

Recent advances in the characterization and analysis of polymers and polymer particles by size exclusion chromatography

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Abstract - Major recent developments in SEC as applied to mainly synthetic polymers, both hydrophobic and hydrophilic, and in the chromatography of latex particles are discussed in this paper. In view of the burgeoning literature in the field, a major review is not attempted. Instead an overview of each subject is given and some topics of particular interest are amplified. In respect of hydrophobic polymers, particular attention is paid to the application of modern, sophisticated multi-detector systems and to advances in calibration techniques. The main slant in the discussion of particle chromatography is a comparison between SEC and hydrodynamic chromatography (HDC); two complementary techniques. The section on aqueous SEC (hydrophilic polymers) focusses mainly on polyelectrolytes. Since cationic polymers have to date received scant attention, a comprehensive review is given in this case.

INTRODUCTION

This is a review of recent published works on the size exclusion chromatography (SEC) of polymer molecules in solution and polymer particles in suspension in the submicron range. This review covers synthetic polymers but virtually neglects the vast amount of published work on the gel filtration chromatography of natural polymers. The main emphasis here is on the characterisation of such polymer and polymer particle types as have been found difficult to examine quantitatively. Examples include heterogeneous copolymers, polymers with long chain branching, polyelectrolytes, microgel and latex particles). The reader is referred to a number of recent texts on SEC for information on advances in the many different aspects of SEC not covered here (separation mechanisms, packing materials, mobile phases and many others (refs. 1-4)).

The review begins with a discussion of detector systems, introduces some general relationships which can be used with detectors based on light scattering, UV and IR spectrophotometry and on viscometry and then refers to recent publications which have employed these detector types. Also considered are special applications of SEC such as for the characterization of heterogeneous copolymers, measurement of microgel content in latex polymers, molecular weight characterization of polyelectrolytes and the measurement of the size distribution of latex particles.

DETECTOR SYSTEMS

Introduction

The most promising and versatile SEC detector systems to date are the low angle laser light scattering photometer (LALLSP) coupled with a mass concentration detector (MCD) and a viscometer (VISC) coupled with an MCD. The MCD may be a differential refractometer or a UV or IR spectrophotometer. These detector systems are unique in that they give a measure of the molecular weight of the contents of the detector cell ($M_w(V,Uc)$ and $M_v(V,Uc)$ or $[\eta](V,Uc)$) and thus provide valid measurements of M_w , M_v and $[\eta]$, the weight-average and viscosity-average molecular weights and intrinsic viscosity of the whole polymer sample. Corrections for peak broadening need not be made and in fact the molecular weight values obtained should be independent of the resolution of the SEC (hence the term absolute detector system). These detector systems may also be used for molecular weight and peak broadening calibration and can detect extremely small quantities of microgel in polymer samples.

Multiple wave-length UV and IR spectrophotometers when properly calibrated, can be used online with SEC to investigate detailed microstructure of polymer chains at different molecular weight/molecular size levels (copolymer composition, sequence lengths, chain ends and others). These analytical techniques will no doubt become of increasing interest as the production of specialty high performance polymers continues to expand.

General relationships

Universal molecular weight calibration.

At this point, it is appropriate to define simple and complex polymers in the context of SEC. A simple polymer is one for which a unique relationship between the size of polymer solute in the mobile phase (radius of gyration, hydrodynamic volume) and its molecular weight exists. All other polymers are complex. Equation (1) is a statement of the universal molecular weight calibration curve with polystyrene as the standard polymer used for its measurement.

$$[\eta](V)M_N(V) = [\eta]_{PS}(V) M_{PS}(V) \quad (1)$$

where $[\eta]_{PS}(V)$ and $M_{PS}(V)$ are the intrinsic viscosity and molecular weight of polystyrene in the detector cell at retention volume V . $[\eta](V)$ and $M_N(V)$ are the intrinsic viscosity and number-average molecular weight of some other polymer. For the special case where the polymer in question is simple, $M_N(V)$ can be replaced by $M(V)$ and one then obtains the original Benoit universal calibration curve. In the derivation of eqn. (1), it is assumed that all of the polymer molecules in the detector cell have the same hydrodynamic volume (same $[\eta]M$) and of course this is true when peak broadening is negligible. When peak broadening is significant the appropriate symbols for intrinsic viscosity and molecular weight are $[\eta](V, U_c)$, $M_N(V, U_c)$, $M_W(V, U_c)$ and so on.

Estimation of molecular weight/molecular size dispersity of polymer in the detector cell

The effective use of molecular weight detectors and detectors for the measurement of polymer chain microstructure requires a knowledge of the dispersity of polymer solute size and molecular weight in the detector cell.

The distribution function $W(V, Y)$ given by eqn. (2) gives a measure of molecular size dispersity of polymer solute in the detector cell.

$$W(V, Y) = \beta(V)C(V, Y) = W(Y)G(V, Y) \quad (2)$$

where $W(V, Y) dVdY$ is the area under the detector response at retention volume due to polymer solute with mean retention volume Y ($C(V, Y)$ is the concentration of this polymer solute and $\beta(V)$ is the detector response factor). The variance of $W(V, Y)$ (or $C(V, Y)$) with respect to Y gives a measure of the dispersity of polymer solute size in the detector cell. $W(Y)$ is the detector response corrected for peak broadening and $G(V, Y)$ is the instrumental spreading function (the normalized detector response for polymer solute with mean retention volume Y). Eqn. (2) clearly applies for a mass concentration detector (MCD) although $W(V, Y)$ can be expressed as a product of $W(Y)$ and $G(V, Y)$ for any detector type.

When peak broadening is not extreme, $W(V, Y)$ can be approximated by a Gaussian distribution function of the form:

$$W(V, Y) = \frac{F(V)}{\sqrt{2\pi\bar{\sigma}(V)^2}} \exp\left[-\frac{[Y - \bar{Y}(V)]^2}{2\pi\bar{\sigma}(V)^2}\right] \quad (3)$$

where $F(V)$ is a normalization factor. The mean and variance of $W(V, Y)$, $Y(V)$ and $\sigma(V)^2$ can be related to the variance of a Gaussian instrumental function as follows (ref. 5):

$$\bar{Y}(V) = V + \frac{1}{D_2(V)} \ln\left[\frac{F(V + D_2(V)\sigma(V)^2)}{\sqrt{F(V - D_2(V)\sigma(V)^2)F(V + D_2(V)\sigma(V)^2)}}\right] \quad (4a)$$

$$\bar{\sigma}(V)^2 = \sigma(V)^2 + \frac{1}{D_2(V)^2} \ln\left[\frac{F(V - D_2(V)\sigma(V)^2)F(V + D_2(V)\sigma(V)^2)}{F(V)^2}\right] \quad (4b)$$

where $\sigma(V)^2$ is the variance of the Gaussian instrumental function and $D_2(V)$ is the slope of the molecular weight calibration curve at retention volume V and is given by the molecular weight calibration curve:

$$M(Y) = D_1(V) \exp(-D_2(V)Y) \quad (5)$$

It is clear that $Y(V)$ and $\sigma(V)^2$ should not depend on $D_2(V)$ and the use of a Taylor series expansion shows this. For ease of calculation $D_2(V)$ is left in eqn. (4a,4b) and with any reasonable choice for $D_2(V)$ correct values for $Y(V)$ and $\sigma(V)^2$ are found. The mean and variance of $W(V, Y)$ and $C(V, Y)$ are the same and it has been shown that eqn. (4a,4b) can be used with either MCD or LALLSP detector responses to give the mean and variance of $C(V, Y)$ (ref. 6). This fact can also be used to investigate detector response distortion when the sample passes from one detector through tubing and into the second detector cell. Should values for $\sigma(V)^2$ obtained from the MCD and LALLSP detector responses using eqn. (4a, 4b) disagree, one might suspect that solute mixing occurs to a significant extent in the detector cells and connecting tubing (ref. 7).

