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S. T. Balke[a]
[a] Xerox Research Centre of Canada, Mississauga, Ontario

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ORTHOGONAL CHROMATOGRAPHY: CHROMATOGRAPHIC CROSS-FRACTIONATION OF POLYMERS

S.T. Balke
Xerox Research Centre of Canada
Mississauga, Ontario L5K 2L1

1. INTRODUCTION

Polymers are generally extremely multi-component materials. Performance of a product is often dictated by the individual concentrations of their wide variety of different molecules. We conventionally consider the importance of molecular weight distribution (i.e. concentration of each molecular weight present) and attempt to characterize it via one or two averages (a number (M_n) and/or a weight average (M_w)) for correlation with performance properties. However, most industrial polymers are copolymers or branched polymers or both. Even *"simple"* linear copolymers often contain molecules which differ from each other by at least three properties: molecular weight, composition and sequence length. [Sequence length here refers to the number of one type of repeating chemical unit in a row within a macromolecule before other types are encountered.] Three simultaneous property distributions are then present. Branched polymers contain the added complication of different branch lengths and/or *"density of branching per molecule"*. Despite the potential importance of all of these distributions,

with the exception of molecular weight distribution, at most one average value can readily be measured to characterize each (e.g. average composition, average sequence length, average branch length). Also, as will be discussed later, molecular weight distribution measurements of such polymers are highly uncertain.

Molecular property analysis is now the rate limiting step in our attempt to tailor polymer molecules in industrial reactors. The situation may be summarized as follows:

i. The property distributions are manipulated variables for a given polymer. In free radical polymerization, for example, these distributions are readily affected by reactor design (e.g. mixing effects [1]).

ii. Performance properties (e.g. mechanical and rheological properties) usually depend upon both shape and range of the distributions. There is some information on molecular weight distribution dependence (e.g. [2, 3, 4]) but much less for the other distributions because of our difficulty in measuring them [1,5].

iii. A single average value cannot adequately characterize changes in distribution shape. Even two averages (e.g. M_w/M_n) can often mislead performance predictions. An example of this is in rheological studies where measurements made on blends of narrow molecular weight distribution polystyrene standards are difficult to compare with those of a unimodal, broad molecular weight distribution [2, 6].

iv. Variations in property distributions can be predicted from kinetic models. However, most predictions cannot be tested beyond single average values and are currently encountering puzzling observations [7] which would be resolved by elucidation of the distributions themselves.

Advances in both fractionation and detection of polymers are required. Gel

Permeation Chromatography (GPC) particularly with dual detectors, is currently the usual method of analyzing complex polymers. However, GPC is conventionally limited to separating macromolecules only by size in solution. High Performance Liquid Chromatography (HPLC) fractionates by chemical properties but is conventionally applied only to small molecules. Orthogonal chromatography (OC) is a recently developed method of synergistically combining recent advances in both GPC and HPLC for analysis of macromolecules. The purpose of this paper is to review the basis for OC and to describe its current status.

2. FUNDAMENTAL PROBLEMS IN THE CHROMATOGRAPHY OF POLYMERS

2.1 Fractionation Interference

2.1.1 Interference Within a Property Distribution:

Even chromatographic fractionation of a linear homopolymer has been the subject of intense research. In that separation, although only a molecular weight distribution is present, a large continuous variety of molecular sizes (each uniquely related to molecular weight for a homopolymer) and the imperfect resolution of the GPC results in the final chromatogram representing literally thousands of unseen, overlapping peaks, one for each different molecular size present in the sample. This is commonly referred to as the *"axial dispersion problem"* in GPC. Attempts to overcome this problem have recently been reviewed [8, 9]. In quantitative GPC interpretation, whether we choose to utilize experimental or theoretical correction methods (or both) for axial dispersion the fundamental difficulty is a lack of knowledge of the shape of the individual unseen curves composing the chromatogram.

The most direct approach to determining the shape of the curves of the individual molecular sizes is injection of a *"monodisperse"* polymer. Then,

the observed chromatogram would be attributable to only one species and, as in HPLC of small molecules, the curve shape observed is the shape function required. The approach avoids numerical instabilities and limitations on admissable curve shapes. However, even the narrowest standards commercially available contain many molecular weights. Collection and reinjection of the fractions from the GPC is one solution [10, 11] but it is tedious and its accuracy is strongly dependent upon the size of the fractions.

In analysis of more complex polymers, this *"axial dispersion problem"* appears as a general *"within distribution interference"*. That is, we may then be concerned with fractionating according to say, composition, rather than molecular size. The overlapping curves are then each characteristic of individual compositions in the sample rather than molecular sizes. Furthermore, for such polymers, *"within distribution interference"* is superimposed upon *"between distribution interference"*.

