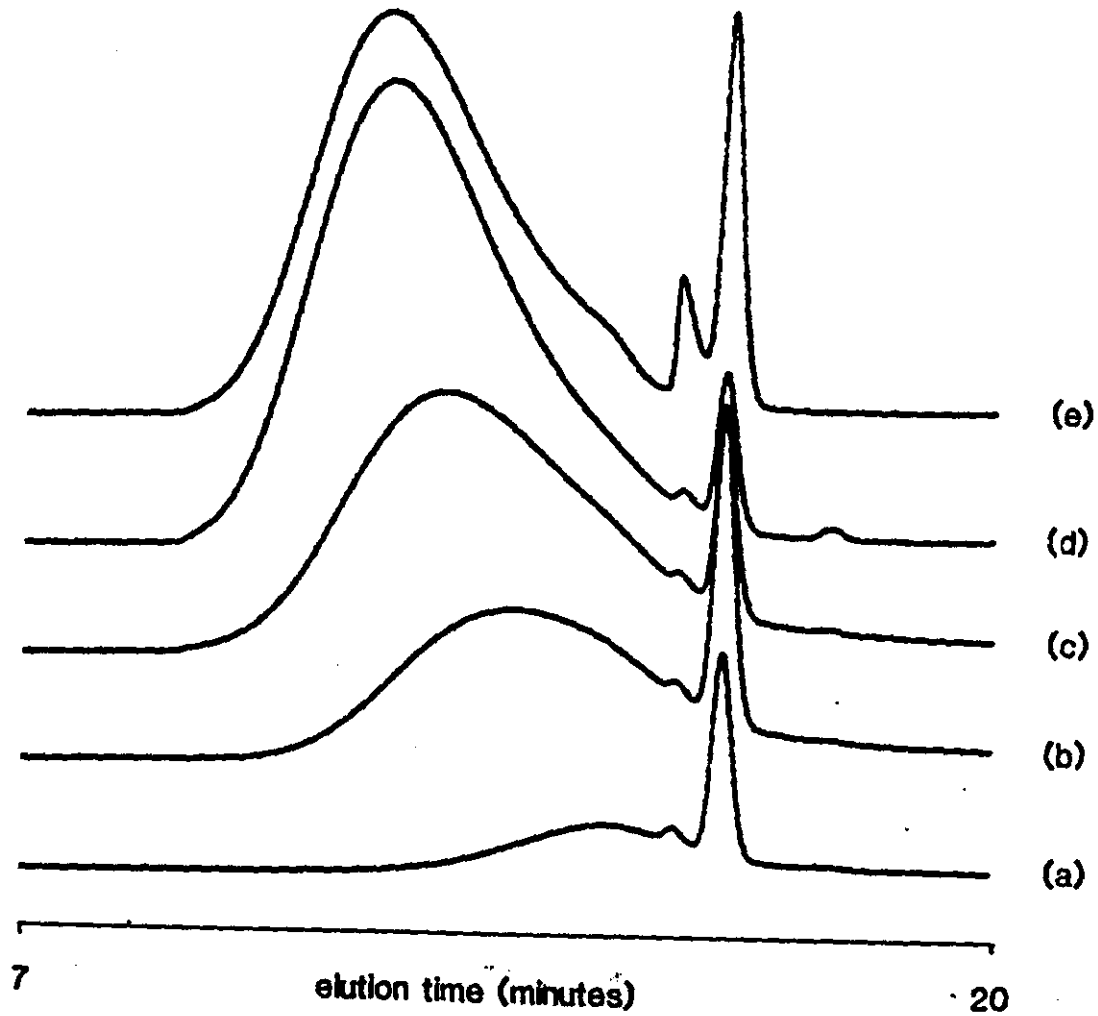
The buffer eluent was further modified by the addition of methanol (80/20 buffer/methanol). This has the supposed effect of reducing any hydrophobic interactions, and producing separations purely according to size [25]. The resultant chromatograms were quite similar to those obtained with buffer eluent, but the response of partially hydrolysed samples increased relative to the fully hydrolysed sample, and the increase in retention time with decreasing degree of hydrolysis was not as great (FIGURE 6). This would suggest that the addition of methanol did reduce hydrophobic interaction, but did not completely inhibit it. An excluded peak was once again only observed with the fully hydrolysed sample suggesting that either the partially hydrolysed samples have a smaller molecular size even with no intra-molecular hydrophobic interaction or that the methanol did not prevent this size reducing phenomenon.

The SEC eluent was changed to 0.05M $NaNO_3$ solution with no pH control, as suggested in the literature [29], and the samples were reanalysed. The resultant chromatograms were very similar to those acquired using buffer eluent, except that for the fully hydrolysed sample there was no excluded peak with the lower salt concentration (FIGURE 7). Therefore ionic modifier not only inhibits the strange behaviour encountered with water but also promotes exclusion, there being an



2 x PL aquagel-OH 40 columns and RI detector

FIGURE 6 SEC chromatograms for PVOH samples with different degrees of hydrolysis: (a) 77.7%; (b) 83.4%; (c) 86.7%; (d) 90.6%; (e) 100%; with 0.2M $NaNO_3$, 0.1M NaH_2PO_4, pH7 buffer/methanol (80/20 v/v) as eluent.



2 x PL aquagel-OH 40 columns and RI detector

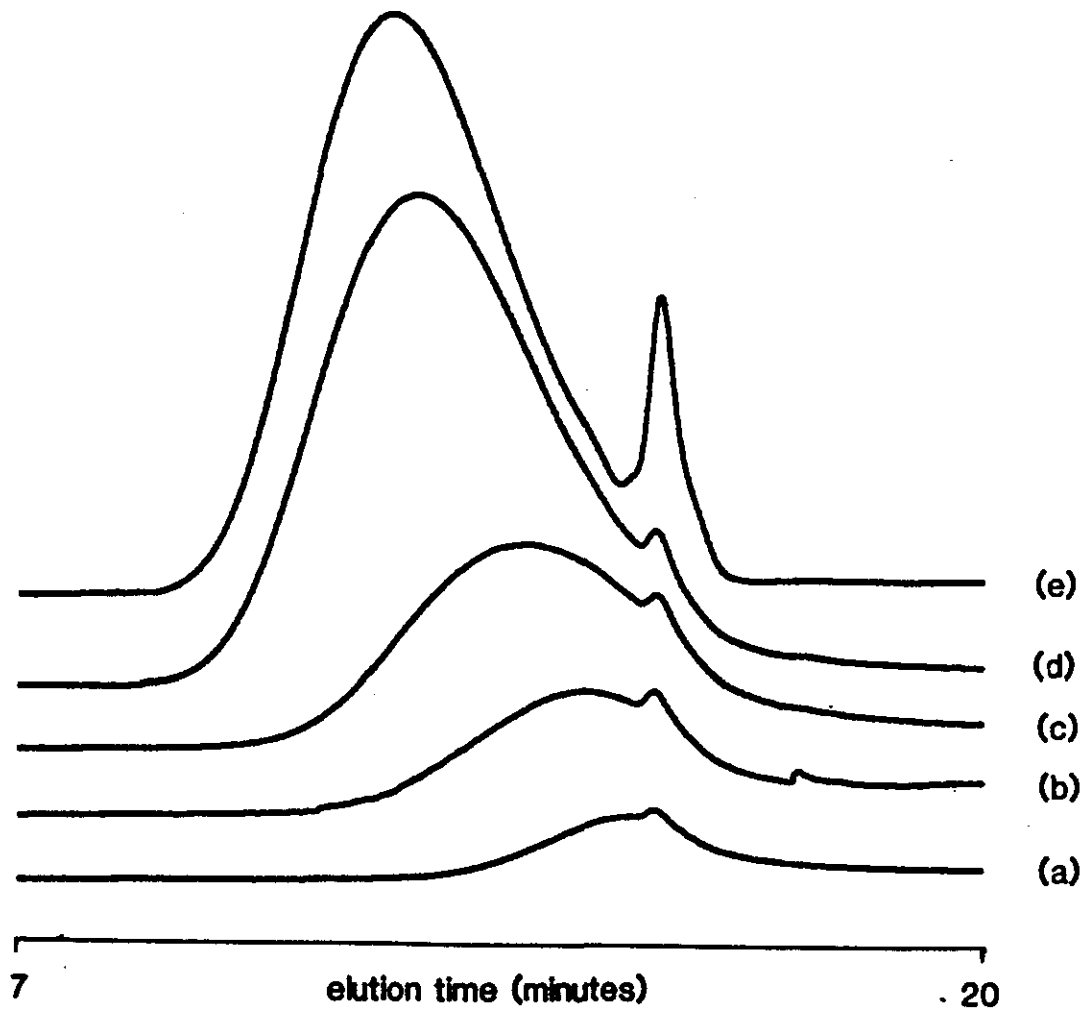
FIGURE 7 SEC chromatograms for PVOH samples with different degrees of hydrolysis: (a)77.7%; (b)83.4%; (c)86.7%; (d)90.6%; (e)100%; with 0.05M $NaNO_3$ as eluent.

optimum salt concentration at which both effects can be controlled.

The SEC eluent was again changed, this time to 0.05M $HCOONH_4$ (ammonium formate) since this is a volatile salt and would not interfere with secondary RPC because it would be evaporated away in the mass detector. However, this produced a series of chromatograms similar to those with sodium nitrate in that response increased and retention time decreased with increasing degree of hydrolysis (FIGURE 8). An increase in concentration to 0.2M $HCOONH_4$ had little effect on the peak positions and also produced an excluded peak with fully hydrolysed polymer (FIGURE 9).

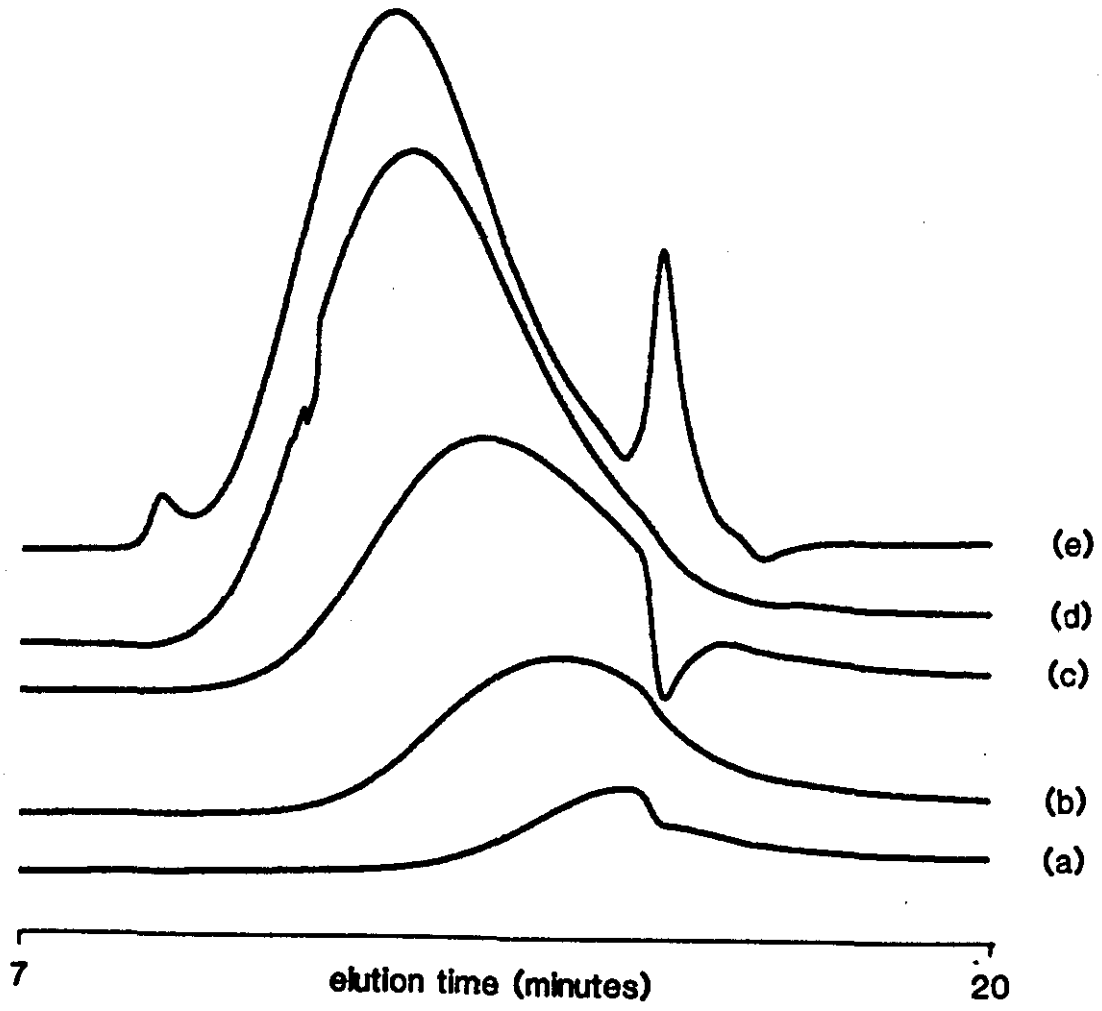
Whatever salt concentration is used, organic modifier is required to suppress any hydrophobic interaction, although both roles can be performed by the use of an ionic surfactant solution as eluent. Sodium dioctyl sulfosuccinate (Aerosol OT) is an anionic surfactant, and was used as eluent (0.1%w/v in water) for the analysis of the PVOH samples. The resultant chromatograms exhibited jagged, distorted peaks for fully and partially hydrolysed grades (FIGURE 10), and repeated injections of the same samples gave different peak shapes. However, this surfactant concentration was found to be greater than the quoted critical micelle concentration (cmc) for Aerosol OT of 0.07%w/v. Above the cmc, the alkyl chains of a number of surfactant molecules tend to cluster together, forming a sphere-like structure called a micelle. The presence of these quasi-macromolecules may have affected detection and produced the curious peaks.