Equations which may be used with either a mass concentration detector response or with a LALLSP detector response to estimate molecular weight dispersity in the detector cell follow:

Mass concentration detector

$$\frac{M_N(V, U_c)}{M(V)} = \frac{F_M(V)}{F_M(V + D_2(V)\sigma^2(V))} \exp\left[-\frac{[D_2(V)\sigma(V)]^2}{2}\right] \quad (6a)$$

$$\frac{M_W(V, U_c)}{M(V)} = \frac{F_M(V - D_2(V)\sigma(V)^2)}{F_M(V)} \exp\left[\frac{[D_2(V)\sigma(V)]^2}{2}\right] \quad (6b)$$

Low angle laser light scattering detector

$$\frac{M_w(V, Uc)}{M(V)} = \frac{F_L(V)}{F_L(V + D_2(V)\sigma(V)^2)} \exp\left[-\frac{[D_2(V)\sigma(V)]^2}{2}\right] \quad (7a)$$

$$\frac{M_z(V, Uc)}{M(V)} = \frac{F_L(V - D_2(V)\sigma(V)^2)}{F_L(V)} \exp\left[\frac{[D_2(V)\sigma(V)]^2}{2}\right] \quad (7b)$$

where $F_M(V)$ and $F_L(V)$ are responses for MCD and LALLSP detector, respectively and $M(V)$ is the molecular weight calibration curve. Eqn. (6a-7b) as written apply for simple polymers with the polydispersity in the detector cell $M_w(V, Uc)/M_n(V, Uc)$ approaching unity as peak broadening approaches zero. For complex polymers, $M(V)$ in eqn. (6a-7b) should be replaced by $M_n(V)$ and $M_w(V)$ and now as peak broadening approaches zero the polydispersity approaches $M_w(V)/M_n(V)$. It should be remembered that for a complex polymer there are an infinite number of molecular weight calibration curves, one for each molecular weight average. The deviation of $M_w(V)/M_n(V)$ from unity is a measure of the deviation from simple behaviour of the polymer in question. This deviation could be due to long chain branching frequency and length and composition variations for polymer molecules which have the same hydrodynamic volume. This of course results in a polydispersity greater than unity even in the absence of peak broadening.

The effect of peak broadening on the intrinsic viscosity of the polymer solute in the detector cell is given by:

$$\frac{[\eta](V, Uc)}{[\eta](V)} = \frac{F_M(V - aD_2(V)\sigma(V)^2)}{F_M(V)} \exp\left[\frac{[aD_2(V)\sigma(V)]^2}{2}\right] \quad (8)$$

and

$$[\eta](V) = KM(V)^a \quad (9)$$

where K, a are Mark-Houwink constants for a simple polymer.

Detectors for molecular weight measurementLALLSP/MCD and VISC/MCD detector systems.

The introduction of light scattering as a technique for polymer characterization (molecular weight and polymer chain dimensions) dates back to the beginning of this century when Steubing (ref. 8) and Mecklenberg (ref. 9) introduced a photometer that could measure the intensity of scattered light of a monochromatic, depolarized light beam. It took 25 years before Putreys (ref. 10) found an empirical relation between the scattered light intensity and the molecular weight of polymer molecules in solution (proteins in aqueous solution in this case). The pioneering theoretical work for light scattering photometry as an absolute method for the determination of molecular weights and sizes was done by Debye (ref. 11) in the early forties and Doty and Zimm (refs. 12-14) applied these theoretical results to polymer solutions. The scattered light intensity is a function of both the angle of measurement and the polymer solution concentration and therefore it was necessary to extrapolate the results to zero angle and concentration (ref. 15) (the Zimm plot) to determine the weight-average molecular weight of the polymer. Likely for this reason it took another 20 years before light scattering could be used to continuously monitor polymer molecular weights. Cantow (ref. 16) used a very sensitive photometer to measure continuously and simultaneously the scattered light intensity at two angles for rather dilute polymer solutions for which extrapolation to zero concentration was not required.

After the establishment of SEC as a valid and powerful tool for molecular weight characterization of polymers, attempts were made to collect fractions and measure the intrinsic viscosity of the collected polymer using Ubbelohde viscometers offline. Meyerhoff (refs. 17,18) and Goedhart and Opschoor (ref. 19) first employed this technique, in which a set of micro Ubbelohde viscometers were filled and emptied periodically by a siphon, to measure polymer molecular weights without the use of a molecular weight calibration curve from a prior calibration. However, with the introduction of high performance SEC with the associated small retention volume range, these offline techniques consumed too large a volume of mobile phase per fraction and thus became impractical. Ouano (refs. 20,21) developed a continuous viscometer that measured the pressure drop of the mobile phase with dissolved polymer solute as it flowed through a micro capillary tube. Although this technique required a flowrate with little variation and a highly sensitive pressure measurement, it has been used successfully by several workers (refs. 22-26, 38, 39). In 1984 the first micro differential viscometer suitable for use as a detector for SEC was made available commercially by Viscotek (ref. 27). A thorough evaluation of this viscometer by SEC users has yet to be published.

The most successful online molecular weight detector using low-angle laser light scattering photometry was developed about 10 years ago by Kaye (ref. 28). This photometer was commercialized by Chromatix and is sold under the trade name Chromatix KMX6. This photometer measures the intensity of scattered light at low angles with adequate signal-to-noise ratio for dilute solutions typical of those used with SEC and therefore extrapolation to zero angle and concentration is not necessary. In collaboration with Kaye, Ouano (refs. 29,30) evaluated this technique and showed that it had potential to be a most effective polymer characterization technique. With the commercialization of the Chromatix KMX6 in the late seventies (refs. 31-33), the SEC/LALLSP/MCD technique has proven to be a very powerful analytical tool with an ever increasing number of applications. It should be mentioned that Millaud and Strazielle (ref. 34) have des-

cribed the modification of a standard light scattering photometer for the continuous monitoring of molecular weight with SEC and Strazielle et al. (ref. 35) continue to evaluate this detector system with a number of typical applications such as the measurement of Mark-Houwink constants for linear polymers and the structure factor for branched polystyrene samples.

(i) Molecular weight and peak broadening calibration

Linear homopolymers and linear copolymers of uniform composition are simple polymers, and have a single molecular weight calibration curve. SEC/LALLSP/MCD and SEC/VISC/MCD are convenient techniques for its determination. This is particularly true for polymers for which narrow MWD standards are not available and whose SEC separation does not closely follow the universal molecular weight calibration curve as is often the case with aqueous SEC. SEC/LALLSP/MCD and SEC/VISC/MCD measure $M_w(V,Uc)$ and $[\eta](V,Uc)$ the weight-average molecular weight and intrinsic viscosity of the polymer solute in the detector cell at retention volume V . When peak broadening is negligible $M_w(V,Uc)$ can be set equal to $M(V)$ the molecular weight calibration curve and $[\eta](V,Uc)$ can be set equal to $[\eta](V)$ the intrinsic viscosity which appears in the universal molecular weight calibration curve (see eqn. (1)). If the universal calibration curve (eqn. (1)) based on polystyrene is known and is applicable to the polymer in question, eqn. (1) can be solved for $M(V)$.

Referring to eqn. (6b) one can see that for the MCD, corrections for peak broadening are likely to be smaller for the high-molecular-weight end (low-retention-volume end) of the detector response. The exponential factor on the right hand side of eqn. (6b) is always greater than unity while the pre-exponential factor is usually less than unity. The opposite is true for the low-molecular-weight end of the detector response. For very narrow MWD polymer samples, the pre-exponential factor is the dominant correction factor because the slopes of the detector response (dF_M/dV) near the point of inflection are quite large. The exponential factor would usually be in the range, 1-1.1 for an SEC with acceptable resolution. This explains why He et al. (ref. 36), who employed narrow MWD polystyrene standards with SEC/LALLSP/MCD to measure the polystyrene molecular weight calibration curve, were unsuccessful. Only when measured near the peak position, where dF_M/dV is small, does $M_w(V,Uc)$ come close to being on the true polystyrene calibration curve, $M(V)$. They found calibration curves whose slopes were always much smaller than that of $M(V)$. At the high-molecular-weight end of $F_M(V)$, $M_w(V,Uc)$ was smaller than $M(V)$ and at the low-molecular-weight end, larger than $M(V)$ in agreement with eqn. (6b). It is clear that a broad MWD polymer sample would have much lower local correction for peak broadening giving $M_w(V,Uc) \approx M(V)$ over a much wider range of retention volumes (or molecular weights). This strategy was employed by Kim et al. (refs. 6,37) who made up very broad MWD samples of dextran and nonionic polyacrylamide by blending existing polymer standards for use with SEC/LALLSP/MCD to determine the molecular weight calibration curve $M(V)$ for these polymers. A further investigation of this strategy was recently made by Hamielec and Meyer (ref. 7). A comparison between $M(V)$ measured using ten narrow MWD PS standards (using molecular weight versus peak retention volume) and $M_w(V,Uc)$ measured by SEC/LALLSP/MCD with a single moderately broad MWD polystyrene sample is given in Figure 1. It is clear that this single moderately broad MWD polymer sample permits SEC/LALLSP/MCD to measure $M(V)$ over a rather wide range of molecular weights.