2.1.2 Interference Between Property Distribution

When more than one property distribution is present, attempts to fractionate with respect to one will usually be interfered with by the presence of the other. When we attempt to measure a molecular weight distribution of a copolymer using GPC, the presence of the composition and sequence length distributions readily interfere with our efforts. This is because the GPC fractionates according to molecular size in solution and many different combinations of molecular weight, composition, and sequence length can provide a particular molecular size. This *"between distribution interference"* means that our calculated molecular weight distribution is only an apparent one which is expected to change as any one of the property distributions change. Attaching several detectors to a GPC to attempt elucidation of copolymer composition distribution cannot overcome the inadequacy in

fractionation [12]. Similar difficulties are encountered in the analysis of branched molecules by GPC using light scattering [13, 14, 15].

Attempts to separate polymer molecules using adsorption or reverse phase HPLC are also generally affected by this problem. If pores within the packing are sufficiently large to admit the molecules, then the desirable adsorption/partition separation can be confounded by a size exclusion mechanism. Molecules of different composition can exit after the same retention time in the chromatograph because some were adsorbed strongly in a few pores while others simply permeated many pores without adsorbing. The complexity of this situation has led to a variety of interpretations and experimental approaches [16, 17].

For example, in efforts to fractionate styrene acrylonitrile copolymer by composition using column adsorption chromatography, ambiguous results were encountered because of the superposition of size exclusion and adsorption effects [18]. In later work using styrene methyl methacrylate copolymers, fractionation was obtained by eliminating the size exclusion effect by using packing with small pores and by employing gradient elution (i.e. time programmed mixing of solvents) to improve resolution [19].More recently, using gradient HPLC, styrene methyl methacrylate copolymer was fractionated according to composition by a system which seemed almost unaffected by pore size [20]. This is in accordance with some Thin Layer Chromatography (TLC) results [21, 22] and polymer adsorption theory [21] that describes the existence of a critical adsorption energy of interaction which could be encountered during gradient elution. However, in gradient elution of oligomers according to molecular weight, pore size did appear important [23]. The poor resolution of the higher molecular weights was attributed to inadequate surface area for adsorption because of their exclusion from small pores.

2.2 Calibration

Calibration methods in GPC have recently been reviewed [24, 25]. Assumption of a molecular size separation and use of molecular size vs. retention time as a universal calibration is most commonly-employed for all types of polymers. However, for other than linear homopolymers, even if a universal calibration curve can be constructed, molecules with different properties are present at each molecular size. The alternative of fractionating with respect to one of the property distributions present (e.g. composition), if it can be performed despite the interference effects discussed above, requires calibration with respect to the distribution examined. However, such calibration standards are not available commercially. Synthesis of standards and use of polymerization kinetics is difficult because of uncertainties in the predominantly untested kinetic models [26]. Furthermore, a significant complication is the occasional predominance of non-exclusion effects in GPC which cause violations of Universal Calibration (separation by other than size exclusion). These effects have recently been reviewed [17]. As will be seen, OC attempts to turn non-exclusion effects to advantage.

Conventional calibration can be avoided by utilizing a detector system which both identifies the property of interest and reports its concentration. For example, a low angle laser light scattering detector has become available to determine directly the molecular weight eluting at any time [27, 28, 29]. However, interpretation of the detector output when the molecules passing through its cell are not of the same composition, is not readily accomplished [30]. Furthermore, a separate refractive index detector is necessary for determination of concentration. The problems associated with attaching more than one detector to a GPC will be discussed in the next section.

2.3 Detection

2.3.1 Ambiguous Detector Response

The conventional purpose of detection is to obtain a measure of concentration of the molecules at any retention time. More recently, more

detectors have been added to identify the macromolecules as well (i.e. to calibrate with respect to molecular weight or with respect to composition) [31]. Ambiguous detector response results, when the number of variables affecting the response, exceed the number of independent detectors available. For example, in analysis of linear copolymers, a refractive index detector followed by a fixed wavelength UV detector can be sufficient to determine concentration and composition averages across the chromatogram only if other microstructure (e.g. sequence length) does not affect the response. For UV detectors, this is often not a good assumption [32, 33, 34, 35]. If only one detector is used, then conceivably three variables (concentration, composition, and sequence length) determine the observed chromatogram. The molecular weight distribution calculated in the usual way from such a chromatogram can then again only be an *"apparent"* one whose change in shape during industrial processing can be easily and expensively misinterpreted.

2.3.2 Inaccurate Chromatogram Pairing

When more than one detector is used, we must somehow superimpose the detector responses for the same molecules. However, not only is there a time delay for transport of the molecules between detectors, but cell sizes and mixing effects in each detector cell are often different. Furthermore, high resolution columns result in extremely steep chromatograms where a small error in retention time results in a large error in interpretation when we attempt to *"overlap"* chromatograms from two detectors.