An alternative anionic surfactant with a much higher cmc (18%w/v) is sodium diisobutyl sulfosuccinate (Aerosol IB45). Samples were analysed using 0.1%w/v



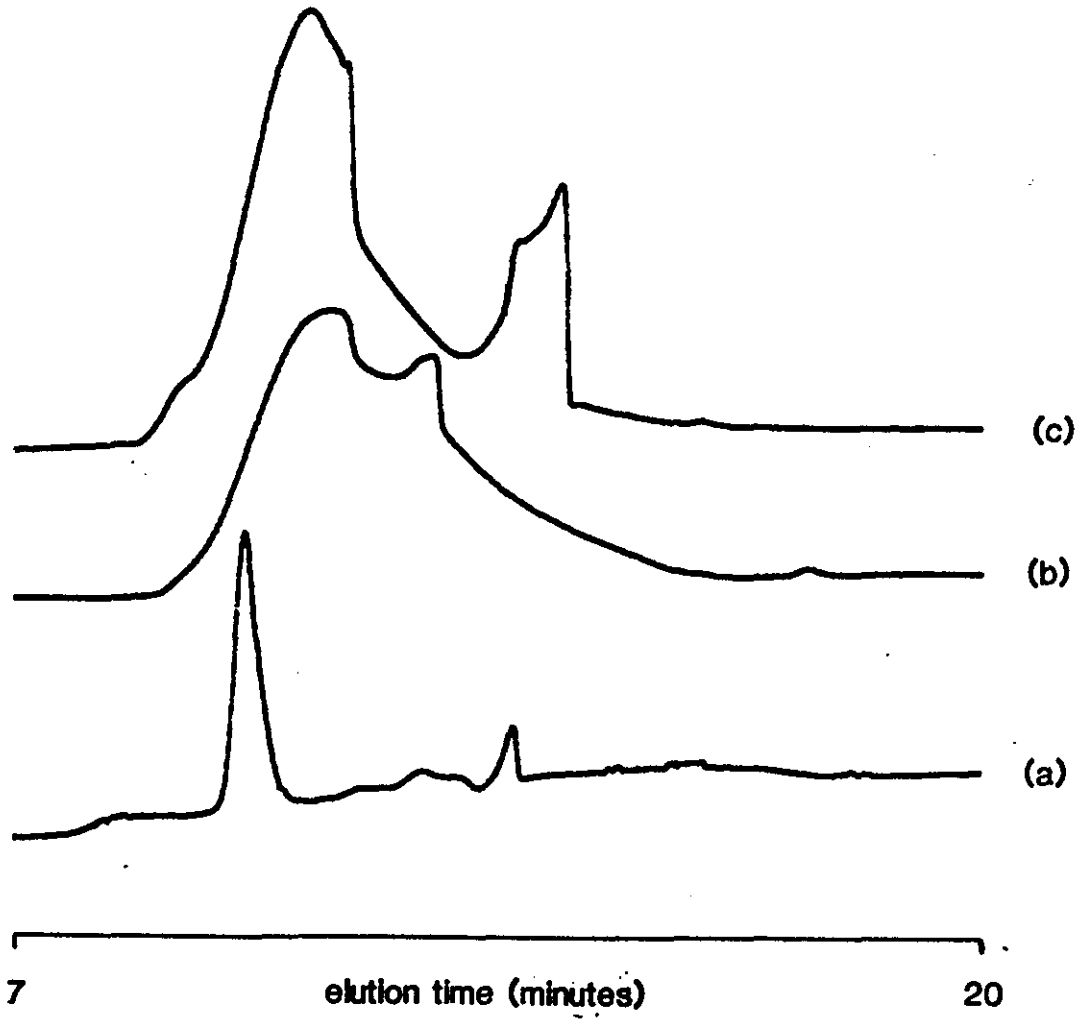
2 x PL aquagel-OH 40 columns and RI detector

FIGURE 8 SEC chromatograms for PVOH samples with different degrees of hydrolysis: (a) 79.6%; (b) 83.6%; (c) 87.3%; (d) 91.8%; (e) 100%; with 0.05M $HCOONH_4$ as eluent.



2 x PL aquagel-OH 40 columns and RI detector

FIGURE 9 SEC chromatograms for PVOH samples with different degrees of hydrolysis: (a)79.6%; (b)83.6%; (c)87.3%; (d)91.8%; (e)100%; with 0.2M $HCOONH_4$ as eluent.



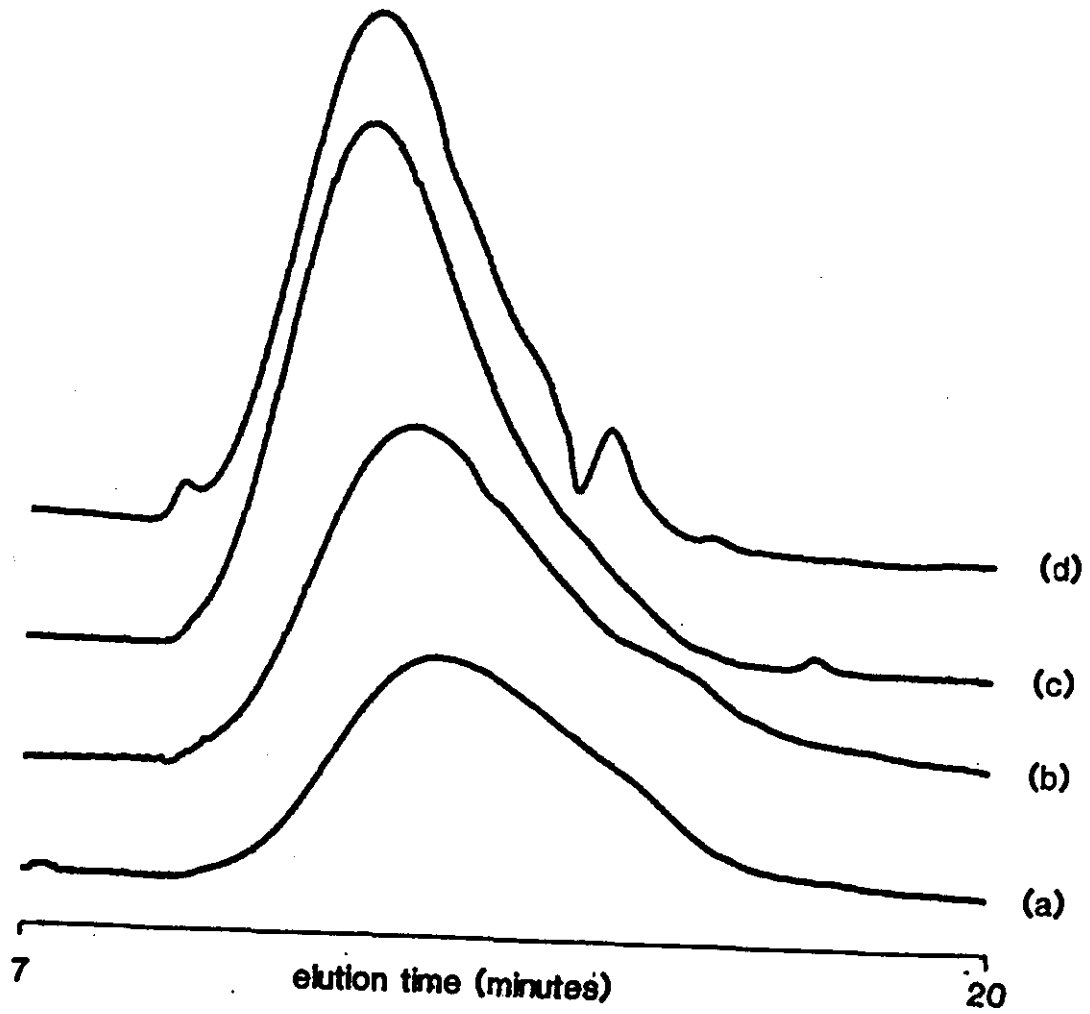
2 x PL aquagel-OH 40 columns and RI detector

FIGURE 10 SEC chromatograms for PVOH samples with different degrees of hydrolysis: (a) 72.2%; (b) 90.6%; (c) 100%; with 0.1% (w/v) Aerosol OT as eluent.

surfactant as eluent and the resultant chromatograms were similar to those obtained with buffer eluent (FIGURE 11). An excluded peak was observed for fully hydrolysed PVOH and retention times increased with decreasing degree of hydrolysis, suggesting that hydrophobic interactions were still occurring at this concentration. Also, partially hydrolysed samples exhibited tailing peaks, a sign of possible adsorption, and the eluent was found to be a poor solvent: polymer less than 80% hydrolysed proved to be insoluble with a sample concentration 0.2%w/v. Increased surfactant concentration would possibly inhibit hydrophobic interaction, but was not found to improve solubility.

Reduced response with decreasing degree of hydrolysis was observed with sodium nitrate and Aerosol IB45 eluent systems. This would normally suggest that the RI of the polymers in these solutions decreases with decreasing degree of hydrolysis. Direct injection of sample solutions into the differential refractometer produced a similar response trend for all (soluble) samples (FIGURE 12), suggesting this theory is correct.

The SEC chromatograms so far obtained were not considered to be a real representation of molecular size distributions of the PVOH samples, since all of the samples had a simlar molecular weight but exhibited different SEC profiles, there being other factors such as ionic and hydrophobic interactions involved in the separation mechanism. The behaviour of the polymer in solution was not fully understood, although it was believed that inter- and intra-molecular interactions affected molecular size. For true size separation an SEC eluent had to be found in which these size-affecting phenomena were minimised. Previously, anionic surfactants have been suggested for such a purpose but



2 x PL aquagel-OH 40 columns and RI detector

FIGURE 11 SEC chromatograms for PVOH samples with different degrees of hydrolysis: (a) 83.4%; (b) 86.7%; (c) 90.6%; (d) 100%; with 0.1% (w/v) Aerosol IB45 as eluent.