Given the molecular weight calibration curve $M(V)$ and in particular its slope ($D_2(V)$), SEC/LALLSP/MCD and SEC/VISC/MCD can be used to measure the peak broadening parameters $\sigma(V)$, $\sigma(V)$ and $Y(V)$ using eqn. (6b,7a,8) and eqn. (4a,4b). In the case of SEC/VISC/MCD the Mark-Houwink constants for the simple polymer must also be known. Figure 2 shows $\sigma(V)$ values for dextrans using aqueous SEC/LALLSP/DRI and CPG packing and the DRI detector response (see eqn. (6b)) (ref. 37). A moderately narrow MWD dextran standard (Pharmacia) was used for the measurements. To accurately measure $\sigma(V)$ one should use quite narrow MWD polymer samples so that the ratio $M_w(V,Uc)/M(V)$

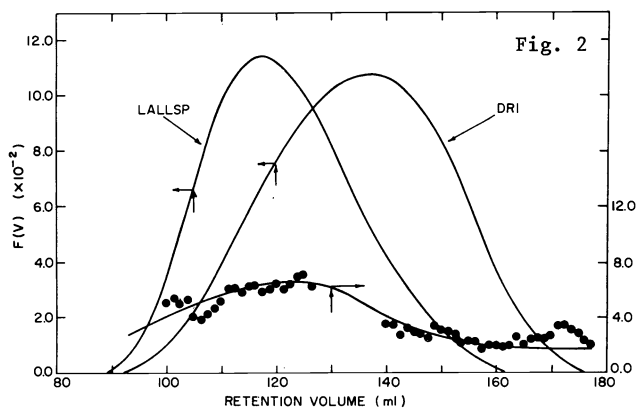
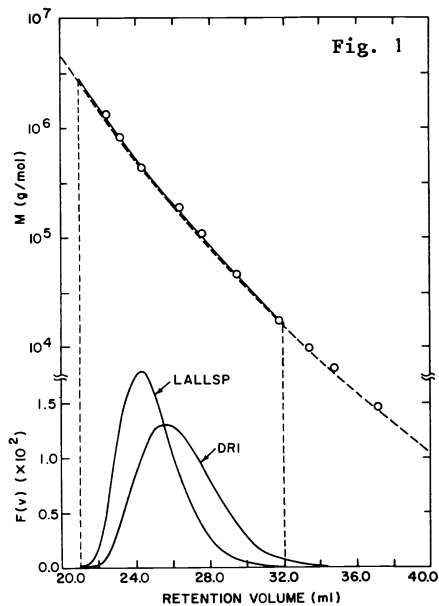


Fig. 2. Peak broadening parameter ($\sigma(V)$) for the Gaussian instrumental spreading function found using SEC/LALLSP/DRI and a moderately narrow MWD dextran sample. [37]

Fig. 1. Molecular weight calibration curve for polystyrene found using narrow MWD PS standards (—O—) and using one moderately broad MWD PS sample (—) with SEC/LALLSP/DRI. [7]

will be very different from unity (large $dF(V)/dV$) indicating sensitivity to peak broadening. To measure $\sigma(V)$ one should use eqn. (6b) at the low-molecular-weight end and eqn. (7a) at the high-molecular-weight end of the detector responses to get maximum signal-to-noise ratios at both ends. He et al. (ref. 36) measured $\sigma(V)$ accurately using SEC/LALLSP/DRI with a number of narrow MWD polystyrene standards. Kim et al. (ref. 6) used aqueous SEC/LALLSP/DRI and a number of moderately narrow MWD nonionic polyacrylamides and measured $\sigma(V)$ over a wide range of molecular weights. They then used eqn. (4a,4b) with both DRI and LALLSP detector responses to estimate $\sigma(V)$ and $Y(V)$ (peak broadening parameters for $C(V, Y)$). Values for $\sigma(V)$ and $Y(V)$ obtained using both detector responses were in excellent agreement showing that additional dispersion in the second detector cell and in the tubing connecting the two detector cells was negligible. A more detailed discussion of this subject may be found elsewhere (ref. 7).

(ii) Polymers with long chain branching.

Polymers with long chain branching should be treated as complex polymers. The SEC/LALLSP/MCD and SEC/VISC/MCD provide measures of $M_W(V, U_c)$ and $[\eta](V, U_c)$. When peak broadening is negligible one gets $M_W(V)$ and $[\eta](V)$ (corrections for peak broadening can be made using the principle of universal peak broadening calibration when necessary). Application of eqn. (1) provides $M_N(V)$ and one thus has the polydispersity $M_W(V)/M_N(V)$. The greater the deviation of the polydispersity from unity, the greater is the number of long branches per polymer molecule, $B_N(V)$. It should be remembered that the detector cell likely contains a mixture of linear and branched chains and the branch lengths and number of branches can vary from chain to chain, even though all the polymer solutes in the detector cell have the same hydrodynamic volume. Jordon et al. (ref. 40) and Jordan and McConnell (ref. 41) have pointed out that from SEC fractions the intrinsic viscosity ratio $g(V)$ at the same hydrodynamic volume is accessible where:

$$g(V) = [\eta]_b(V)/[\eta]_l(V) \quad (10)$$

According to the universal molecular weight calibration curve (Eqn. (1)):

$$g(V) = M_\ell(V)/M_N(V) \quad (11)$$

where the subscripts b and ℓ stand for branched and linear. The ratio of the radius of gyration of branched and linear polymer of the same molecular weight g_M is given approximately by:

$$g_M = \left([\eta]_b / [\eta]_l \right)_M^{\frac{1}{\epsilon}} \quad (12)$$

with the branching structure factor ϵ lying in the range, $0.5 < \epsilon < 1.5$. Expressing the intrinsic viscosity of branched polymer as

$$\ell n[\eta]_b = \ell n K + a \ell n M + b(\ell n M)^2 + c(\ell n M)^3 \quad (13)$$

where K, a are Mark-Houwink constants for linear polymer, we have

$$g_M^\epsilon = \left([\eta]_b / [\eta]_l \right)_M = \exp \left[b(\ell n M)^2 + c(\ell n M)^3 \right] \quad (14)$$

It can readily be shown that

$$g_M = \left(M_\ell(V) / M_N(V) \right)_M^{\frac{1+a}{\epsilon}} = g(V)^{\frac{1+a}{\epsilon}} \quad (15)$$

Eqn. (15) is the correct equation to use with SEC when employing the branching equations after Zimm and Stockmayer. SEC/VISC/MCD can provide a measure of $M_N(V)$ which then can be used with the Zimm-Stockmayer branching models to estimate $B_N(V)$ and then B_N for the whole polymer sample. Most previous workers including Foster et al. (ref. 42) who investigated branching in HPLDPE used eqn. (10) with the Zimm and Stockmayer branching model and of course this is not correct. Comparing long chain branching frequencies measured by NMR and SEC/DRI they found a branching structure factor of $\epsilon = 0.75$. When the correct eqn. (12) is used these data give $\epsilon = 1.28$ for the HPLDPE samples investigated. A further study of long chain branching in polyethylene has recently been carried out by Rudin et al. (ref. 43).

To fully characterize polymers with long chain branching one should use SEC/LALLSP/VISC/MCD. In principle, with this detector system one can measure $C(V)$, $[\eta](V)$, $M_N(V)$, $M_W(V)$ and $B_N(V)$ when used along with the universal calibration curve. These quantities, when integrated with respect to retention volume V , provide these same molecular properties for the whole polymer sample.

Detectors for microstructure measurement

(i) Copolymer composition

Garcia-Rubio et al. (refs. 44-46) have recently reviewed the use of SEC/UV/DRI and reported composition and styrene sequence lengths for SAN copolymers. Another recent review is given by Quivoron (ref. 47).

For homogeneous copolymers the measurement of copolymer composition as a function of molecular weight (or hydrodynamic volume) using SEC/UV/DRI or SEC/IR/DRI is straightforward. However, for heterogeneous copolymers (complex polymers) copolymer molecules having the same hydrodynamic volume can have varying compositions and molecular weights. This can present serious problems with detector response factors which may depend on copolymer composition and sequence-length distribution. It is not clear how these problems can be solved with traditional SEC. However, Balke et al. (ref. 48) recently proposed a new "cross or orthogonal" chromatography to measure copolymer composition by connecting two SEC instruments, each running with a different mobile phase. Copolymer molecules are first separated on the basis of size using one mobile phase and then cuts are injected into a second SEC with another mobile phase and a second size separation is done. If the copolymer molecules in the cut from the first SEC are heterogeneous in composition the second mobile phase, if properly selected, will provide a size distribution which depends on the composition spread of the copolymer.

(ii) Copolymer sequence length distribution

Although SEC/UV/MCD and SEC/IR/MCD of copolymers for composition measurement have been extensively used, it is with the development of new and powerful techniques (infrared lasers, FTIR, multiple-scan IR(MIR) and others) that the analysis of SEC effluents has become a more useful technique. IR spectroscopy is more general than RI or UV since almost every organic molecule will show some form of absorption in the IR region. A necessary step in the use of spectroscopy data is the assignment of absorption bands to specific functional groups. If multiple wavelength UV or IR detectors are used with SEC the measurement of copolymer composition and sequence-length distribution across the detector responses and thus as a function of molecular weight (or hydrodynamic volume) are possible in principle. Garcia-Rubio et al. (refs. 44, 45) using SEC/UV/DRI have demonstrated this for styrene/acrylonitrile copolymers.