3. DEVELOPMENTS PROVIDING THE BASIS FOR OC

3.1 Synergism in Chromatography Mechanisms

There is an accelerating tendency to unify HPLC and GPC into one integral approach to separation and to consider the *"between distribution*

interference" problem mentioned above as an opportunity to enhance separation. For example, a size exclusion/normal phase partition chromatography mode has been used in separation of small molecules [36]. Gradient adsorption liquid chromatography using GPC columns packed with silica effected separation of high molecular weight polystyrene standards according to molecular weight [37, 38]. Examples of attempts to apply HPLC to more complex macromolecules in measurement of composition distributions were mentioned above with respect to the problem of *"between distribution interference"*. Application of size exclusion chromatography *"alone"* to small molecules is now commonplace and has recently been reviewed [39].

The three primary mechanisms of concern in the analysis of macromolecules are size exclusion, adsorption and partition. A few authors have noted that these effects can combine to result in a much more effective separation [16, 31, 39, 40, 41] although there are many concerns regarding *"between distribution interference"*. The work of White and Kingry [16, 41] and of Klein and Treichel [42] are particularly important with respect to showing the interaction of these mechanisms.

White and Kingry [16, 41] showed that the retention volume can be expressed by the following equation:

$$\frac{V_i}{V_c} = \alpha + \chi^E (1-\alpha) \lambda_i + \varphi_i \chi^I_i (1-\alpha) \lambda_i + \gamma_i (1-\alpha) \varphi_i \qquad (1)$$

where

V_i = retention volume of polymer i

V_c = empty column volume

α = void fraction

χ^E = external surface area per unit packing volume

λ_i = amount of polymer i adsorbed per unit available surface area

φ_i = partition coefficient for polymer i distributed between solvent inside and outside of packing

χ^I_i = internal surface area available to polymer i per unit packing volume

γ_i = pore volume available to polymer i per unit packing volume

The notable quality of this equation is that it clearly shows that every term beyond the first (*"void volume"*) term on the right is an interaction term. The retention volume then cannot simply be considered as a sum of individual adsorption, partition and exclusion contributions. For example, the adsorption coefficient interacts with the partition coefficient and with the internal surface area available for adsorption (which, in turn, depends upon the available pore volume). Even if adsorption is negligible, the retention volume is affected by both available pore volume and partition coefficient. Thus, even to accomplish the desired fractionation of a macromolecule let alone to synergistically enhance separation, this formulation shows that we must deal with several interacting mechanisms.

Klein and Treichel [42] derived a limiting form of equation (1) without partition. However, they incorporate the idea that even if the macromolecules can enter all the pores, the volume available to a macromolecule depends upon its hydrodynamic volume since its centre cannot approach closer to the wall than its hydrodynamic radius. Consequences of this are that the fraction of pore volume available to a molecule is really the product of two terms, the fraction of pore volume which the molecule can enter and the fraction of that volume which it can occupy. This means that according to this theory, we cannot eliminate

steric exclusion effects by choosing columns with pores sufficiently large to admit all macromolecules analyzed.

3.2 Multidimensional Chromatography

Multidimensional chromatography is a very general term describing methods whereby fractions from one chromatographic system are each transferred to another for further separation. The techniques encompass column switching, multiphase, multicolumn and coupled column chromatography. They have recently been reviewed by Majors [43] and by Freeman [44]. In terms of actual hardware, these systems may employ common components (e.g. pumps, detectors or even columns). The important aspect is the increased information gained by the step-wise separation process [45].

Of particular interest to the development of OC were systems in which GPC was coupled to HPLC. Johnson, Gloor and Majors [46] used what they termed Coupled Column Chromatography to analyze small molecules by separating them from macromolecules in a GPC and injecting them into an HPLC operating in a reverse phase mode with conventional C_{18} columns. This was actually performed *"on-line"* in that the effluent line of the GPC was directly connected to the injection valve of the HPLC. They were not successful in analyzing polymers this way because of solubility problems. However, conclusions relevant to the development of OC were obtained and are as follows:

- concentration of injected sample is a compromise between column capacity of the first chromatograph and detectability of amounts exiting from the second;
- solvent compatability of the mobile phases of each instrument with respect to both miscibility and strength is important (e.g. large injections

of tetrahydrofuran into the second instrument can modify the separation by deactivating adsorption sites).