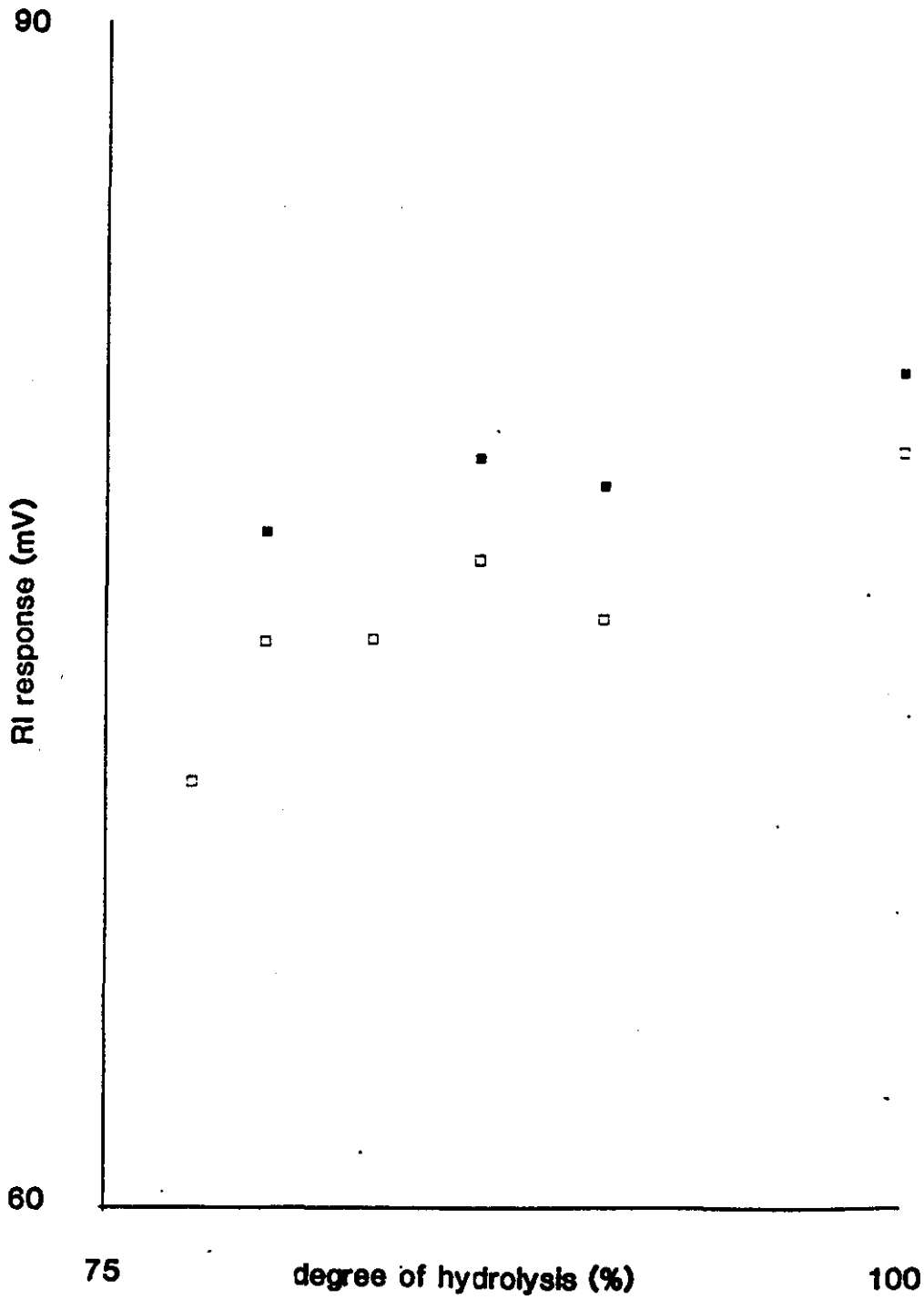
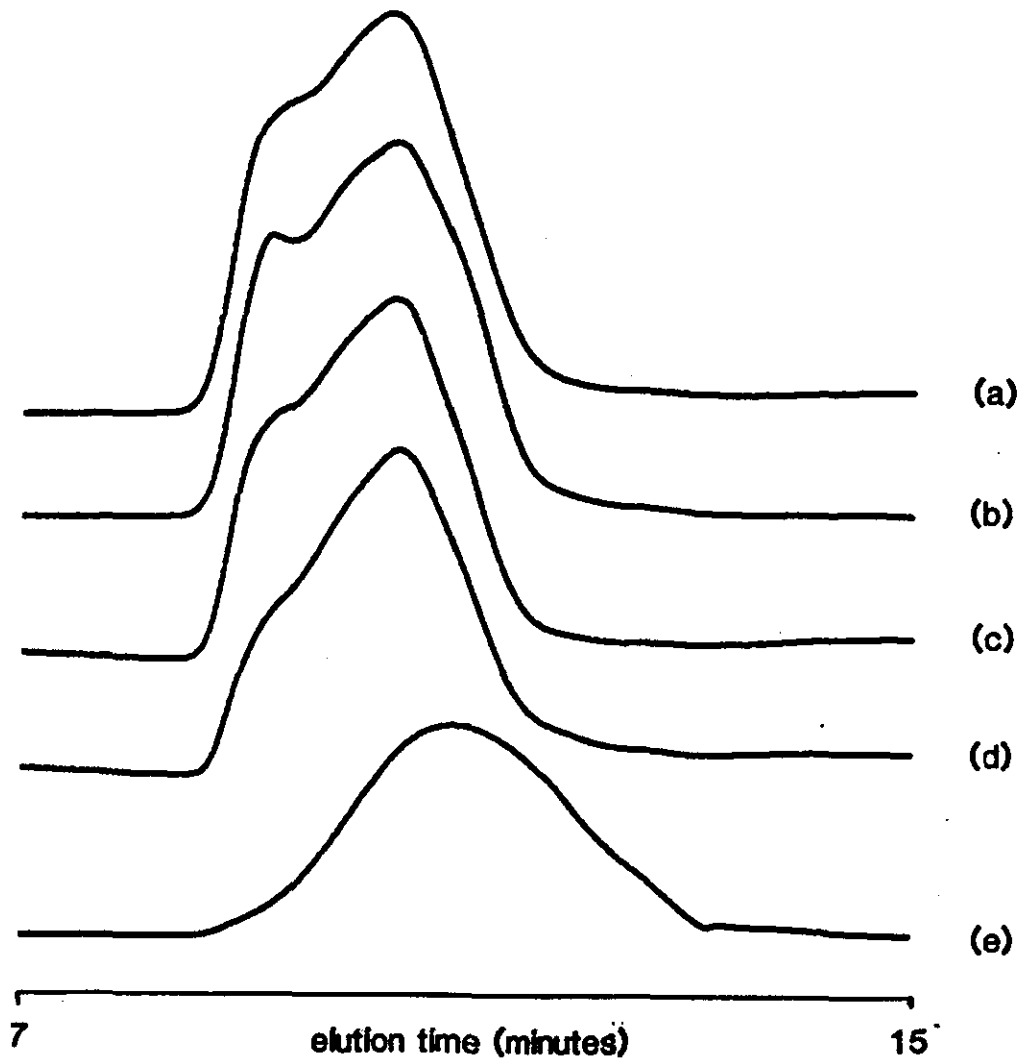


FIGURE 12 RI response as a function of degree of hydrolysis for PVOH samples dissolved in (□) 0.05M $NaNO_3$ and (■) 0.1%(w/v) Aerosol IB45.

results were poor. However these poor results were due to micellization (with Aerosol OT) and poor sample solubility (with Aerosol IB45); an alternative anionic surfactant, sodium lauryl sulphate (SLS), exhibited good sample solubility with a relatively high cmc (0.26%w/v). It had been shown to bind readily to PVOH polymer and dissociate multimers [16], and by the same mechanism it would inhibit any intra-molecular interactions which tend to reduce molecular size. One problem encountered with an SLS solution was its tendency to cloud at low temperature. It had been reported that this was caused by surfactant association and could be avoided by maintaining the solution at a temperature slightly above room temperature [16].

Chromatograms obtained with 0.2%(w/v) SLS as eluent were completely different to those obtained with the other anionic surfactants. Partially hydrolysed grades exhibited large exclusion peaks, the size of which increased with decreasing degree of hydrolysis, indicating increasing high molecular weight species content. Peak elution times now increased with increasing degree of hydrolysis, for all samples, showing that molecular size decreased in this range (FIGURE 13). However, the elution time of the fully hydrolysed polymer was shorter than in other eluent systems showing that it, and hence all samples, had increased in molecular size in SLS compared to other eluents. This size increase was due to a combination of the ionic and hydrophobic effects of SLS which reduced intra-molecular interactions, causing the molecules to "open out". The increase in size was greater with decreasing degree of hydrolysis, i.e. increasing hydrophobicity; fully hydrolysed PVOH, which had no



2 x PL aquagel-OH 40 columns and RI detector

FIGURE 13 SEC chromatograms for PVOH samples with different degrees of hydrolysis: (a)79.6%; (b)83.6%; (c)87.3%; (d)91.8%; (e)100%; with 0.2%(w/v) SLS as eluent.

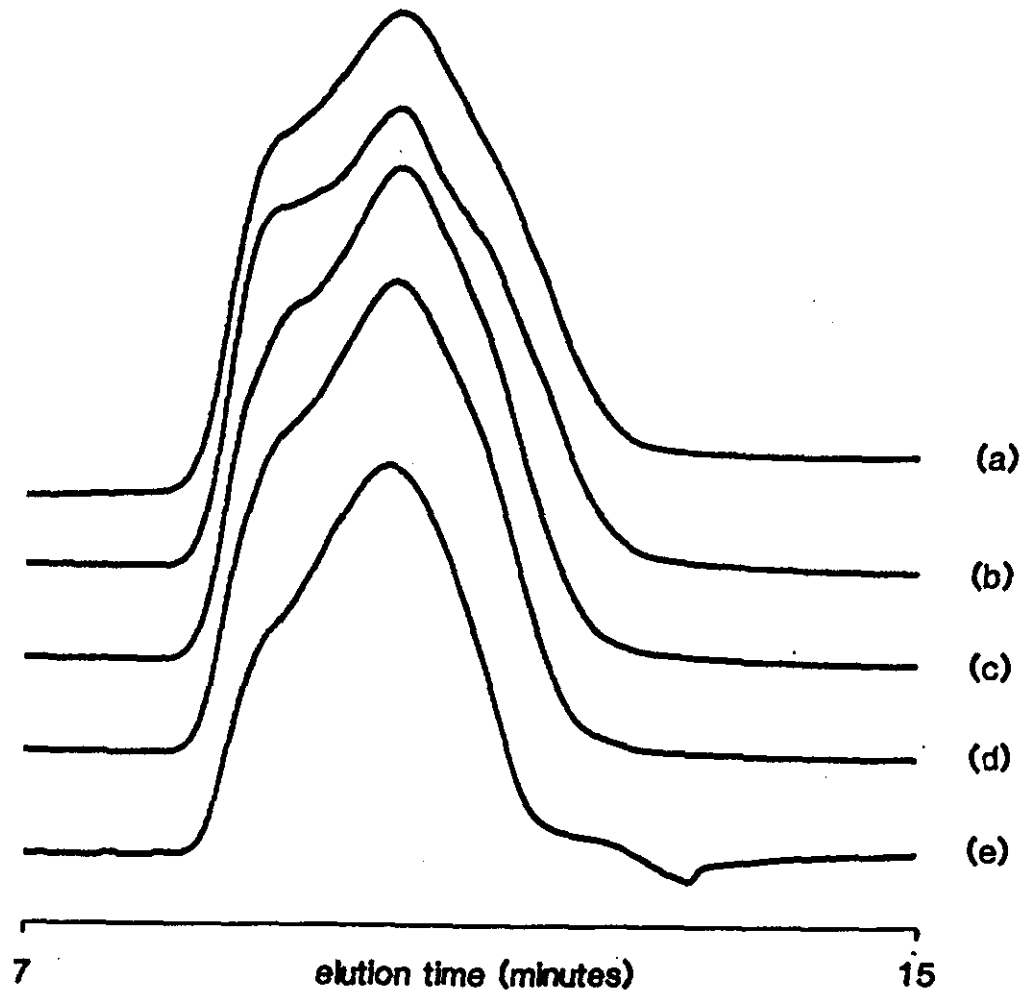
hydrophobic content, was the least affected, which suggested that the ionic character of SLS was not as strong as its hydrophobic character at this concentration. Eluent concentration was increased to 0.25%w/v SLS (just below the cmc) and the resultant chromatograms showed that all of the samples had the same elution time. Peak shapes were very similar for all the samples (FIGURE 14).

Although these chromatograms appeared to represent good size separation, each had an excluded peak. To investigate the nature of this excluded material, the samples were analysed using columns with a higher exclusion limit (PL aquagel-OH 50). All of the samples eluted at the same elution time, with no exclusion, indicating a similar molecular size (FIGURE 15).

4.2 REVERSED PHASE CHROMATOGRAPHY

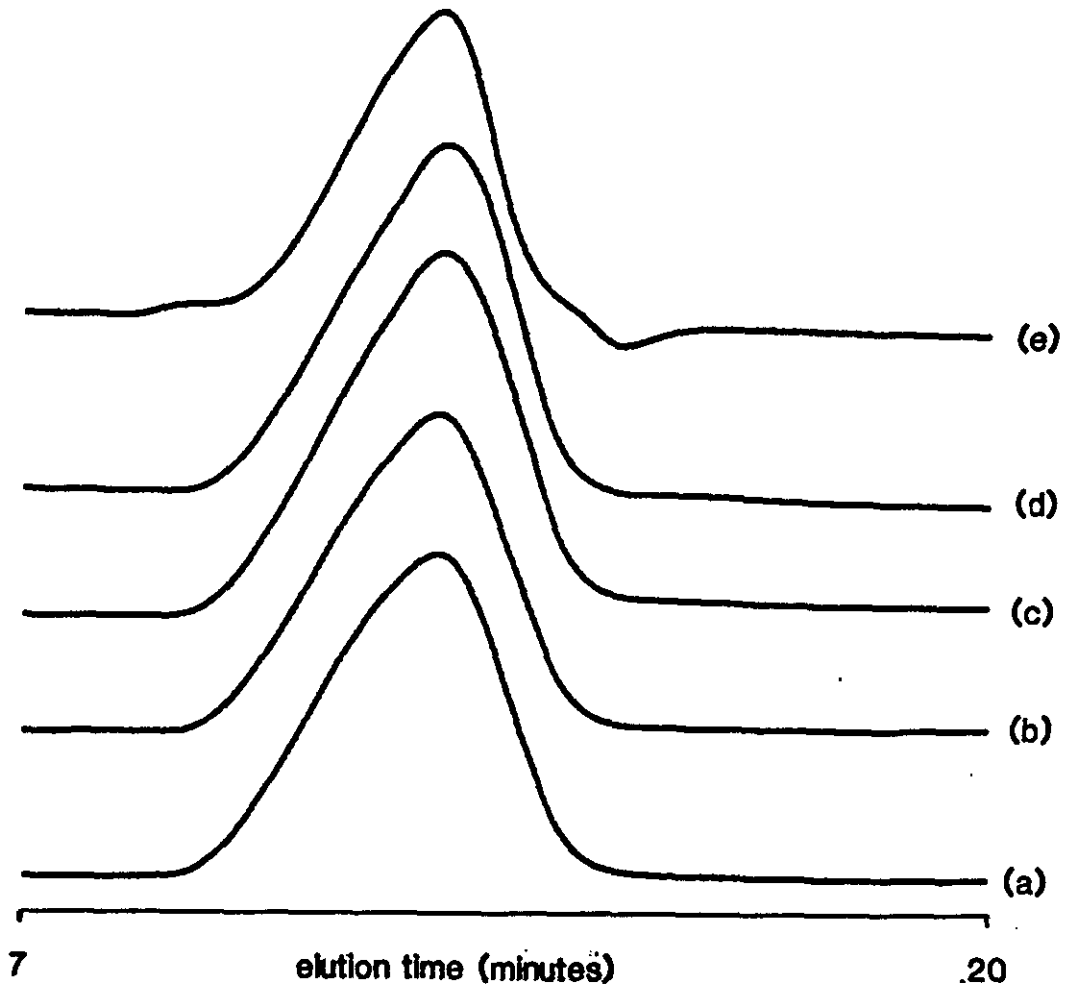
The samples were also analysed by RPC with gradient elution from 99/1 to 30/70 water/THF (v/v) in 5 minutes. The resultant chromatograms were inherently different for fully and partially hydrolysed grades: the former exhibited a sharp, early eluting peak whereas the latter produced broader later eluting peaks. The retention time variation indicates increased column interaction with decreasing degree of hydrolysis, which confirms the increasing hydrophobicity with increasing acetate content. However, the variation in peak width indicates that there are other differences between fully and partially hydrolysed grades (FIGURE 16).