MACROMOLECULAR CHAINS AND PARTICLES IN AQUEOUS ELUANTS

General introduction

The first reported chromatographic separation of macromolecules according to their size (ref. 49) was on an aqueous system; polydextrans were chromatographed using a semi-rigid, porous polysaccharide gel. The subsequent development of aqueous size-exclusion chromatography (SEC) to the point of being capable of providing rapid and accurate estimates of average molecular weights (MWs) and distributions (MWDs), as is feasible for many synthetic polymers in organic solvents, has, however, been relatively slow. The main reason for this is that, superimposed on the basic steric-exclusion mechanism which has become accepted for conventional SEC, are a number of non-steric effects. In order that the column packing materials be compatible with aqueous eluants, charged or polar groups are required at the packing surface. Such polar groups are also those which confer water-solubility on macromolecules, as well as stability on hydrophobic colloids. Interactions between the charged/polar groupings give rise to the non-steric-exclusion effects which fall into the following four basic categories: adsorption and, specifically in the case of ionic polymers, ion exchange, ion exclusion and ion inclusion, sometimes referred to as Donnan effect. These phenomena are well documented and explained in the several review articles covering the field of aqueous SEC (refs. 50-54). A further complicating factor in the aqueous SEC of polyelectrolytes is their highly salt-sensitive hydrodynamic properties. Eluant ionic strength and the degree of ionisation of the polymer molecule influence very strongly the degree of expansion of the polymer chains. The conformation of certain nonionic macromolecules, such as polyethylene oxide (ref. 55) is also affected by the presence of salts. Since each of these above effects varies in relative magnitude from system to system, the basic tactics employed in achieving successful separation of species according to their size involve the "tailoring" of chromatographic conditions, viz. choice of packing materials, eluants and modifiers, to suit the particular case. With this in mind, the following sections discuss the aqueous SEC of the various classes of compounds. Basic concepts are introduced and some recent developments are described.

PARTICLE CHROMATOGRAPHY

Introduction

Until relatively recently, chromatographic techniques, including SEC, have been concerned with the separation of matter at the molecular level. The chromatographic separation of aqueous colloidal suspensions was first reported by Krebs and Wunderlich in 1971 (ref. 56), using columns packed with porous glasses. Small demonstrated in 1974 that such systems could also be separated by passage through columns packed with non-porous spherical beads which were much larger than the latex particles (ref. 57). The term hydrodynamic chromatography (HDC) has been coined for the case when non-porous packings are used. The two methods, HDC and SEC may be regarded as complementary in the sense that solute particles normally elute in order of decreasing size. More recently still, two further chromatographic techniques, capillary chromatography (CPC) (ref. 58) and field-flow fractionation (FFF) (ref. 59) have been developed. The relative merits and demerits of SEC vs. HDC vs. FFF as methods of particle separation have been discussed by Yau and Kirkland (ref. 60). It is, however, the intention of this article to concentrate on SEC and HDC. A comprehensive review of the field has recently been published by Penlidis, et al. (ref. 61).

The mechanisms of separation involved in the two techniques are best explained with reference to Figure 3. In SEC, larger particles are more excluded from the pores of the packing material than smaller ones, thereby eluting ahead. In HDC, a capillary flow regime is assumed to exist in the interstitial flow channels. The eluant velocity tends to zero as the capillary wall is approached. Since larger particles can access the faster moving flow channels away from the walls, they once again elute ahead of the smaller ones.

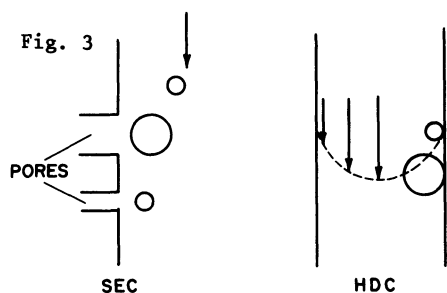


Fig. 3. Illustration of the mechanisms of separation involved in SEC and HDC.

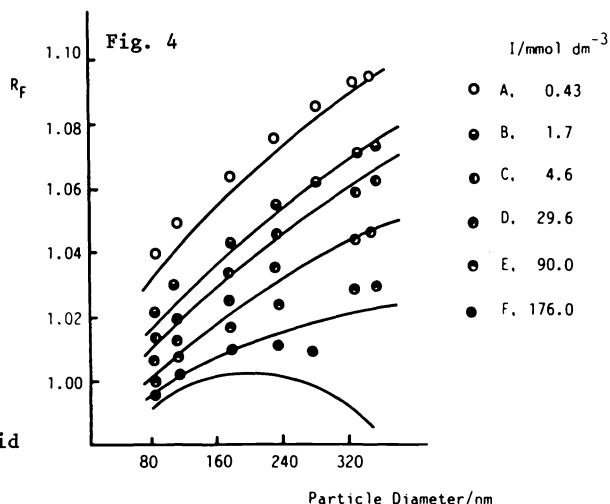


Fig. 4. Comparison between calculated (solid lines, [69]) and measured (points, [57]) values of R_F in HDC.

Separation on both non-porous and porous packings has been shown to be a function of eluant ionic strength (ref. 57, 62-66). This ionic-strength dependence of elution volume arises from the repulsive interactions between the electrical double layers which surround the colloidal particles and the column packing materials; both of which carry electrical charges at the surface.

HDC

HDC was discovered in the laboratories of Dow Chemical Co. (ref. 57) as a result of efforts to satisfy a perceived need (ref. 67), i.e. to reduce the time involved in measuring the size of colloids. The idea of extending SEC to colloids was considered, but discarded in view of the low diffusivity ($D \approx 10^{-9} \text{ cm}^2 \text{ s}^{-1}$) of the $1 \mu\text{m}$ solute particles that were of interest at the time. Poor mass transfer of colloids into particles of the requisite porosity would, it was argued, lead to unacceptable peak broadening and hence difficulties in estimating particle-size distributions (PSDs). With this motivation of direct industrial application in mind, rather more effort has been expended on the development of HDC applied to colloids than SEC. The technique was patented by Small (ref. 68) in 1975 and licensed to the Micromeritics Corporation in 1977 for commercialisation. A mathematical model has been developed by Silebi and McHugh (ref. 69), which successfully accounts for the ionic-strength dependence of the elution parameter R_F in HDC. The original experimental data of Small (ref. 57) are shown together with the curves predicted by the model in Figure 4. R_F is defined as:

$$R_F = \frac{\text{Rate of transport of colloid through columns}}{\text{Rate of transport of marker through columns}} \quad (16)$$

The initial decrease in R_F with increasing ionic strength, I , is a result of the decrease in effective particle diameter due to shrinkage of the double layers. At high values of I however, the double layers become so compressed that van der Waals attractive interactions between solute and packing begin to occur. This qualitatively explains the behaviour observed in Fig. 2 for the data obtained at $I = 0.176 \text{ M}$.

The method used so far to obtain particle-size and PSD-estimates by HDC has been the following. First a calibration curve is established by chromatographing colloidal standards, typically polystyrenes of narrow PSD, at a fixed ionic strength. The universality of such a calibration for spherical colloids of differing chemical types has been reported (refs. 69,70). An unknown sample is then chromatographed at the same ionic strength. Methods for calculating the PSD directly from the chromatogram using the polystyrene latex calibration have been disclosed. This method is further commented on in the section pertaining to SEC.

Recent improvements in the technology of HDC have led to the design of instruments which are capable of routinely analysing colloidal samples within 6 min. (ref. 73), compared with approximately 1.5 hr. which was previously required. The integrated, computerised HDC then calculates the actual PSD of the sample in an additional 3-5 minutes.

In the decade or so which elapsed between the early observations of Porath and Flodin (ref. 49) and the final acceptance of the equilibrium mechanism (ref. 2) of solute retention in the SEC of polymer molecules, the "separation-by-flow" mechanism forwarded by DiMarzio and Guttman (ref. 74) enjoyed a brief period of serious consideration. This mechanism, which is essentially that occurring in HDC, was rejected on the basis of the results of static mixing experiments, and dynamic experiments in which polymer molecules were not observed to be separated according to their size upon passage down a column packed with non-porous material. This has long been regarded in a general sense as one of the limitations of HDC. However, recent publications by Prud'Homme et al. (refs. 75-77) disclose the use of HDC to size-separate various water-soluble polymers of high MW. Their interest has focussed upon xanthan gums, partially hydrolysed poly(acrylamides) ($\text{MW} = 2 \times 10^5 - 1.8 \times 10^7 \text{ g.mol}^{-1}$) and dextrans ($\text{MW} \approx 2 \times 10^6 \text{ g.mol}^{-1}$). Successful separations have been achieved. The data obtained from the xanthan separations have been interpreted in such a manner as to predict "apparent hydrodynamic volumes", based on a polystyrene latex calibration (ref. 76). Similar experiments have been performed by Lecourtier and Chauveteau (ref. 78) on two xanthan gum samples of approximate average MW 2 and $3 \times 10^6 \text{ g.mol}^{-1}$. Estimates of MWD were also reported, with good reproducibility.