3.3 Cross-Fractionation

Cross-fractionation is a fractional solvent/non-solvent precipitation technique for copolymers [31]. The procedure involves first a fractionation with respect to molecular weight. Then, each molecular weight fraction is itself fractionated according to composition by using a different solvent-non-solvent system. This method is not often used because of the labour involved. However, it is important because it begins to deal with the increased dimensionality of the fractionation problem of complex polymers. Chromatographic cross fractionation using first a size fractionation by GPC and then a composition separation by TLC has been very successful for some complex polymers [21, 47]. However, even for one-dimensional fractionations, much effort has been focussed on replacing TLC by HPLC [18, 19, 20] for improved quantitative accuracy and control.

3.4 Detection Development

Stop-flow operation and use of scanning IR detectors have been used to identify and quantify composition of copolymer fractions by conventional GPC operation [48]. Use of such detectors eliminates problems associated with attempting to superimpose outputs from two separate detectors. Rapid diode array scanning detectors have been used for many years in HPLC [49, 50]. They avoid the necessity of stop-flow operation since they are now capable of accomplishing a complete 200-800 nm UV scan in approximately 2 seconds [51].

4. SYNTHESIS OF OC

The development of this method was stimulated by the need to develop high conversion copolymerization kinetics for reactor design. The insight

provided by the kinetics clearly showed that dual-detector GPC applied to linear copolymers was providing data which could not be compared with kinetic predictions because it was based only upon a molecular size separation. A cross-fractionation was required before detection. In OC one GPC is connected so that its effluent passes through the injection valve of the second. Both instruments utilize GPC columns. However, whereas the first is operated so as to achieve conventional molecular size separation, the second attempts to fractionate by composition or sequence length by utilizing a solvent mixture to encourage adsorption and partition effects as well as size exclusion.

The first paper describing OC was presented at the Polymer Reaction Engineering Session of the 29th Canadian Chemical Engineering Conference [52]. It was later published in the ACS Symposium Series [53] and a short summary in the Journal of Polymer Science [54]. This initial work accomplished the following:

- The feasibility of the method was demonstrated by using styrene n-butyl methacrylate copolymers and parent homopolymers to show a composition based separation.

- OC was shown to provide a direct approach to elucidating the *"shape function"* (shape of the chromatogram of a single molecular weight). That is, if a commercially-available narrow polymer standard was injected into the first GPC and sampled at its peak for injection into the second GPC, the chromatogram from the latter instrument is that of a very narrow fraction of polymer. This approach then enables HPLC measures of resolution for GPC which have been heretofore prohibited because of the lack of sufficiently narrow molecular weight standards.

- A consistency test to assess errors caused by inadequate resolution in the first GPC was developed. It involved running both instruments with the same GPC solvent and obtaining a size fractionation in both.

- Equations from free radical polymerization kinetics for property distributions of linear copolymers polymerized to high conversions were developed so as to provide predictions which could be compared to anticipated OC quantitative results. This enables developments in polymerization kinetics to assist OC development and vice-versa.

This early work also showed that several problems had to be overcome before OC could provide reliable quantitative information. These problems were:

- As anticipated, the concentration of the injected solution was a critical variable. To obtain detectable outputs on the second instrument while avoiding poor resolution in the first, as many as 12 GPC columns were used in the first instrument. This resulted in very long analysis times.

- Fixed wavelength UV detectors provided minimal information for identification of the different compositions exiting from the GPC. Complex samples and errors inherent in dual-detector operation resulted in significant interpretation difficulties.

At the first presentation of the method at the ACS Conference [55], a Hewlett Packard 8450 Diode Array UV/Vis Spectrophotometer was used to identify the peaks as they eluted. Also, analysis times were significantly reduced when it was realized that only 3 high resolution columns and comparatively low injection concentrations were needed. The reason for this was that dilution and axial dispersion within a large number of columns nullified the desirable aspects of high concentration injections. The consequence of this change was a 50% decrease in analysis time (from 1 hour to 30 minutes for the first analysis). Furthermore, very recently [56], in examining the effect of concentration on the separation of two homopolymers (polystyrene and poly n-butyl methacrylate), it was demonstrated that concentration tolerance was much higher than expected. However, at the same time added

complications were encountered in this recent work in that it was found that the tetrahydrofuran (THF) used in the first GPC significantly influenced the separation obtained in the second. The THF effectively provided a solvent gradient that enhanced separation. This effect may account for the observed concentration tolerance since gradient elution is known to permit "*preconcentration*" of solute [21, 22, 43, 45]. However, with polymers this also meant that classical approaches to calibrate referencing retention volumes were very difficult to implement. The quantitative use of the rapid scanning UV/Vis detector was shown to provide a dynamic method of calibrating. That is, each sample essentially provided its own composition versus retention time relation. A strategy for dealing with quantitative interpretation by utilizing such detectors based on absorbance ratioing was developed and exemplified by calculation of differential copolymer composition distribution.