These samples were prepared by alcoholysis in the presence of methanol/methyl acetate, which has been shown to yield polymer with a wide distribution of degree of hydrolysis [3]. This wide distribution may



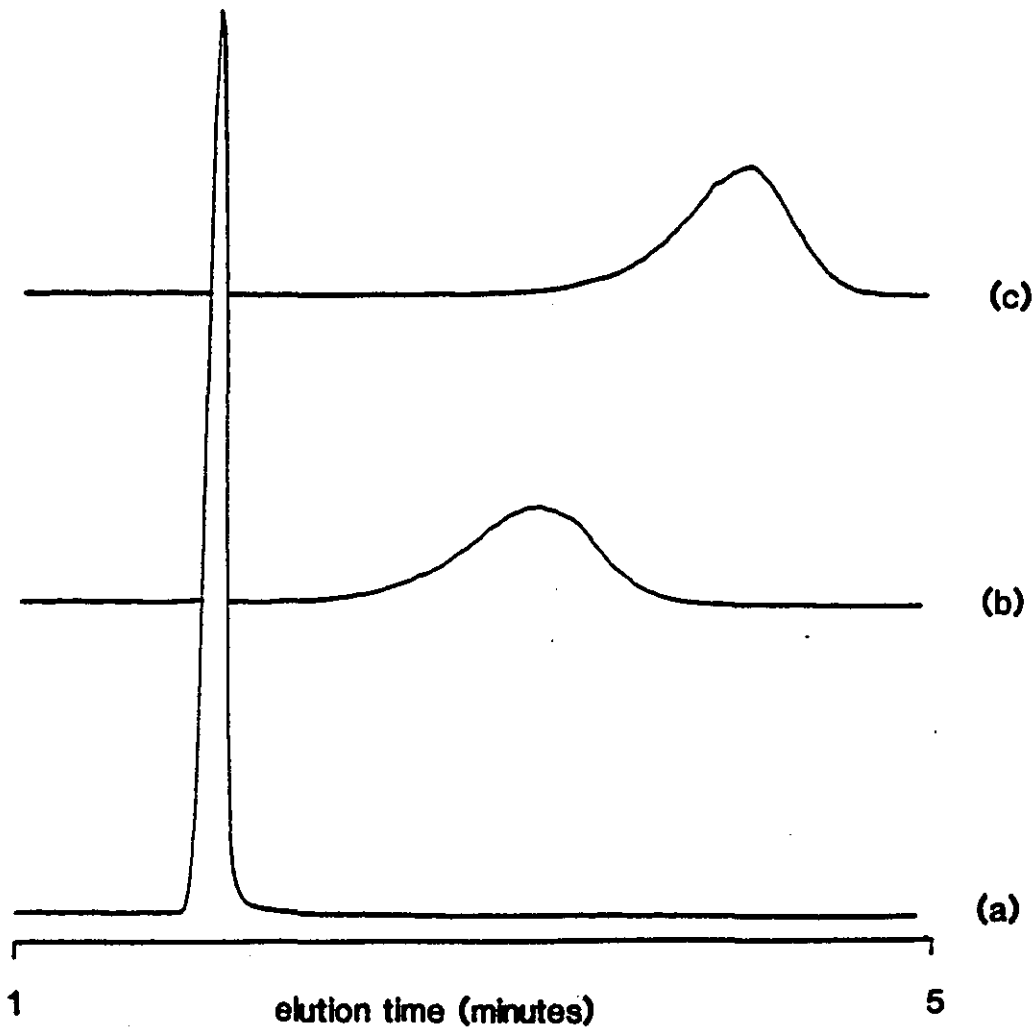
2 x PL aquagel-OH 40 columns and RI detector

FIGURE 14 SEC chromatograms for PVOH samples with different degrees of hydrolysis: (a) 79.6%; (b) 83.6%; (c) 87.3%; (d) 91.8%; (e) 100%; with 0.25% (w/v) SLS as eluent.



2 x PL aquagel-OH 40 columns and RI detector

FIGURE 15 SEC chromatograms for PVOH samples with different degrees of hydrolysis: (a) 79.6%; (b) 83.6%; (c) 87.3%; (d) 91.8%; (e) 100%; with 0.25% (w/v) SLS as eluent.



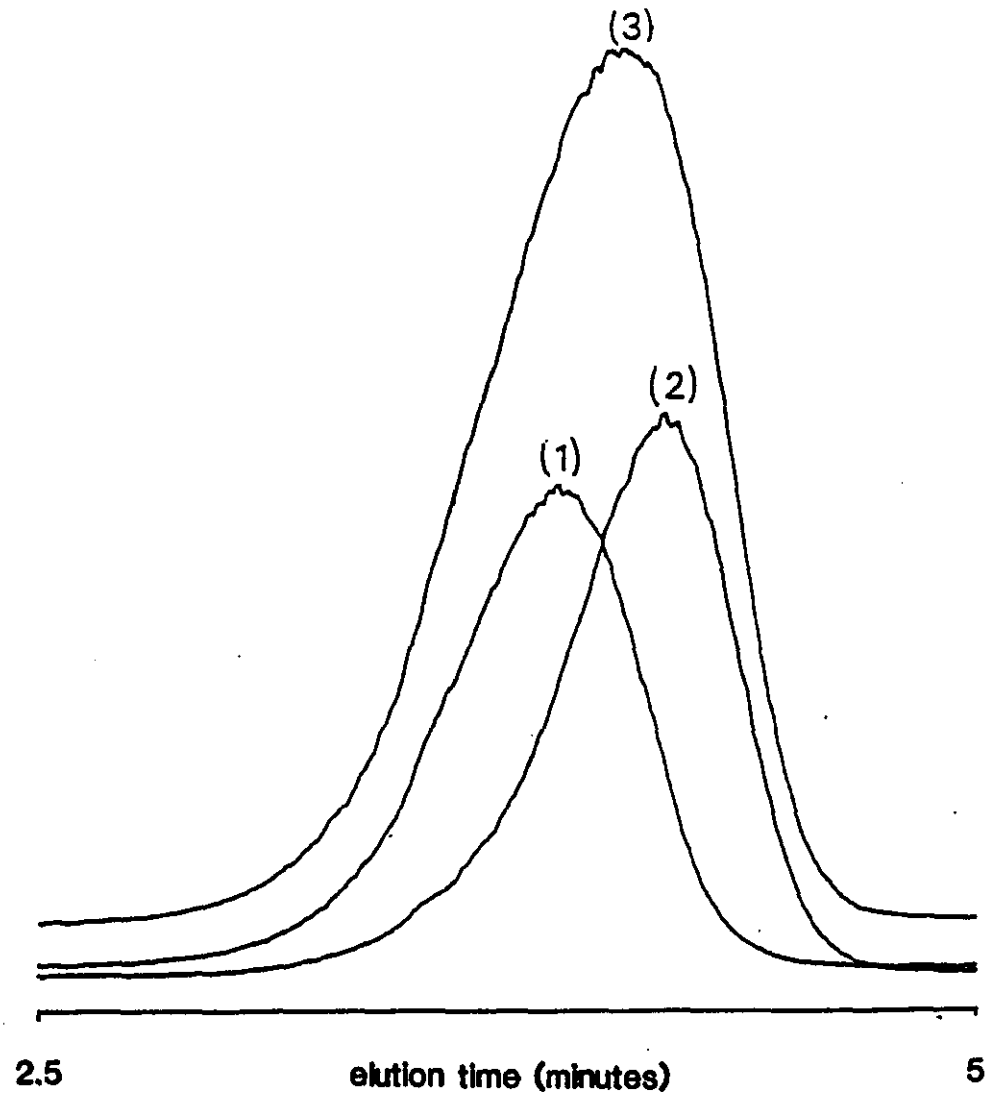
1 x PLRP-S 8µm 4000Å column and mass detector

FIGURE 16 RPC chromatograms for alkaline hydrolysed PVOH samples with different degrees of hydrolysis: (a)100%; (b)87.3%; (c)73.7%; with gradient elution from 99/1 to 30/70 water/THF (v/v) in 5 minutes.

account for the broad peaks observed for partially hydrolysed grades, although other compositional variations may also play a part. The sharp peak observed with the fully hydrolysed sample would suggest no such compositional variation, as would be expected after a completed hydrolysis reaction.

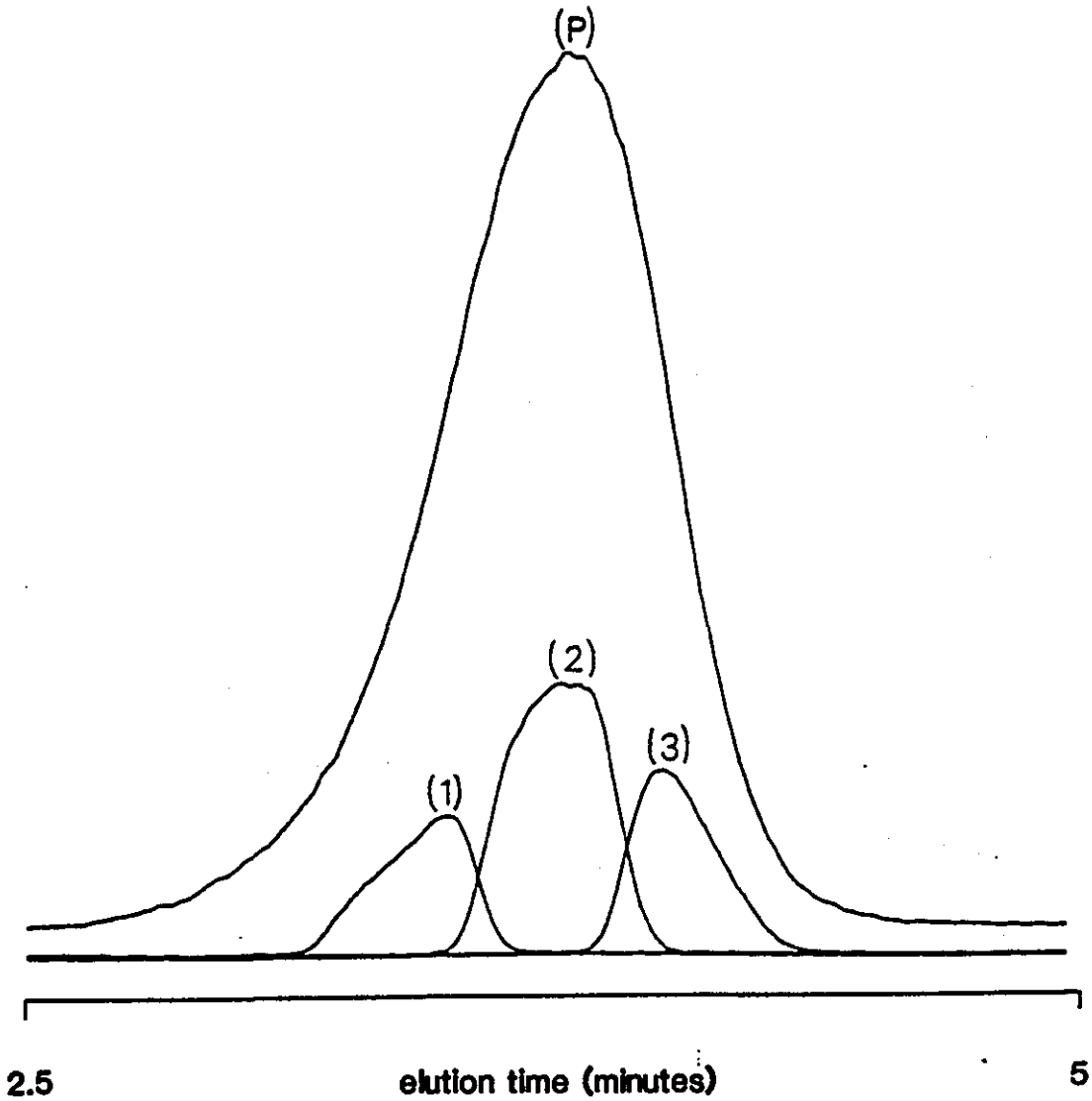
To investigate this peak broadening effect further, 50/50 v/v blends of alkaline hydrolysed sample solutions were prepared. The chromatogram for the blend was compared with those of the constituent samples. For two samples of similar degree of hydrolysis, 72.2% and 77.7%, the individual chromatograms revealed incomplete resolution of the two peaks. The chromatogram obtained after blending exhibited a single, broadened peak such that it enveloped the two constituent peaks (FIGURE 17). These observations would indicate that a relatively small increase in the distribution of degree of hydrolysis results in peak broadening and that the peak position, that is the maximum response, represents the average degree of hydrolysis for a given sample.

Confirmation of this proposal was achieved by fractionation of the PVOH samples as they eluted from the HPLC column. Based on previously observed elution times, three fractions were collected across the original whole sample peak after removal of the mass detector from the system. The three fractions,when reinjected, display individual peaks which all elute within the peak envelope of the original polymer which in this case was 79.6% hydrolysed (FIGURE 18). These results would suggest that the peak elution time is dependent on the degree of hydrolysis and that this sample, which was typical of all partially hydrolysed samples studied, exhibits a distribution of degree of



1 x PLRP-S 8μm 4000Å column and mass detector

FIGURE 17 RPC chromatograms for alkaline hydrolysed PVOH samples: (1)77.7% hydrolysed; (2)72.2% hydrolysed; (3) 50/50 (v/v) blend of (1) and (2); with gradient elution from 99/1 to 30/70 water/THF (v/v) in 5 minutes.