A series of articles of relevance to this field have been published in the Japanese language by Shiibashi et al. (refs. 79-81). At the time of writing they are not available in the English language. In the first (ref. 79), the HDC of latex particles on columns packed with ion-exchange resins was proposed for determining particle sizes within 30 minutes. In the second (ref. 80), non-spherical latices were chromatographed on a similar instrument and their average diameters evaluated, based on the calibration obtained for spherical polystyrene latices. In the third (ref. 81), successful separations of the flexible-chain polymers sodium poly(styrene sulphonate), NaPSS, and polyethylene oxide were reported on the same non-porous packings.

SEC

Research into the use of SEC for latex particle-size determination, as was mentioned earlier, has been somewhat low key relative to endeavours in the field of HDC. This has been due at least partly to the peak broadening inherent in the technique. (The literature pertaining to the subject is well-covered by Penlidis et al. (ref. 61) up to 1983). However, SEC does have one distinct advantage over HDC, namely much better resolution. For example in one HDC system used by Silebi (ref. 82), comprising three columns packed with spherical poly(styrene-co-divinyl benzene) beads, the typical peak elution volume for a latex was approximately 60 cm³, whilst the peak separation between a latex of diameter 380 Å and one of 3570 Å was approximately 1.5 cm³. However, in the SEC system employed by Styring et al. (refs. 65,66) comprising four columns packed with porous borosilicate glass gels, latex elution volumes varied between approximately 95 and 120 cm³ depending upon ionic strength. The peak separation between a latex of 300 Å diameter and one of 900 Å diameter was some 20 cm³ at an ionic strength of 107.1 mmol.dm⁻³. The purpose of the study by Styring et al. was a thorough investigation of the ionic-strength dependence of elution volumes for a series of well characterised, monodisperse polystyrene latices. The elution behaviour of the latices was shown to be consistent with the concept of an effective exclusion radius, r_e , which is the sum of contributions from the core diameter of the latex (measured by transmission

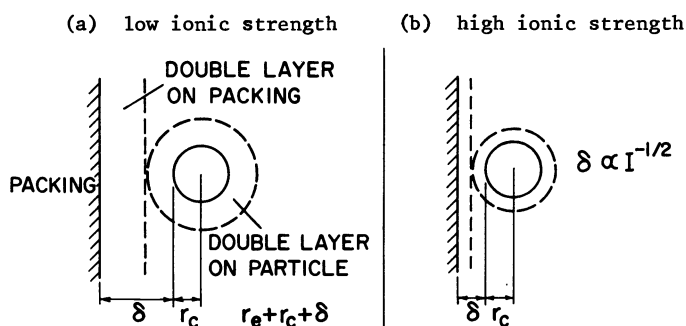


Fig. 5. Illustration of the contribution of the electrical double layers on both solute and packing to the effective exclusion radius (r_e) of the solute particle.

electron microscopy), r_c , plus a contribution, δ , from the double layers surrounding both particle and packing. This situation is illustrated in Fig. 5. Application of the Gouy-Chapman approximation predicts that δ should be proportional to $I^{-1/2}$ and further, assuming r_e to be the steric-exclusion parameter, that V_R , the latex retention volume should vary linearly with $I^{-1/2}$ (V_R increases as I increases). This was well borne out by experimental observations. It was inferred from this that the core radius will determine separation only when the double-layer thickness is zero, i.e. when $I^{-1/2} \rightarrow \infty$. This condition cannot, of course, be approached experimentally, but may be examined by extrapolation of data obtained at finite values of I to $I^{-1/2} = \infty$. Acceptance of these tenets leads to a reconsideration of the technique of calibration of both SEC and HDC systems. The value of δ , the composite double-layer thickness (i.e. packing plus solute) depends not only on I , but also upon the surface potentials, ψ_0 , on both packing and solute. The surface coverage of latices by ionic surfactants is well known to vary with the chemical nature of the latex (refs. 83,84). The area per surfactant molecule increases with increasing hydrophilicity. Correspondingly the surface charge density and hence ψ_0 decrease. Thus, if all other factors are kept constant, the double layer surrounding, say, a latex particle of poly (vinyl acetate) will not be of the same thickness as that around a poly (styrene) particle. A universal calibration can only then strictly apply at the limit of infinite ionic strength. This could be approached by extrapolation of elution data obtained at finite ionic strength to $I^{-1/2} = 0$. The "universal" calibrations of particle diameter against RF reported in some articles referring to HDC (refs. 69,70) must therefore be regarded as fortuitous. However, in practical applications, errors in particle-size determination of around, say, 10% are perfectly acceptable. If measurements on latex samples are made at high ionic strength then the double layers will be relatively thin and the error incurred in assuming that all latices have equal double-layer thickness at fixed I will be correspondingly small. The very fact that both SEC and HDC have been used to obtain reasonable particle-size estimates in many instances demonstrates that theoretical considerations need not impair practical ones.

Analysis of microgel content in polymers by SEC

Although aqueous eluants are not employed in the techniques described in this section, the similarity in configuration between microgels and latices suggests that their discussion at this point in our article is appropriate.

Cross linking of polymer chains frequently occurs in emulsion polymerisation processes. Upon mixing dried latex particles, prepared by emulsion techniques, with appropriate organic solvents, the uncrosslinked polymer chains usually dissolve quite quickly. Those latex particles which contain cross links will, however, from a swollen matrix rather than dissolving; the degree of swelling depending upon the extent of cross linking. It was predicted and verified by Gaylor et al. (ref. 85) that the solvent-swollen gel particles, being considerably larger than dissolved molecules, should elute more quickly through an SEC apparatus. Their system comprised typical nitrile elastomers, dissolved in methyl ethyl ketone and passed through a bed of controlled-pore glass with a differential refractometer as the detector. Their chromatographically-measured gel contents were highly reproducible in the range 30-80% gel and compared favourably

with static measurements, i.e. leaching out polymer chains with solvent, followed by filtration of the gel and subsequent exhaustive drying. Apart from ease of operation, one great advantage of the chromatographic technique is its speed. Analysis times of 1 hour, including sample preparation, are common as compared with 2-3 days for the static technique.

Further applications have been disclosed by Williamson et al. (ref. 86) and by Hellman et al. (ref. 87). The most recent publication in the field, by Malihi et al. (ref. 88) describes the determination of gel content in emulsion-polymerised butyl acrylate/methyl methacrylate copolymers, dissolved in Tetrahydrofuran (THF). CPG was again the chosen support.

SEC OF WATER-SOLUBLE POLYMERS

Introduction

SEC of water-soluble polymers is a rapidly expanding area, especially since the introduction of high-performance packings which have potential for rapid, accurate determination of MW and MWD. Development of the technique has, however, been hampered not only by the various non-steric-exclusion phenomena mentioned in the general introduction, but also by the lack of polymer standards of narrow MWD which are usually required for successful calibration.

In this section, the three classes of water-soluble polymer (nonionic, anionic and cationic) will be examined in turn, although there is some overlap between the classifications, as will become apparent.

Since the literature pertaining to nonionics and anionics has been subject to several reviews (refs. 50-54), details of available column packings and much of the earlier work are omitted here. The aqueous SEC of polycationics has only recently become feasible, however, and no review has yet been published. For this reason, cationics are discussed in greater depth.

Nonionics

Polyethylene oxide (PEO), unhydrolysed poly(acrylamide) (PAM) and dextrans are among the few water-soluble polymers which can either be obtained commercially or readily fractionated to yield standards of narrow MWD.

PEO is well known to adsorb onto many unmodified glass or silica-based packings (refs. 50-54). For this reason, SEC analysis of PEO is often more conveniently carried out in conventional SEC using organic solvents and packing materials. Letot et al. (ref. 89) however, have reported a means of overcoming the adsorption of PEO to unmodified silica packings, through pre-emptive adsorption of poly(vinyl pyrrolidone) (PVP) of MW $\approx 10,000$ g.mol⁻¹. Some PVP was also required at low concentration in the eluant to maintain the coating stability. A cross-linked hydrophilic vinyl polymer gel, trade name TSK Gel PW has recently become available from the Toyo Soda Co., Japan. One advantage of this packing is that the surface groupings are neither charged, nor highly polar. Adsorptive and electrostatic interactions between this gel and many solutes are thus negated, or at least reduced. A study by Kato et al. (ref. 90) has demonstrated the efficacy of such columns for the chromatography of PEO, dextran and pullulan. The purpose was to compare the three types of solute as calibration standards. A universal hydrodynamic volume calibration (ref. 91) was shown to apply in an eluant of 0.1M aqueous sodium chloride solution. It was concluded that PEOs were the best calibration standards.

Optimum conditions for the SEC of PVP samples have been identified by Malawer et al. (ref. 92). Using diol-derivatised silica gels and a mobile phase of 1/1 (volume/volume) MeOH/H₂O containing 0.1M LiNO₃, samples eluted without adsorption to yield a log-linear calibration curve.