5. STATUS OF OC

5.1 Fractionation

Figs. 1 and 2 show schematic presentations of an OC system. The outlet of the first GPC is connected to the injection valve of the second. At any time, flow of the first instrument can be stopped and a "*slice*" of the chromatogram from the first GPC can be injected into the second. To-date tetrahydrofuran has been the most effective solvent run in the first with blends of tetrahydrofuran and n-heptane run in the second. Fig. 3 shows the best fractionation obtained to-date. The azeotropic copolymer (53.3% styrene n-butyl methacrylate, with a very narrow composition distribution) is shown to be clearly separated from each of its homopolymers. The results were obtained by OC analysis of a solution of the three polymers injected into the first GPC. The power of the solvent composition in the second GPC to affect the separation is aptly demonstrated. Our rationale for the basis of such OC fractionation of linear copolymers is discussed in the following

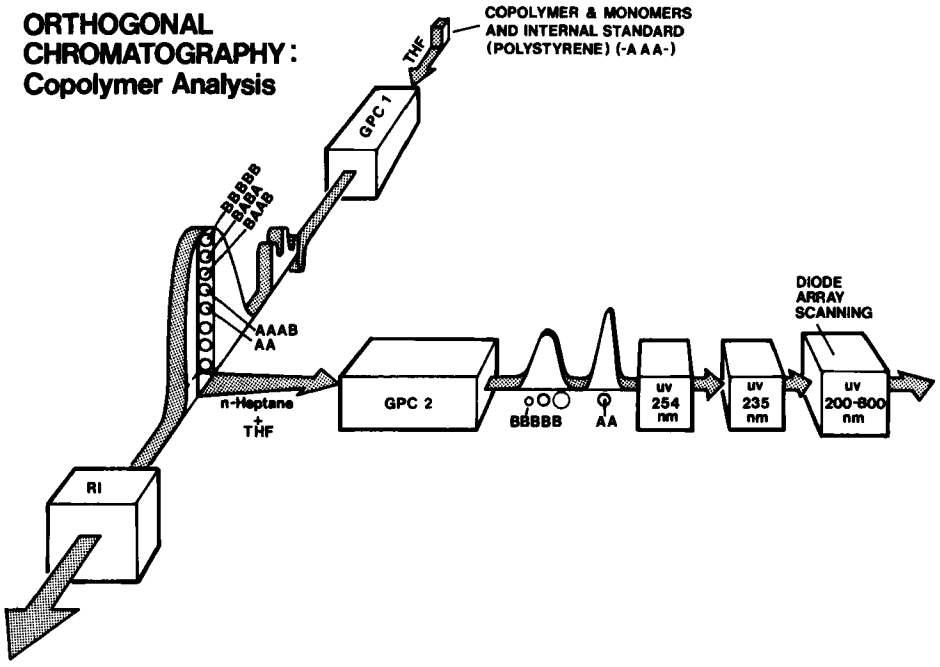

FIGURE 1

Schematic presentation of an OC System showing size fractionation of a linear copolymer by GPC #1 and the variety of molecules of the same molecular size within a chromatogram *"slice"* (in this case A refers to styrene units and B to n-butyl methacrylate units).[56]

paragraphs. If we assume that in GPC #1 conventional molecular size separation and adequate size resolution is obtained, then for molecules within the chromatogram *"slice"* (i.e. in the injection loop of the second GPC):

- a variety of molecular weights, copolymer compositions, and/or sequence lengths may be present;
- all molecules are the same molecular size;