1 x PLRP-S 8µm 4000Å column and mass detector

FIGURE 18 RPC chromatograms for an alkaline hydrolysed PVOH sample and fractions collected from it: (P)79.6% hydrolysed (whole polymer); (1),(2),(3) are associated fractions; with gradient elution from 99/1 to 30/70 water/THF (v/v) in 5 minutes.

hydrolysis consistent with alcoholysis in the presence of methyl acetate.

All of the samples so far studied had been prepared by alkaline alcoholysis of PVAc, producing relatively blocky polymer. Alternative samples were prepared with mineral acid as catalyst, to produce more random polymer, and hence give some idea of the comparative material properties.

Both acid and alkaline hydrolysed samples were analysed using ^{13}C-NMR in order to ascertain their relative blockiness [5]. This was of limited use due to insolubility of samples greater than 80% hydrolysed in dimethylformamide (DMF), the solvent used in the NMR measurements. Spectra were produced for samples less than approximately 80% hydrolysed and the peak characteristics were interpreted with regard to the literature [5], (TABLE 2; FIGURE 19). These results confirmed that the alkaline hydrolysed samples were blocky, and also showed that blockiness decreased with increasing degree of hydrolysis (since the blockiness factor, [n], increases with decreasing blockiness); the blockiness factor calculated for both soluble acid hydrolysed samples was typical of a random copolymer.

A comparison of two polymers having the same nominal degree of hydrolysis but different blockiness characteristics was made by RPC with an eluent gradient of 99/1 to 30/70 (v/v) water/THF in 5 minutes. The random, acid hydrolysed sample displayed a narrower peak than the blocky alkaline hydrolysed sample (FIGURE 20); this could be due to a narrower degree of hydrolysis distribution, although there is no literature evidence to support this, or could be associated with a narrower sequence length distribution.

HYDROLYSIS TECHNIQUE	DEGREE OF HYDROLYSIS (%)	BLOCKINESS FACTOR [n]
ALKALINE	69.6	0.39
ALKALINE	73.7	0.41
ALKALINE	79.6	0.43
ACID	72.2	0.83
ACID	75.6	0.83

TABLE 2 Blockiness factors for various PVOH samples calculated from NMR spectra.

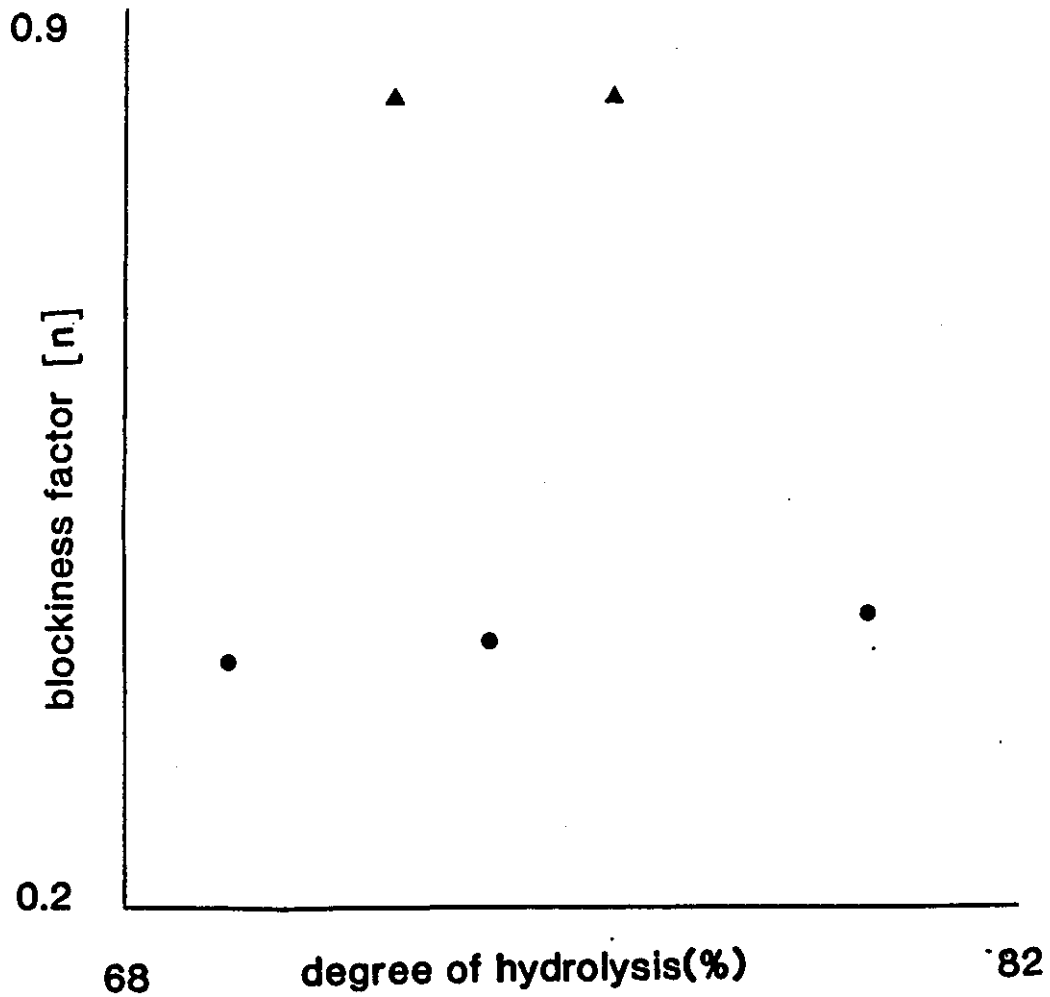
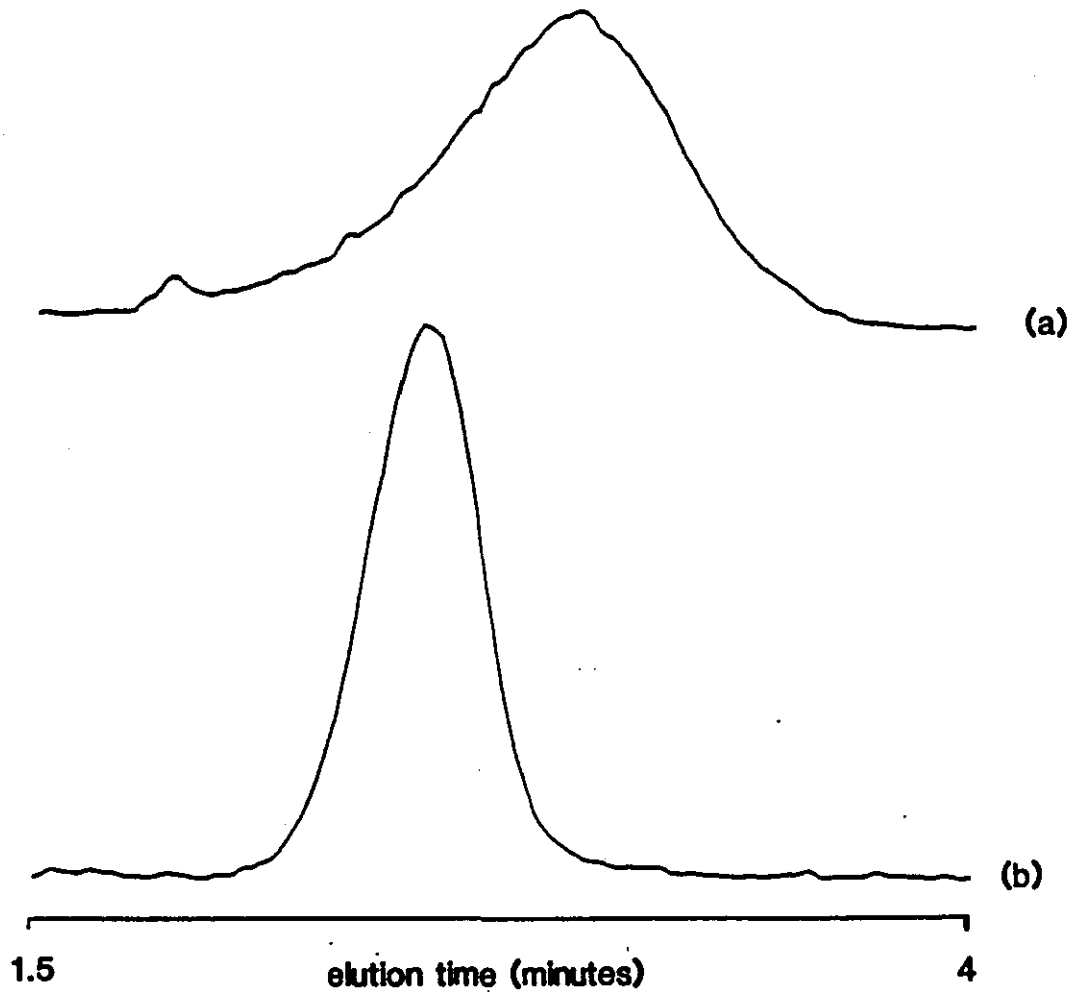


FIGURE 19 Blockiness factor as a function of degree of hydrolysis for (●) alkaline and (▲)acid hydrolysed PVOH samples calculated from NMR spectra.

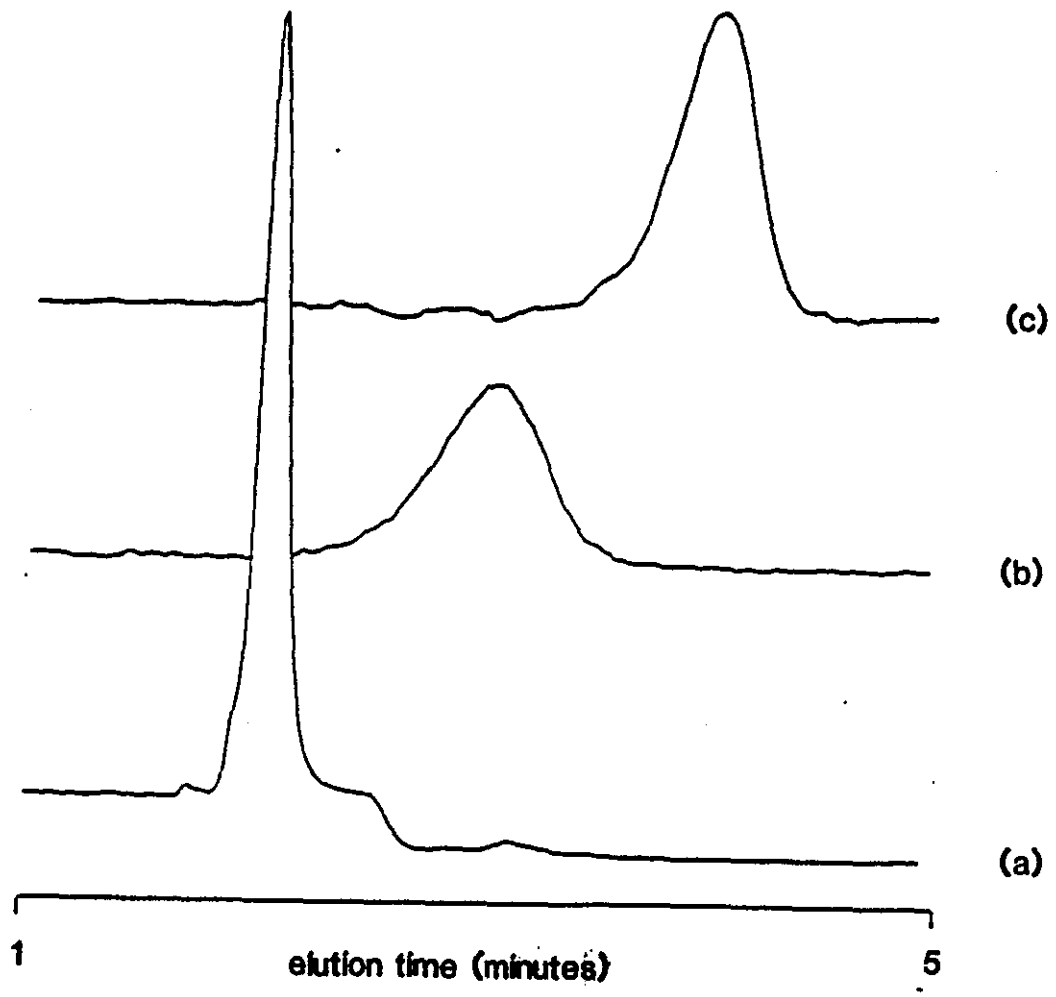


1 x PLRP-S 8µm 4000Å column and mass detector

FIGURE 20 Comparison of RPC chromatograms for PVOH sample of degree of hydrolysis 89.5%: (a) alkaline hydrolysed; (b) acid hydrolysed; with gradient elution from 99/1 to 30/70 water/THF (v/v) in 5 minutes.