Much attention has been paid to the aqueous SEC of PAM. The utility of controlled-pore (borosilicate) glass (CPG) in this application is well established (refs. 93,94). Klein and Westerkamp (ref. 95) used eighteen PAM samples varying in MW from 1.4×10^5 to 5.5×10^6 g.mol⁻¹. (M_w) to obtain a calibration to which a cubic B-spline function was fitted. CPG columns were used, with an eluant of 0.1M Na₂SO₄ (aq.); $I = 0.3$. A more sophisticated study of the aqueous SEC of PAM was carried out by Kim et al. (ref. 6), using a coupled differential refractive index/low angle laser light scattering photometer (DRI/LALLSP) detection system. CPG was again the porous substrate. The eluant was 0.2M Na₂SO₄ with Tergitol NPX at 0.01 g dm⁻³. Methodology was developed to determine both the MW calibration curve and the peak broadening parameter, σ^2 , over a wide range of MW. The method was based on the injection of a single broad MWD standard and a generalised analytical solution of Tung's integral equation for the detector response, corrected for peak broadening. The mathematics of the methodology were more fully discussed earlier in this article. An analogous method for the characterisation of dextrans was also developed by the same authors (ref. 37).

Very recently reports on the successful chromatography of PAMs on silica supports have appeared. Narrow fractions of PAM were chromatographed on porous silicas having a chemically-bonded aminopropyl phase by Biran and Dawkins (ref. 96). Successful separations of samples having MWs from 10^4 to 10^6 g.mol⁻¹ were obtained using a mobile phase of formamide:water (1:5). Nesterov et al. (ref. 97) recommended the use of a 92.9:1.02:6.0 mixture of distilled water:glycerol:tartaric acid for the chromatography of PAMs on unmodified silica sorbents.

Anionics

As well as adsorption, factors such as ion exchange, ion exclusion, ion inclusion and polyelectrolyte chain expansion, fully described in several excellent reference works (refs. 98-100) must be considered as complicating factors in the aqueous SEC of anionics. In common with nonionics, relatively few narrow-MWD standards are available. Exceptions are NaPSS salts, obtainable from the Pressure Chem. Co., Pittsburgh, Pennsylvania and ionic PAM, prepared by hydrolysis of nonionic fractions.

Apart from the identification of suitable conditions for the chromatography of individual anionic solutes, the aim of much of the work so far published on aqueous SEC of anionics has been to establish the validity of calibration techniques, particularly Benoit's "universal" ($[\eta]M$) technique (ref. 91), which has gained wide acceptance in the field of conventional ("organic") SEC (ref. 2). Most such investigations have involved the elution of both anionics and nonionic solutes from the same column set. The fundamental assumption behind this approach is that for each column/eluant system, there exists a certain ionic strength above which both polyelectrolyte expansion effects and ion exclusion due to the electrical double layers on both packing and solute become insignificant. Once such conditions are achieved, a direct comparison between the elution behaviour of nonionics and of polyelectrolytes becomes permissible. Several groups of workers have used this methodology. Spatorico and Beyer (ref. 101) examined the behaviour of samples of NaPSS ($M_w = 0.8 - 985 \times 10^3 \text{ g.mol}^{-1}$), dextran ($M_w = 53 - 1230 \times 10^3 \text{ g.mol}^{-1}$) and fractions of a copolymer of acrylic acid and ethyl acrylate ($M_w = 29.6 - 5.68 \times 10^3 \text{ g.mol}^{-1}$). Columns were packed with CPG gels and eluants containing Na_2SO_4 at 0.2 M and 0.8 M were chosen. Intrinsic viscosities $[\eta]$, were measured in the same solvents. In both cases, single linear plots of $\log [\eta] M_w$ against V_R were obtained for the four polymer types and so the universal calibration procedure was deemed to be applicable.

An investigation into the effect of solvent ionic strength in the aqueous SEC of NaPSS, dextran and PAM was undertaken by Cooper and Matzinger (ref. 102). CPG was the column packing material and ionic strengths (phosphate buffer, pH7) of 0.01, 0.1 and 1.0 were examined. The large dependence of V_R on I was demonstrated. It was asserted that the universal calibration procedure should be valid at high I .

Two studies using unmodified silica gels as packings have been reported by Rochas, et al. (ref. 103) and by Desbrieres et al. (ref. 104). Ion exclusion effects were assumed to be negligible at values of I (NaNO_3) ≥ 0.05 . In the first study (ref. 103), NaPSS, dextran and Na polyglutamate samples were examined. At $I = 0.1$, plots of $\log [\eta] M$ vs V_r fell on a single line. In the second (ref. 104), Na PSS and dextran standards were eluted and a 3-detector system (refractometry, conductimetry and viscometry) was employed. In 0.1 molar NaNO_3 solution, universal calibration was once again reportedly successful.

Bose et al. (ref. 105) used a support of Sepharose CL-6 to separate NaPSS and dextran samples over a wide range of ionic strengths, using both NaCl and NaOH, separately, as the simple electrolytes. The universal calibration procedure was invalid for all experimental conditions examined here. A modified calibration procedure which accounts for excluded-volume effects, based on the excluded-volume theory of Ptitsyn and Eizner (ref. 106) was successful in describing the data obtained in NaOH solutions of moderate and high ionic strength. At low NaOH concentrations and all NaCl concentrations the method was again unsuccessful. The authors rationalised their observations in terms of ion-exclusion effects at low I and the possibility that (i) NaCl causes interaction between the polyelectrolyte and the Sepharose or (ii) some supermolecular structure exists in high-ionic strength NaCl solutions. It is also possible that the pore geometry of the semi-rigid gel is affected by salt concentration and/or type.

A subsequent publication from the same laboratory has recently appeared (ref. 107). Fractogel TSK gels were chosen as supports. These are similar to the TSK Gel PW materials mentioned earlier, except that they are specifically intended for low-pressure SEC applications. The lack of surface charges on these hydrophilic gels with consequently reduced ion-exclusion properties renders them an interesting alternative to the commonly-used glass gel packings. Using columns packed with gels of three different porosities, the universal calibration procedure was successfully applied to NaPSS, dextran and poly(ethylene glycol) (PEG) samples in 0.42 M NaOH solution. The detector system was a coupled DRI/online viscometer arrangement.

By contrast with the foregoing authors, none of the studies on aqueous SEC of water-soluble polymers undertaken by Hamielec et al. (refs. 6,37,93,94,108) have involved tests of the applicability of the universal calibration procedure. Their efforts have instead concentrated upon optimisation of chromatographic conditions (i.e. elimination of non-steric effects, minimisation of peak broadening and maximisation of peak separation) for each particular type of polymer studied. For example, using CPG supports, Omorodion et al. (ref. 108) showed that optimal conditions for the aqueous SEC of NaPSS were achieved using an eluant of pH ~ 6 and $I \sim 0.05$, with addition of a few ppm of neutral surfactant to prevent adsorption to the packing surface. Since each water-soluble polymer system examined in the same series of studies (refs. 94, 109) required a somewhat different mobile phase for optimal resolution, the universal calibration procedure was deemed inappropriate.

The validity of the procedure for polyelectrolytes is further put in question as a result of the recent findings of Styring et al. (refs. 66, 110). Eight NaPSS samples were chromatographed on CPG in eluants similar to those of Omorodion et al. (ref. 108) over a wide range of I values from ≈ 0.008 to ≈ 0.25 . MWs of the samples varied from 3.1 to $3,200 \times 10^3 \text{ g.mol}^{-1}$. Intrinsic viscosities were measured as a function of I . Linear plots of $[\eta]$ versus $I^{-1/2}$ were obtained for all samples, which is a general phenomenon for polyelectrolytes (ref. 111). One aim of the study was a comparison of the SEC data with those obtained for polystyrene latices under similar experimental conditions (refs. 65, 66). At each value of I , the elution data for both types of solute were compared on a $[\eta] M$ basis. For example, see Fig. 6. For polymers of MW between 3.1 and $182 \times 10^3 \text{ g.mol}^{-1}$ the data points fitted on the $[\eta] M$ calibration established for the latices at the same value of I , although the values of $[\eta] M$ did not overlap. For the higher -M.W. polymers whose $[\eta] M$ values did overlap with those measured for the latices there was a very large departure from the latex calibration. A comparison was then made of the latex calibration, constructed by linear extrapolation of latex elution volumes to $I^{-1/2} = 0$, with polysulphonate elution data. The extrapolation to infinite ionic strength was less straightforward than that for the latex data (refs. 65, 66). It was found necessary to assume constant surface potential on the polyelectrolyte coil, irrespective of ionic strength, in order to obtain a mathematically tractable extrapolation procedure. In the event, the $[\eta] M$ calibration at $I = \infty$ was found to behave in the same way as that shown in Fig. 6 for $I = 0.107$. The main conclusion drawn was that the universal calibration procedure was not valid for this system. The double-layer interactions between the packings and latices could be quantitatively accounted for. However, the lack of an adequate theory predicting the variation in polyelectrolyte surface potential (and hence double-layer thickness) with I precluded a similarly successful treatment for the polysulphonates.