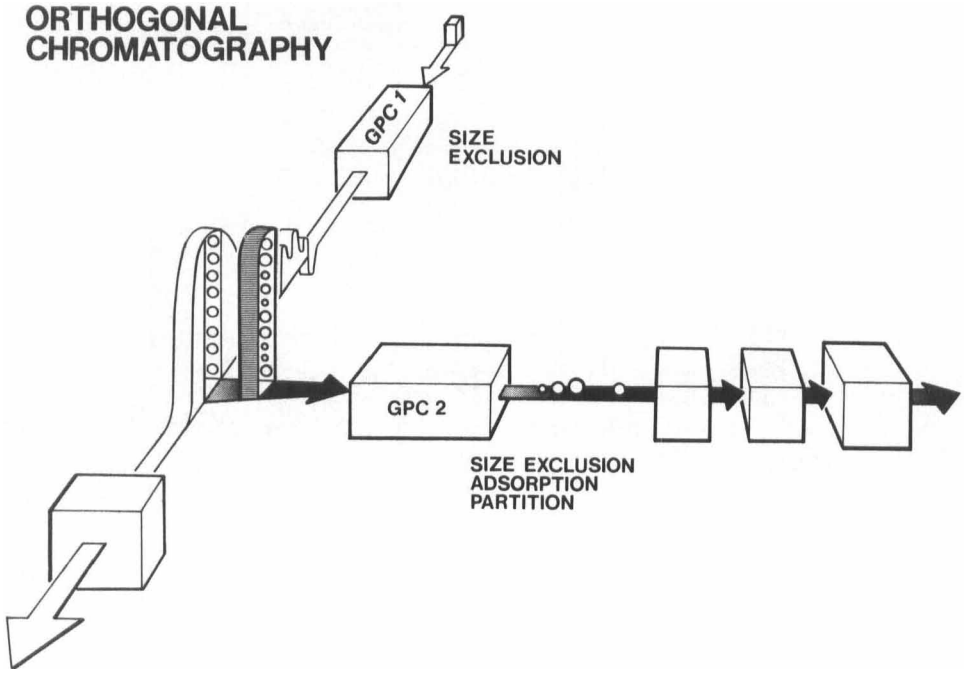

FIGURE 2

Schematic presentation of an OC System showing the separation mechanisms involved.[56]

- if differences in molecular weight are present, they are always associated with a difference in composition and/or sequence length.

Evidence for the validity of the last point is as follows:

- If we consider injection of blends of different homopolymers, two homopolymers would result in at most 2 different molecular weights within the slice, 3 homopolymers would result in at the most 3 different molecular weights and so on;

- The relationship between molecular size (hydrodynamic volume and molecular weight) is

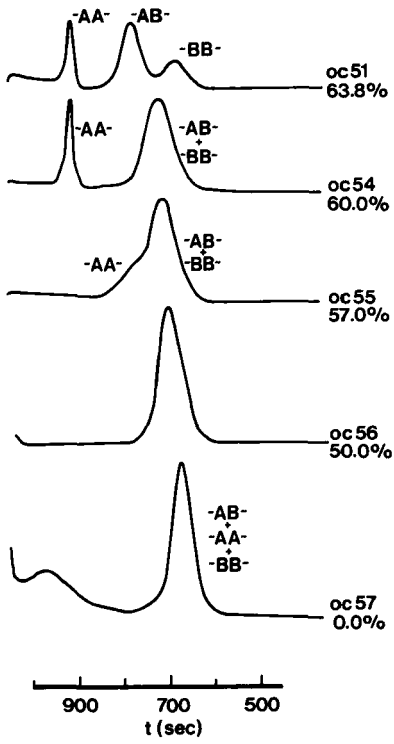

FIGURE 3

Fractionation of a 3-polymer mixture by OC showing the effect of % n-heptane in the solvent of GPC #2 (AA: polystyrene, AB: polystyrene n-butyl methacrylate, BB: poly n-butyl methacrylate).[56]

$$V = KM^{a+1} \quad (2)$$

where

V = molecular size (hydrodynamic volume) in solution

K,a = Mark-Houwink constants

M = molecular weight

Since changes in composition and/or sequence length are expected to affect the Mark-Houwink constants, and hydrodynamic volume is constant within the slice, changes in these constants will be reflected by a change in molecular weight.

This last point explains how one dimension of the analytical problem, molecular weight, is removed even though the first GPC is separating on the basis of molecular size rather than molecular weight.

With the solvent combination run through GPC #2, molecular size exclusion, adsorption and partition are assumed to be present. Then, in that GPC, for a specified column packing:

- the pore volume available to a given polymer molecule and the surface area which it sees for adsorption depends upon its molecular size in solution;
- the size of the molecule in solution and its adsorption coefficient as well as its solubility are functions of its molecular weight, composition and sequence length as well as the characteristics of the mobile phase in the second GPC.

As a result, when molecules of the same size are injected into the second instrument, they change in size in the new solvent and create a size distribution (note Figure 2) which influences the separation in the second GPC. In the analysis of linear polystyrene n-butyl methacrylate described above since n-heptane is a non-solvent for polystyrene, when injected into the second GPC the styrene rich molecules will shrink from their original size in pure THF while n-butyl methacrylate rich molecules will be relatively unaffected. Furthermore, if the THF preferentially tends to fill the pores in the packing and to coat surface area used for adsorption, then styrene rich molecules would be more attracted to the stationary phase than would n-

butyl methacrylate rich molecules. A synergistic effect then can result among separation mechanisms since the styrene rich molecules are smaller, permeate more pores and see more surface area than do the n-butyl methacrylate rich molecules. This can be carried one step further if the solvent in GPC #1 is chosen such that styrene rich molecules are smaller in molecular weight than n-butyl methacrylate rich molecules of the same molecular size in THF. In this case, if in pure THF the styrene rich molecules within the *"slice"* of chromatogram of GPC #1 are smaller in molecular weight than n-butyl methacrylate rich molecules, then the difference in molecular *size* in THF/n-heptane (the GPC #2) solvent will be even greater and will contribute to the synergism. This rationale can readily be expressed mathematically by using Equations (1) and (2).