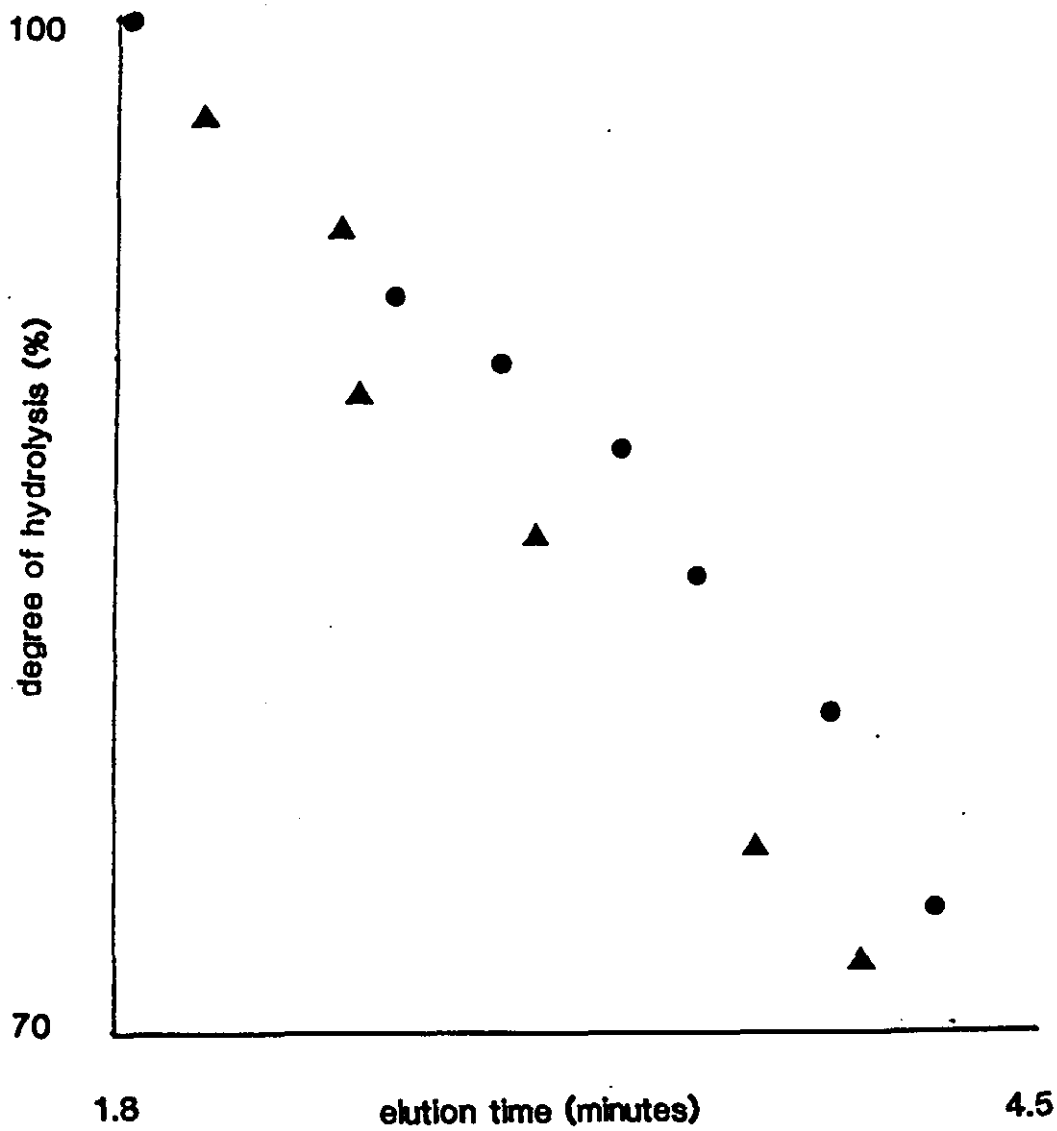
The basis of sample retention in RPC was explored by studying partially hydrolysed polymers covering a wide range of degree of hydrolysis for both acid and alkaline hydrolysed PVOH. Typical chromatograms for acid hydrolysed samples exhibited a similar trend to alkaline hydrolysed samples in that peak elution time increased with decreasing degree of hydrolysis (FIGURE 21). At decreasing degrees of hydrolysis, the increasing acetate content enhances the hydrophobicity of the polymer causing it to interact more strongly with the non-polar PS-DVB packing material at the start of the gradient. Thus a higher concentration of THF is required to release the sample from the column, resulting in increased elution time. It would appear that a specific water/THF composition is required to desorb polymer of a particular degree of hydrolysis. The elution characteristics observed suggested that the separation mechanism was adsorption rather than precipitation/redissolution **[36][37]**.

A strong correlation was observed between elution time and degree of hydrolysis for both acid and alkaline hydrolysed samples (FIGURE 22). In general, the blocky alkaline hydrolysed polymers eluted later than the random acid hydrolysed polymers of the same degree of hydrolysis. A more blocky distribution of acetate groups, i.e. a longer sequence length, presents a more hydrophobic site for column attachment and hence requires a correspondingly higher THF content to elute the sample. This difference is not observed at high degrees of hydrolysis, greater than 90%, since the sequence lengths in random and blocky polymers are similar **[49]**.



1 x PLRP-S 8µm 4000Å column and mass detector

FIGURE 21 RPC chromatograms for acid hydrolysed PVOH samples with different degrees of hydrolysis: (a) 97.2%; (b) 84.6%; (c) 72.2%; with gradient elution from 99/1 to 30/70 water/THF (v/v) in 5 minutes.



1 x PLRP-S 8μm 4000Å column and mass detector

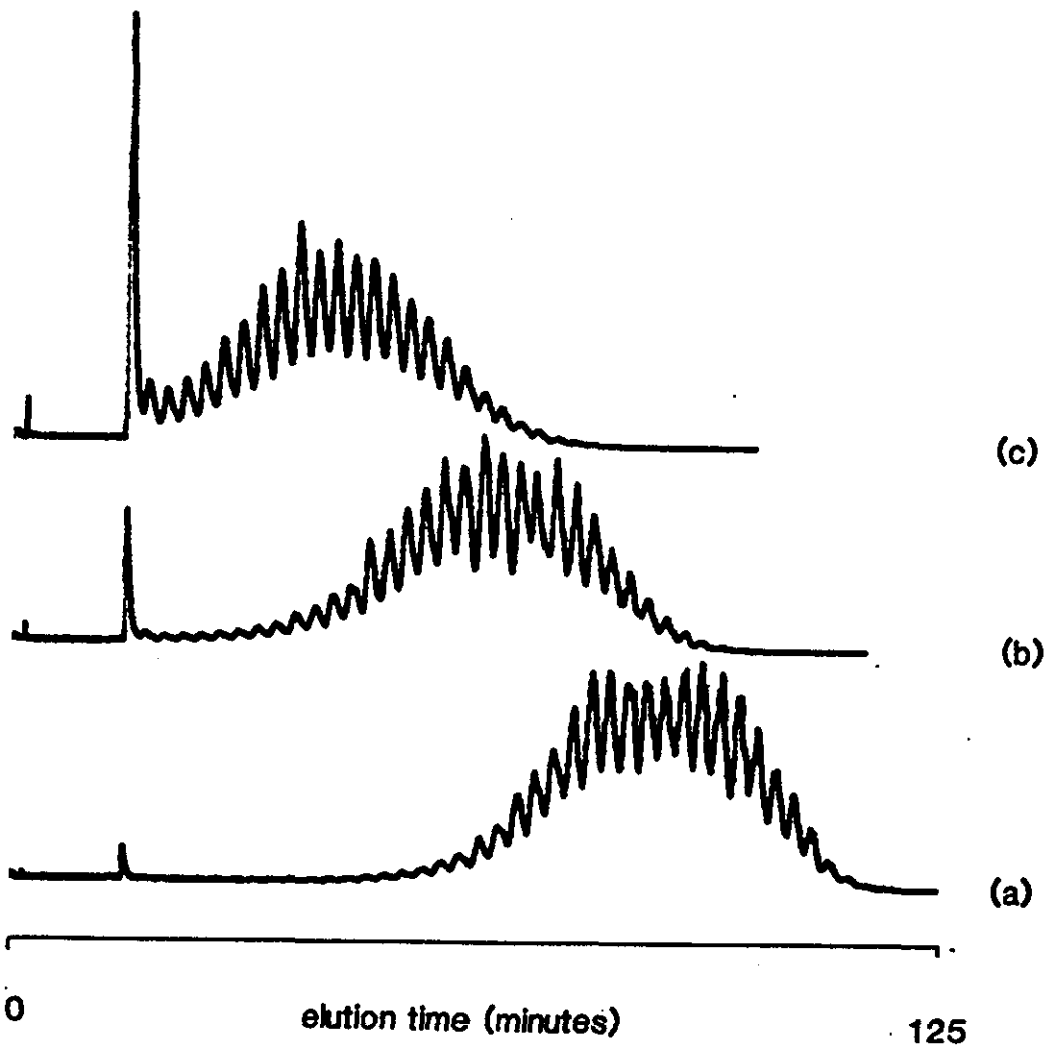
FIGURE 22 RPC elution time as function of degree of hydrolysis for (●)alkaline and (▲)acid hydrolysed PVOH samples; with gradient elution from 99/1 to 30/70 water/THF (v/v) in 5 minutes.

In a coupled system, the secondary separation must be capable of successfully analysing a series of fractions. For an on-line system this requires a very short analysis time relative to the primary separation. All RPC separations so far performed had utilised fast gradients with this in mind; however, by greatly reducing the rate of change of THF composition, samples could be separated according to hydrophobicity as a primary separation.

Acid and alkaline hydrolysed samples were analysed by RPC over an eluent gradient of 99/1 to 40/60 (v/v) water/THF in 150 minutes. The resulting chromatograms exhibited a series of sharp multiple peaks for all partially hydrolysed samples (FIGURES 23,24), which suggested a separation of species based on hydrophobicity, which could be associated with acetate content, sequence length or a combination of the two. Fully hydrolysed polymer exhibited a single sharp peak; once again indicating no compositional distribution.

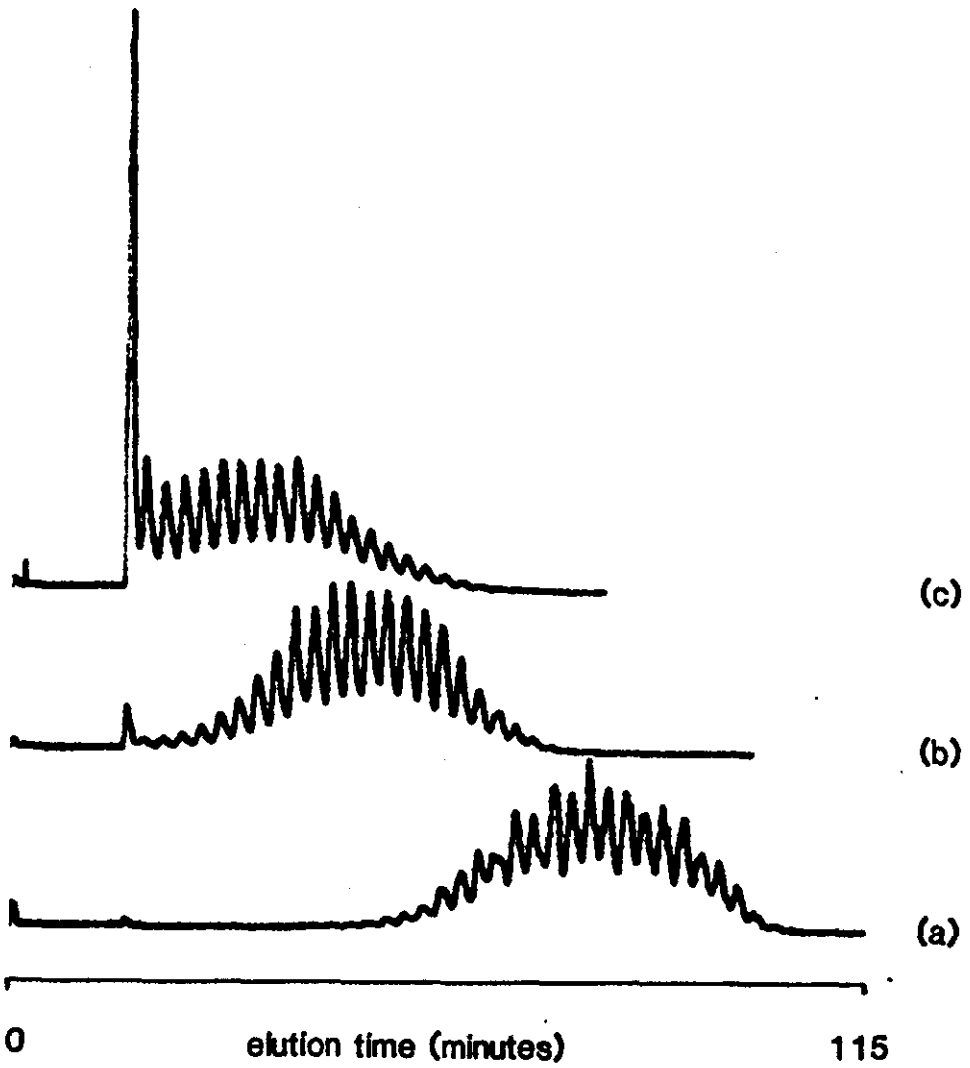
The elution times for the series of multiple peaks was dependent upon the average degree of hydrolysis and blockiness as with a fast gradient, but with this more selective separation, a peak was observed corresponding to that of the fully hydrolysed sample, for all samples. The size of this peak increased with increasing degree of hydrolysis and blockiness, and suggests that all PVOH examined contains some fully hydrolysed material.

This method of RPC resulted in fractionation of PVOH samples according to hydrophobicity and hence some form of chemical composition distribution. To understand the exact nature of this CCD, re-analysis of collected fractions by NMR could be performed. However, to produce a molecular size profile of a discrete hydrophobicity, secondary analysis consisted of aqueous SEC.