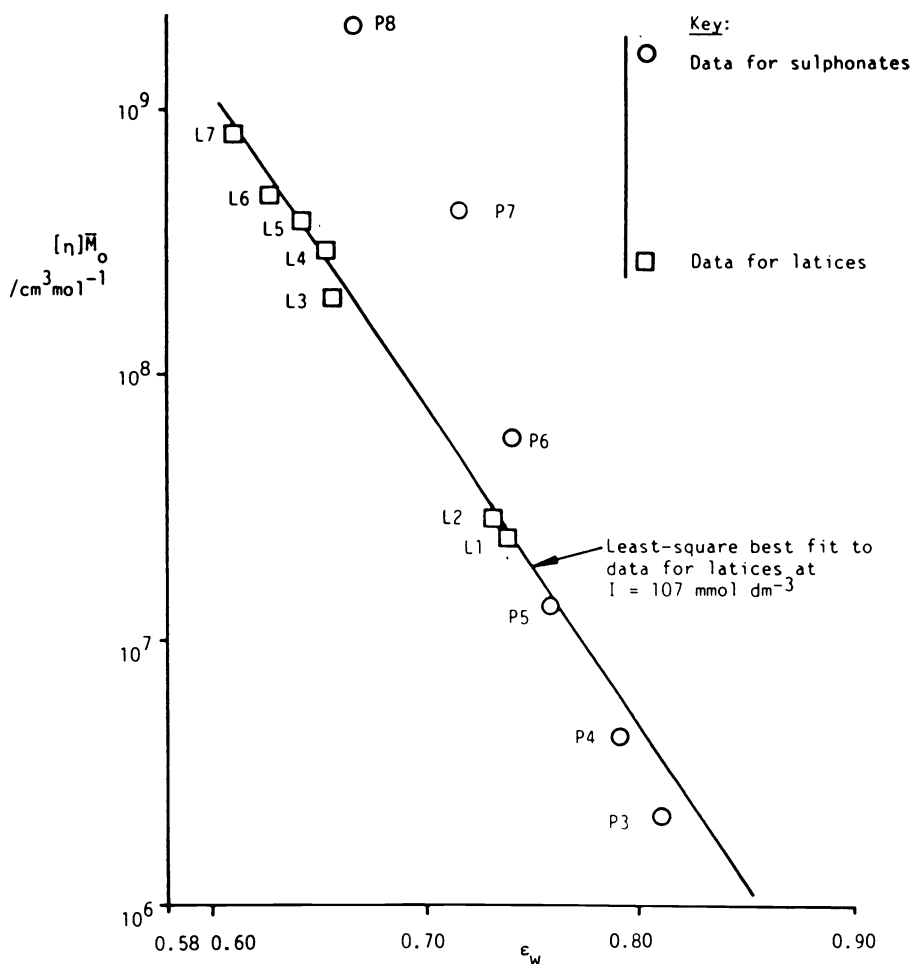


Fig.6: Attempted Universal Calibration [66] of NaPSS and polystyrene latex samples. Polysulphonates: $M_0(P3) \approx 100,000 \text{ g mol}^{-1}$
 $M_0(P8) \approx 3.1 \times 10^6 \text{ g mol}^{-1}$
 Latices : $D_0(L1) \approx 290 \text{ \AA}$
 $D_0(L7) \approx 900 \text{ \AA}$

ϵ_w is the weight-average elution volume of the solute relative to that of the totally-permeating solute benzyl alcohol (for which $\epsilon_w = 1.00$)

It is worthy of note before moving on to the next section that several workers have performed aqueous SEC experiments on anionic polyelectrolytes for purposes other than estimating MWs and MWDs. Determination of the Donnan equilibrium, i.e. the distribution of a simple salt over a membrane that is impermeable to the polyelectrolyte offers an important insight into the electrochemical behaviour of a polyelectrolyte. A typical SEC porous gel matrix may be regarded as a semi-permeable membrane (permeable to simple ions but impermeable to macro ions, provided the latter are sufficiently large). Recent papers in this field have come from Rinaudo and Desbrieres (ref. 112) and from Kadokura et al. (ref. 113). The former determined Donnan equilibrium data for the NaPSS-NaCl system on silica gels, whilst the latter measured the Donnan salt exclusion parameter, Γ for the simple salts NaCl, Na₂SO₄ and Na₃(1,3,6-naphthalene trisulphonate), eluted with aqueous NaNO₃ from Sephadex G-10. On a Sephadex G-25 column, the Γ value for the heparin-NaCl system was determined.

Cationics

The greatest challenge to "ideal" SEC, i.e. adsorption-free SEC of water-soluble polymers, has been posed by cationic polyelectrolytes. This is partly due to their intense and irreversible interaction with the glass and silica supports commonly employed in aqueous SEC. Development of suitably modified silica packings and the appearance on the market of TSK Type PW gels (having an uncharged surface) have occurred only relatively recently. For this reason the literature pertaining to aqueous SEC of cationics is very sparse at the time of writing.

The first report of prevention of adsorption of (unspecified) polycations to silica supports was that of Buijtenhuis and van der Maeden (ref. 114). Tetramethylammonium chloride (TMAC) was included in the eluant and the TMA⁺ ion was presumed to neutralise the negatively-charged silanol groups on the packing. The first successful process for bonding a surface coating to porous silicas, rendering them suitable for aqueous SEC of cationics, was patented by Talley et al. in 1978 (ref. 115). The process entails reaction of the silica glass with 3-aminopropyltriethoxysilane followed by reaction of

the terminal-NH₂ group with 3-chloro-2-hydroxytrimethylammonium chloride. Such quaternised supports were used to successfully size separate samples of dextran and poly (2-vinylpyridine) (P2VP) (ref. 116). The eluant chosen was 0.1 M HNO₃ + 0.1M NaNO₃. pH ≤ 3 is required to protonate the otherwise neutral P2VP chain and thus render it polycationic. Plots of ln [η] M vs V_R for the two types of solute gave two parallel lines, i.e. the universal calibration procedure did not apply. In a more recent publication by Domard and Rinaudo (ref. 117), a wider variety of solute types, including dextrans, PEGs, poly(trimethylacrylate) (PTMAOAc), PVP and quaternised anylopectin. The quaternised silica support was Spherosil QMA (Quaternary Methyl Ammonium). In a manner directly analogous to that described by workers from the same laboratory who examined the behaviour of anionics and nonionics in aqueous SEC (refs. 103, 104) plots of log [η] M vs V_R for many types of solute fell on common lines. Note that this observation is at variance with the findings of Talley and Bowman (ref. 116), who used nominally identical supports in their investigation. This is indicative of the degree of uncertainty pervading the field of aqueous SEC in general.

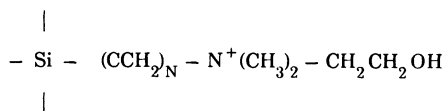
The elution behaviour of P2VP from a silica packing with a different surface-bonded coating (SynChropak CATSEC) was disclosed by Gooding et al. (ref. 118). This type of support has a thin surface-bonded layer of polyamine, polymerised with a neutral hydrophilic crosslinker to increase stability. When experiments were performed using uncoated silicas, considerable tailing of the chromatograms was observed. However, when the CATSEC-bonded silicas were employed, the tailing disappeared, linear recovery of P2VP was obtained and a log-linear MW calibration curve was constructed, indicating exclusion from the support on a purely steric basis.

Guise and Smith (ref. 119) have evaluated the performance of five separate columns, containing four different packing materials, in the separation of samples of P2VP, poly(ethyleneimine) (PEI) and a commercial polyamide-epichlorohydrin resin called Hercosett 125. The four packing types were

- I) Hydrophilic ether-bonded silica (μ Bondagel E-Linear)
- II) Glyceryl ether-bonded silica (TSK Gel-3000 SW)
- III) Quaternary ammonium-modified silica (Zorbax SAX)
- IV) Silica modified according to the method patented by Talley (ref. 115)

Surprisingly, types I and II were reportedly superior in performance. However, since single-porosity columns of type IV were used, thus having limited separation ranges, such a comparison is perhaps unfair.

The most recent report in this field in which the use of cationic-modified silica supports is that by Stickler and Eisenbeiss (ref. 120). The bonded phase was a dimethylammonium derivative, shown below



This stationary phase, used in conjunction with an eluant 0.1M HNO₃ + 0.1M NaNO₃ was shown to control non size-exclusion effects. The cationic standards employed were samples of poly (2-trimethylammonium ethyl methacrylate chloride) (PTMAC) varying in M_w from 7.7 to 11,000x10³ g.mol⁻¹. Owing to the high polydispersity of the standards, a non-linear effective MW calibration procedure was applied to the broad chromatograms, based on the diffusion-separation theory of Yau and Malone (121). A reliable methodology for the determination of MW and MWD in these polymers was thus obtained.