Although the fractionation mechanism described above represents a very useful working hypothesis in agreement with much previous work several uncertainties can readily be identified. The affinity of THF for the packing, the role of the solvent injected with the polymer into GPC #2 and recent adsorption theory [57] emphasizing the importance of small pores, all represent future areas for investigation.

The fractionation which we are attempting to accomplish utilizing OC can also be explained by representing a linear copolymer as a contour map on a ternary diagram. Figure 4 shows such a diagram. Each contour represents a different concentration. Composition, sequence length and molecular size in solution are plotted on each axis. For convenience, this figure is shown as a triangle with the individual property distributions plotted along each side. However, it should be noted that no constraints on the range of each variable is implied. In other words, in a real situation some points could be plotted beyond the boundaries of the triangle. In conventional GPC, fractionation proceeds vertically down the ternary diagram beginning at the vertex marked "*maximum size*". When the contour map of the sample is

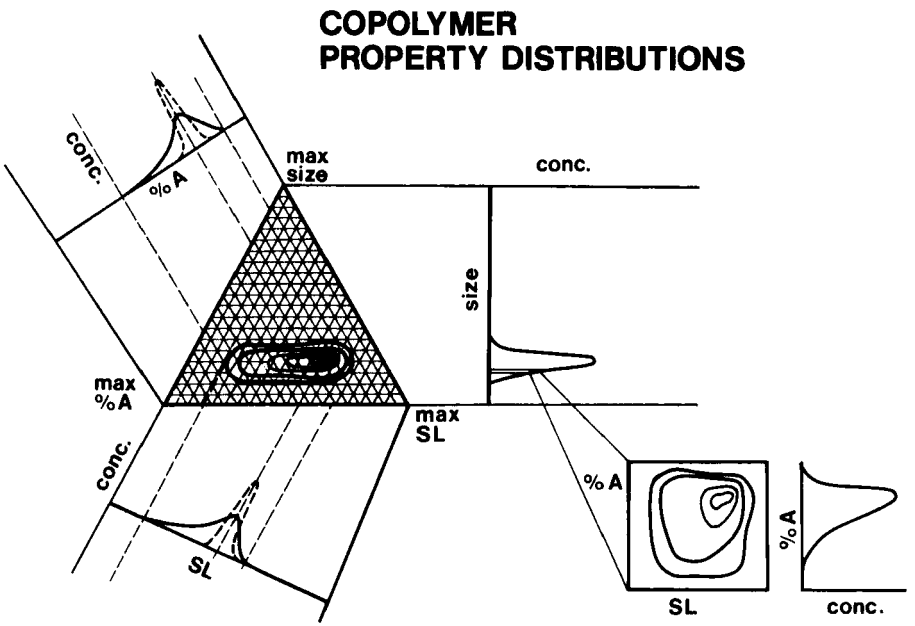

FIGURE 4

Property Distributions in a Linear Copolymer.[56]

encountered, it is evident that, at each molecular size, there is a variety of sequence lengths and compositions (and implicitly molecular weights associated with these variations). Multiple detectors viewing this variety of molecules would be forced to represent all of them at each value of hydrodynamic volume by average property values (e.g. average composition or average sequence length). OC attempts to remedy the situation by further fractionating the molecular size distribution. A slice of this latter distribution is represented as a two-dimensional plot of composition as a function of sequence length. The second fractionation in OC is currently aimed at fractionating the latter two-dimensional situation according to composition. As previously mentioned, the molecular weight dimension is removed because of its association with the other properties present.

Conditions for a successful first fractionation (with respect to molecular size) have been established for linear homopolymers since standards of known molecular size are available [17]. However, for other polymers, notably copolymers, standards are unavailable commercially and more uncertainty exists [5, 58]. Despite this situation, we can infer conditions from homopolymer results and OC allows the testing of the effect of changing column packings to examine possible violations of Universal Calibration using the consistency test for inadequate resolution mentioned previously (Section 4). Fractionation with respect to composition is the source of much greater concern since sequence length can interfere with both the composition fractionation in the second GPC and with detection for quantitative results.