1 x PLRP-S 8µm 4000Å column and mass detector

FIGURE 23 RPC chromatograms for alkaline hydrolysed PVOH samples with different degrees of hydrolysis: (a) 73.7%; (b) 83.6%; (c) 89.8%; with gradient elution from 99/1 to 40/60 water/THF (v/v) in 150 minutes.



1 x PLRP-S 8μm 4000Å column and mass detector

FIGURE 24 RPC chromatograms for acid hydrolysed PVOH samples with different degrees of hydrolysis: (a)72.2%; (b)84.6%; (c)91.8%; with gradient elution from 99/1 to 40/60 water/THF (v/v) in 150 minutes.

4.3 COUPLED COLUMN CHROMATOGRAPHY

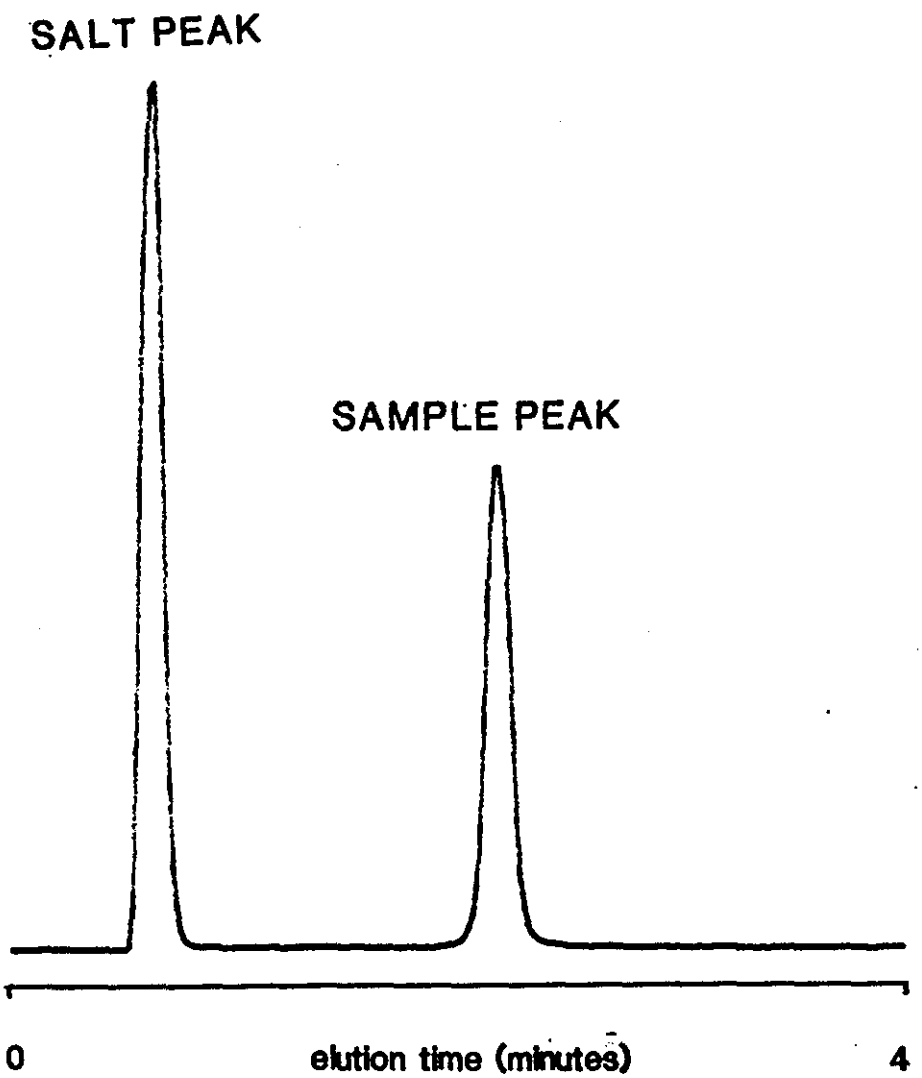
The first problem encountered with a coupled system incorporating RPC followed by SEC concerned the redirection of eluent between the two techniques. The method of detection for RPC used an evaporative light scattering device, in which the eluent is evaporated away; in the coupled system there must be some means of fractionation, hence either the flow must be split prior to detection or the detector must be removed. The use of a flow splitter introduces further dead volume into the system and hence increases time discrepancies between the two separations; removal of the detector from the CCC system requires separate, preliminary RPC with detection in order to evaluate switching times. Although this second method involves increased analysis time, it yields more accurate cross-fractionation times and is therefore the preferred option.

Switching times for cross-fractionation were calculated from an identical RPC analysis and the detector then replaced by the aqueous SEC equipment. However, a major problem was encountered in the SEC of these fractions. A very large peak was observed in the SEC chromatogram of the first fraction due to THF. The baseline recovery of this peak took such a long time that further fraction studies were severely limited, and even then further THF peaks interfered with those of the sample. Alternative detection of the SEC eluent was attempted using an evaporative light scattering device in order to remove the THF. However this proved unsuccessful due to saturation of the device by salts in the SEC eluent.

Successful CCC incorporating RPC followed by aqueous SEC would require an alternative SEC eluent which accommodates the use of an evaporative light scattering detector whilst still separating according to size. This eluent would also be useful in the alternative CCC system of aqueous SEC followed by RPC.

The use of various salt and surfactant solutions as SEC eluent had produced non-perfect chromatograms due to ionic and hydrophobic interactions, but re-analysis of fractions from SEC by off-line RPC could yield some further information on the samples. Unfortunately, a large unretained peak was observed on all RPC chromatograms when using 0.05M $NaNO_3$ as SEC eluent; this peak was caused by salts in solution (FIGURE 25). This peak restricted the speed of the RPC gradient, due to the time required for baseline recovery: the THF content required for sample elution could not be approached until after this time, which was equal to the time for one column volume of eluent to elute (approx. 0.6 minutes). In addition to this, as with all gradient systems, a finite time was required between each RPC analysis to allow reconditioning of the eluent composition to the gradient start composition. These two factors would decidedly limit the number of fractions which could be analysed on-line, hence this method of CCC would only be suitable for off-line analysis. Since gradient elution always requires reconditioning time, to improve the applicability of the SEC-RPC system, an SEC eluent would have to be found which did not produce a peak on the coupled RPC chromatogram, whilst maintaining good size separation.

PVOH polymer was dissolved in various salt solutions and analysed by RPC to imitate the secondary analysis. A known volatile salt, ammonium formate ($HCOONH_4$), produced



1 x PLRP-S 8µm 4000Å column and mass detector

FIGURE 25 RPC chromatogram of a fraction taken from the SEC of a fully hydrolysed PVOH sample using 0.05M $NaNO_3$ as eluent; with gradient elution from 99/1 to 1/99 water/THF (v/v) in 10 minutes.

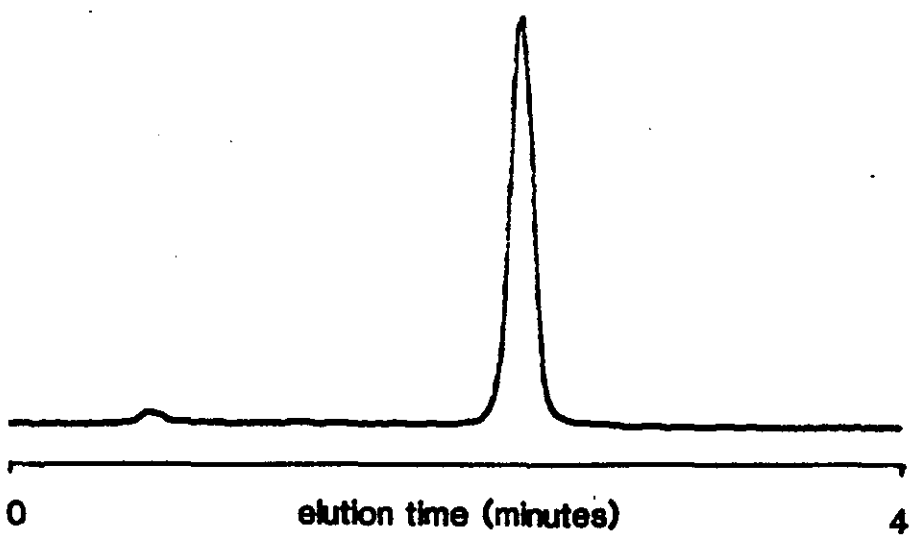
good, clear polymer solutions and went undetected on an RPC chromatogram (FIGURE 26). However, aqueous SEC using $HCOONH_4$ as eluent had been shown to produce poor SEC chromatograms and hence although it appeared to be a successful eluent in terms of non-interference in RPC, it was less than successful in separating according to size.

The only eluent system found to yield good size separation was 0.25%(w/v) SLS and so a coupled system incorporating SEC with this eluent followed by RPC was investigated.

However, before a coupled system could be set up, the compatibility of the eluent systems had to be considered. Samples prepared in 0.25%(w/v) SLS were analysed by RPC over a gradient of 99/1 to 1/99 (v/v) water/THF in 10 minutes. The chromatograms showed that in addition to a sample peak, there were two sharper peaks, which co-eluted with some of the sample peaks (FIGURE 27). These two peaks were shown to be associated with the SLS solution and the smaller, later eluting of the two was thought to be associated with a hydrophobic impurity in the SLS.

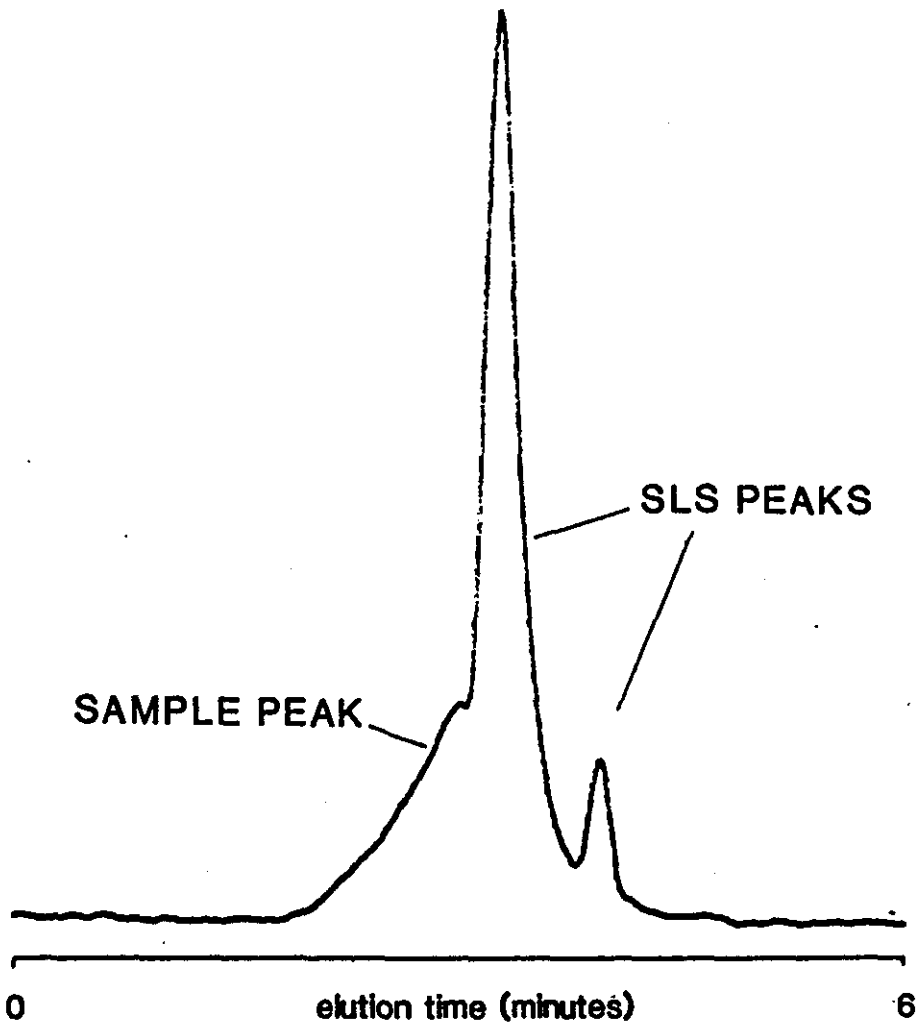
Whatever had caused these peaks, it was obvious that a coupled system incorporating SEC with 0.25%(w/v) SLS as eluent as the primary separation would be somewhat limited by their appearance on the secondary chromatogram.

Alternative ionic surfactants were considered as possible SEC eluents, but also produced peaks on the secondary chromatogram. With the SLS, the peak eluted early enough to allow detection of some hydrophobic material, and so an on-line coupled system was constructed with SEC using 0.25%(w/v) SLS as eluent as the primary separation followed by RPC.



1 x PLRP-S 8µm 4000Å column and mass detector

FIGURE 26 RPC chromatogram of a fraction taken from the SEC of a fully hydrolysed PVOH sample using 0.05M $HCOONH_4$ as eluent; with gradient elution from 99/1 to 1/99 water/THF (v/v) in 10 minutes.



1 x PLRP-S 8µm 4000Å column and mass detector

FIGURE 27 RPC chromatogram of a 91.8% hydrolysed PVOH sample dissolved in 0.25%(w/v) SLS; with gradient elution from 99/1 to 1/99 water/THF (v/v) in 10 minutes.

There were two possible methods of operation of the coupled system, namely continuous cross-fractionation from a single SEC injection, or singular cross-fractionation from repeated SEC injections. However, the applicability of this coupled system was limited to polymer less than 80% hydrolysed (or equivalent hydrophobicity) due to the SLS peaks.

A polymer sample, 73.7% hydrolysed, was analysed by CCC, in discontinuous mode (i.e. cross-fractionation from repeated SEC injections). The benefits of this method were that the time for the RPC gradient and reconditioning was not limited, nor was the number of fractions which could be analysed. The RPC chromatograms obtained from this method showed that for this degree of hydrolysis, the sample and SLS peaks could be differentiated (FIGURE 28). The large peak eluting at between 2.5 and 4.0 minutes was associated with the SLS, and the peak associated with PVOH eluted between 5.0 and 6.0 minutes. The position of this latter peak remained similar for all of the fractions, although closer examination revealed a trend that RPC elution time decreased with increasing SEC elution time. This indicated that increasing molecular weight corresponded to decreasing degree of hydrolysis within the sample. The other two, smaller peaks eluting between 4 and 5 minutes differed slightly from one fraction to another and could be associated with the SLS solution, the PVOH or a combination of the two.

This discontinuous method allowed multi-fraction analysis and the use of long gradients. However it also resulted in long analysis times and needed a high level of operator-interaction due to repeated injections (the long term objective of this project had been to design a quick, semi-automatic coupled system). Continuous

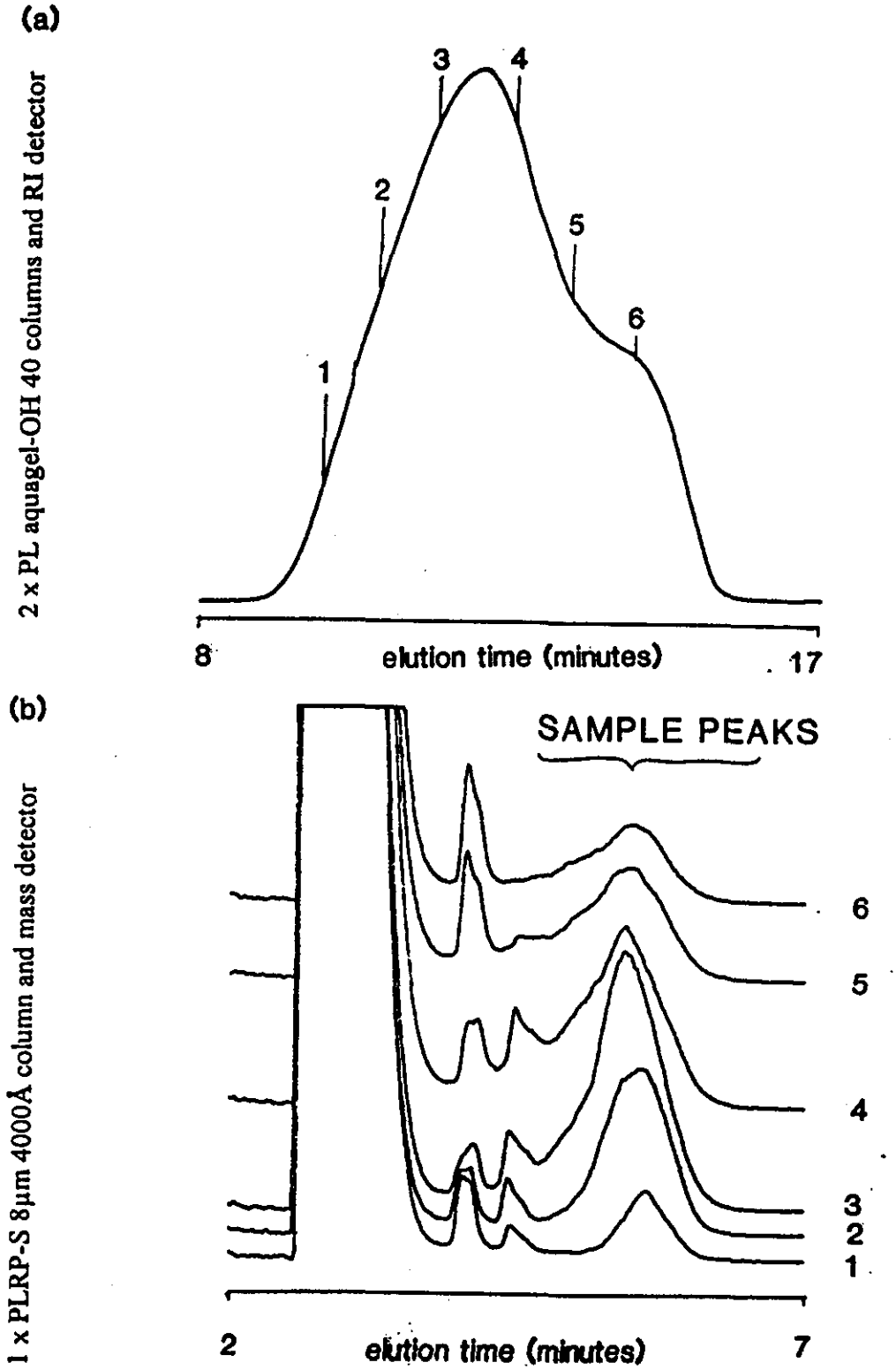


FIGURE 28 CCC chromatograms for a 73.7% hydrolysed PVOH sample (discontinuous mode): (a) typical SEC chromatogram from which fractions 1-6 were collected, with 0.25%(w/v) SLS as eluent; (b) RPC chromatogams of fractions 1-6 with gradient elution from 99/1 to 1/99 water/THF (v/v) in 10 minutes.

analysis on the other hand would involve less manual interaction and shorter analysis times. Also, each fraction would be extracted from the same injected sample, there being no problems associated with sample variation.

Before a continuous method could be used however, the experimental conditions of the two separations had to be altered such that the SEC peak covered a sufficient time for several fractions to be analysed. This was achieved by reducing the SEC flow rate and shortening the RPC gradient.

Reducing the SEC flow rate increased the time range covered by the peak, however further reduction may have introduced band broadening effects due to longitudinal diffusion.

To reduce the RPC gradient time, the start and finish compositions were altered and the rate of change of THF content was increased. The start composition was set such that SLS was not retained at all and flowed straight through the column. This composition was maintained long enough for the SLS peak to recover to baseline , at which time the gradient was started.

A polymer sample, 73.7% hydrolysed, was analysed with this continuous coupled system. The whole analysis took less than half of the time required for the discontinuous method, but was restricted to only four fractions. The RPC chromatograms were similar to those from the discontinuous method in that later eluting fractions from SEC exhibited slightly earlier eluting peaks. Unlike the other method, however, only two peaks were observed since the two smaller peaks exhibited in the other method co-eluted with the SLS peak in continuous mode (FIGURE 29).

Samples greater than 80% hydrolysed were also analysed but no second peak was observed since any polymer would have co-eluted with the SLS.

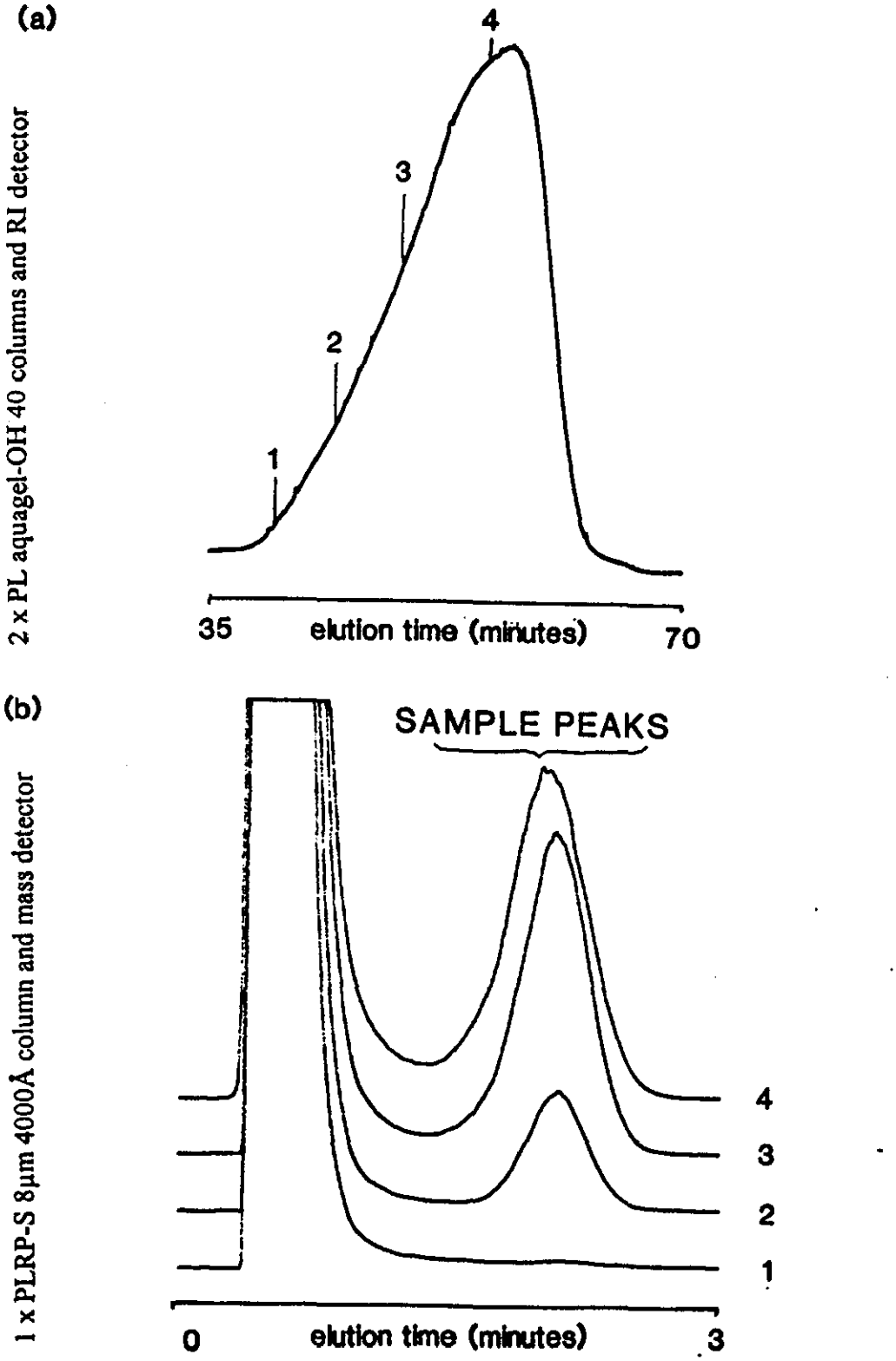


FIGURE 29 CCC chromatograms for a 73.7% hydrolysed PVOH sample (continuous mode): (a) SEC chromatogram from which fractions 1-4 were collected, with 0.25%(w/v) as eluent; (b) RPC chromatograms of fractions 1-4 with gradient elution from 65/35 (held for 0.5 minutes) to 30/70 water/THF (v/v) in 1.5 minutes.

CHAPTER 5 - CONCLUSIONS

A CCC system for the analysis of PVOH polymer has been developed incorporating SEC and RPC techniques.

Aqueous SEC using 0.25%(w/v) SLS as eluent resulted in what appeared to be good separation according to molecular size. The use of this method as the primary separation in a coupled system was limited to polymer with less than 80% hydrolysis due to the appearance of a surfactant associated peak in the secondary RPC chromatogram. However, for such a polymer it was shown that for increasing molecular weight, degree of hydrolysis decreased slightly.

RPC gradient elution using water/THF resulted in the retention of solutes based on their hydrophobicity. Polymer appeared to be separated according to composition, there being a strong correlation between elution time and degree of hydrolysis, and characteristic differences between blocky and random polymers. Broad peaks obtained using fast gradients indicated a distribution in composition, and the use of a much slower gradient resulted in a series of sharp multiple peaks, dependent upon hydrophobicity. However, the use of this slow gradient as the primary separation of a coupled system proved unsuccessful due to the appearance of a THF associated peak in the secondary SEC chromatogram which obscured any sample behaviour.

Although more data could be collected from a discontinuous coupled system, similar results were obtained from the much quicker continuous on-line technique.

CHAPTER 6 - FUTURE WORK

The major problem of the SEC-RPC coupled system was the appearance of an interfering peak in the secondary chromatogram due to the non-volatility of SLS. An alternative eluent which is volatile but still yields good SEC chromatograms is needed in order to be able to analyse all PVOH samples. Ammonium laurate is an ionic surfactant similar to SLS but is also volatile at the operating temperature of the mass detector. It was found to give good SEC chromatograms at a concentration of 0.45%(w/v) but produced a peak at high THF content in the RPC chromatogram; this peak may not appear with the use of a purer source of ammonium laurate.

Ammonium formate, a volatile standard electrolyte, produced no peak in the RPC chromatogram, although it also produced poor SEC chromatograms. The addition of methanol to this eluent would give it some hydrophobic character, and possibly result in SEC similar to SLS; however unlike SLS it would still not show a peak in the RPC chromatogram.

If either of the above SEC eluents proved successful, the coupled system could be switched around with RPC preceding SEC, the latter using a mass detector, in which case no SEC eluent or THF peaks would appear.

The existing SEC-RPC coupled system can operate in continuous mode in which case analysis is quick but limited to only four or five fractions. Methods to increase the number of fractions would invariably increase analysis time, whether it be by reducing the flow rate (which could introduce band broadening),increasing the number of SEC columns or

using multiple primary injections; however, there is the possibility of using multiple detectors in parallel, thus increasing the number of fractions without greatly increasing analysis time.

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