5.2 Quantitative Detection & Interpretation

Figure 5 shows a copolymer composition distribution of polystyrene n-butyl methacrylate containing 0.235 wt. fraction styrene measured using OC by summing the distributions obtained from the second GPC for each *"slice"* across the molecular size distribution. The distribution peak centres on the expected styrene content and the component curves are in an order consistent with kinetic considerations. However, the curve shape is not at all the skewed shape expected from kinetic models. Major uncertainties exist in both untested kinetic model assumptions and in the new analytical measurement. The measurement uncertainties now centre about development of detector interpretation.

Our ability to fractionate polymers according to a given property distribution first depends upon our ability to detect the fractionation efficiency involved. The use of the rapid scanning diode array UV/Vis spectrophotometer is capable of providing sufficient information to resolve this problem for many linear copolymers. There is now increasing evidence that, depending upon

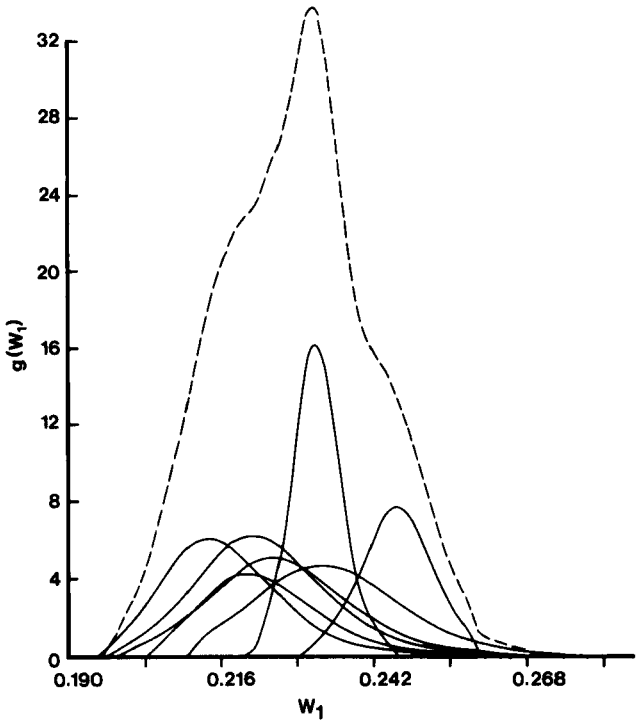

FIGURE 5

Calculated copolymer composition distribution of polystyrene n-butyl methacrylate (23.5% styrene) showing component distributions obtained for different slices of the chromatogram from GPC #1.[56]

the solvent used, and the polymer involved, the complete UV scan can elucidate concentration, composition and sequence length [59, 60, 61]. The present method [56] of utilizing this detector in OC involves isolating regions of the spectra, determining what properties affect these regions, and averaging over the region to increase precision at low absorbances and absorbance ratioing. With the polymers examined to date, a sequence length effect was undetectable and the scan was used to provide information only on concentration and composition.

6. FUTURE OF OC

For many years, detector development and the use of several detectors attached to a GPC has been the major thrust in chromatographic analysis of complex macromolecules. The development of OC marks an attempt to emphasize fractionation as well as detection. It integrates recent advances in HPLC with those of GPC along with knowledge from the synthesis of macromolecules to formulate a general approach to the problem. In particular, it is hoped that the concept will further encourage the transformation of traditional liabilities into advantages. For example, based upon our understanding of the fractionation mechanism, in OC the presence of molecular weight distribution can be employed to enhance composition separation. Also, the confounding of detector response by sequence length can be unravelled to provide information on sequence length as well as on the other properties when complete detector scans are available at each retention time from a well-fractionated macromolecule. Recent developments in UV interpretation are particularly applicable [5, 59, 60, 61] as are investigations of size fractionation requirements for copolymers [5, 58] and further focus on developing polymerization kinetic models to predict information in the form generated by new chromatographic techniques [5, 61]. Furthermore, the detector interpretation advantages of obtaining the desired fractionation before detection are becoming increasingly evident [57]. Light scattering applied to copolymers is a notable example where interpretation when all the molecules in the cell are identical is a much more tractable problem than the usual GPC situation [30].

In future, extension of OC to other than linear copolymers should provide some exciting new insights. OC analysis of branched macromolecules to determine distribution of branch lengths or branching density appears very feasible. Biological macromolecules and other water soluble macromolecules form a whole other area for investigation. Furthermore,

since GPC is also used to fractionate particles ("*Hydrodynamic Chromatography*"), OC could be employed to cross fractionate latex particle fractions.

More complex versions of OC are also easy to visualize. Use of gradient elution techniques or a third cross fractionation both appear as future developments in tailoring fractionation. In detection, further use of scanning detectors, sometimes with *"stop flow"* operation is anticipated.

OC was formed by the association of many previous advances in Separation Science. It widens our perspective of many analytical problems and represents one more step towards integrating large classes of separation methods.

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