The most active group of researchers in aqueous SEC of cationics have been Dubin and co-workers (refs. 122-126). They have used exclusively TSK Gel PW type columns for their investigations, in common with Kato and Hashimoto (ref. 127). A wide range of polycations have been chromatographed on this substrate with little apparent adsorption. Examples include poly (dimethyldiallylammonium chloride) (PDMDAAC) (ref. 122), ionenes (ref. 123), poly(methacryl-amino-propyltrimethylammonium chloride) (ref. 124), and quaternised poly(N-methyl 2-vinyl pyridine), (ref. 127). The absence of adsorption of polycations on PW gel was inferred from the universal calibration established for PDMDAAC, PEO, dextran and poly (N-vinylacetamide) in 0.2M NaCl (ref. 124). However, other observations, such as the late elution of PEI at low ionic strength and the complete retention of poly (N-vinylamine) suggested some form of polymer-packing interaction. A form of hydrophobic bonding was suggested (125) to explain these latter two anomalies, i.e. PW gels contain some apolar sites that bind hydrophobic moieties.

CONCLUDING REMARKS

The efficacy of SEC in determining molecular weights and distributions of simple polymers in many organic solvents has been well demonstrated over the past two decades. Substantial advances have more recently been made in two important areas. First the mathematical basis for the construction of calibration curves for both molecular weight and peak broadening, given relatively few samples of known molecular weight and distribution, has been established. Previously, narrow distribution standards in a wide range of molecular weights were required for this purpose. Secondly, the improvements in chromatographic hardware, particularly in detector systems, has opened the door to extending the utility of SEC to the analysis of complex polymers. The use of multi-detector systems allows, in principle, such properties as long-chain branching in homopolymers and sequence lengths in copolymers to be examined.

Owing to non-size-exclusion effects in aqueous SEC, such sophisticated hardware is not yet generally applicable to water-soluble polymers. Most of the recent advances in this field have come with the development of chromatographic supports which negate, or at least reduce these effects. However, reliable methodologies have been developed for the successful SEC of several commercially important water-soluble polymers. Aqueous particle chromatography, too, with certain provisos, has developed to the stage where good estimates of particle size may be made. By comparison with the "state of the art" in non-aqueous SEC, it is clear that much work, both theoretical and practical, needs to be done in the field of aqueous SEC. The stimulus for such research will grow with the increasing commercial importance of water-soluble polymers in such fields as tertiary oil recovery, sewage flocculation and dewatering and viscosity modification in diverse products. The authors for their part hope to be able to resolve some of the conflicting observations thus far made in this field.

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REFERENCES

1. "Steric Exclusion Liquid Chromatography of Polymers", ed. J. Janca, Chromatographic Science Series, vol. 25, Marcel Dekker, Inc., New York (1984).
2. W.W. Yau, J.J. Kirkland and D.D. Bly, "Modern Size Exclusion Liquid Chromatography", Wiley-Interscience, New York (1979).
3. "Modern Methods of Particle Size Analysis", ed. H.G. Barth, Chemical Analysis, vol. 73, Wiley-Interscience, New York (1984).
4. S.T. Balke, "Quantitative Column Liquid Chromatography", Elsevier, New York (1984).
5. A.E. Hamielec, "Correction for Axial Dispersion", p. 144 in "Steric Exclusion Liquid Chromatography of Polymers", ed. J. Janca, Chromatographic Science Series, vol. 25, Marcel Dekker, Inc., New York (1984).
6. C.J. Kim, A.E. Hamielec and A. Benedek, *J. Liquid Chromat.*, **5**, 1277 (1982).
7. A.E. Hamielec and H. Meyer, "Online Molecular Weight and Long Chain Branching Measurement Using SEC and Low-Angle Laser Light Scattering", in "Developments in Polymer Characterization-5", Applied Science Publishers Ltd., London - in press (1985).
8. W. Steubing, *Ann. Physik*, **26**, 329 (1908).
9. W. Mecklenberg, *Kolloid Z.*, **14**, 172 (1914).
10. P. Putreys and J. Brosteaux, *Trans. Farad. Soc.*, **31**, 1314 (1935).
11. P.P. Debye, *J. Appl. Phys.*, **17**, 392 (1946).
12. P.M. Doty, B.H. Zimm and H. Mark, *J. Chem. Phys.*, **12**, 144 (1944).
13. P.M. Doty, B.H. Zimm and H. Mark, *J. Chem. Phys.*, **13**, 159 (1945).
14. P.M. Doty, W.A. Affens and B.H. Zimm, *Trans. Farad. Soc.*, **42B**, 66 (1946).
15. B.H. Zimm, *J. Chem. Phys.*, **16**, 1009 (1948).
16. H.-J. Cantow, E. Siefert and R. Kuhn, *Chem. Ing. Tech.*, **38**, 1032 (1966).
17. G. Meyerhoff, *Makromol. Chem.*, **118**, 265 (1968).
18. G. Meyerhoff, *Sep. Sci.*, **6**, 239 (1971).
19. D. Goedhart and A. Opschoor, *J. Polym. Sci A2*, **8**, 1227 (1970).
20. A.C. Ouano, *J. Polym. Sci. A1*, **10**, 2169 (1972).
21. A.C. Ouano, D.L. Horne and A.R. Gregges, *J. Polym. Sci., Polym. Phys.*, **12**, 307 (1974).
22. A. Peyrouset and R. Prechner, French Patent 2,268,262 (1974).
23. L. Letot, J. Lescq and C. Quivoron, *Analisis*, **4**, 456 (1976).

24. J. Lesec and C. Quivoron, *J. Liquid Chromat.*, **3**, 427 (1980).
25. D. Lecacheux, J. Lesec and C. Quivoron, *J. Appl. Polym. Sci.*, **27**, 4867 (1982).
26. F.B. Malihi, C. Kuo, M.E. Koehler and T. Provder, "Development of a Continuous Gel Permeation Chromatography Viscosity Detector for the Characterization of Absolute Molecular Weight Distribution of Polymers", p. 281 in "Size Exclusion Chromatography", ed. T. Provder, *ACS Symp. Series 245* (1984).
27. Technical Bulletin, VISCOTEK, 1030 Russell Drive, Porter, Texas 77365.
28. W. Kaye, *Anal. Chem.*, **45**, 221 (1973).
29. A.C. Ouano and W. Kaye, *J. Polym. Sci.*, **12**, 1151 (1974).
30. A.C. Ouano, *J. Chromat.*, **118**, 303 (1976).
31. Application Notes, Chromatix 1977-1979.
32. M.L. McConnell, *Am. Lab.*, **10**, 63 (1978).
33. R.C. Jordan, *J. Liquid Chromat.*, **3**, 439 (1980).
34. B. Millaud and C. Strazielle, *Makromol. Chem.*, **180**, 441 (1979).
35. L. Beltzung and C. Strazielle, *Makromol. Chem.*, **185**, 1145, 1155 (1984).
36. Z.D. He, X.C. Zhang and R.S. Cheng, *J. Liquid Chromat.*, **5**, 269 (1982).
37. C.J. Kim, A.E. Hamielec and A. Benedek, *J. Liquid Chromat.*, **5**, 425 (1982).
38. W.S. Park and W.W. Graessley, *J. Pol. Sci. Pol. Phys. Ed.*, **15**, 85 (1977).
39. C.E. Lundy and R.D. Hester, *J. Liquid Chromat.*, **7**, 1911 (1984).
40. M.C. Jordan and M.L. McConnell, "Characterization of Branched Polymers", pp. 107, in "Size Exclusion Chromatography", ed. T. Provder, *ACS Symp. Series 138* (1980).
41. R.C. Jordan, S.F. Silver, R.D. Sehon and R.J. Rivard, "Size Exclusion Chromatography with Low-Angle Laser Light-Scattering Detection: Application to Linear and Branched Block Copolymers", pp. 295 in "Size Exclusion Chromatography", ed. T. Provder, *ACS Symp. Series 245* (1984).
42. G.N. Foster, A.E. Hamielec and T.B. MacRury, "The Molecular Weight and Branching Distribution Method", p. 131 in "Size Exclusion Chromatography", ed. T. Provder, *ACS Symp. Series 138* (1980).
43. A. Rudin, V. Grinshpun and K.F. O'Driscoll, *J. Liquid Chromat.*, **7**, 1809 (1984).
44. L.H. Garcia-Rubio, A.E. Hamielec and J.F. MacGregor, "UV Spectrophotometers as Detectors for Size Exclusion Chromatography of Styrene-Acrylonitrile (SAN) Copolymers", p. 151, in "Computer Applications in Applied Polymer Science", ed. T. Provder, *ACS Symp. Series 197* (1982).
45. L.H. Garcia-Rubio, J.F. MacGregor and A.E. Hamielec, "Size Exclusion Chromatography of Copolymers", p. 311, in "Polymer Characterization: Spectroscopic, Chromatographic and Physical Instrumental Methods", ed. C.D. Craver, *ACS Advances in Chemistry Series 203* (1983).
46. A.E. Hamielec, *Pure and Appl. Chem.*, **54**, 293 (1982).
47. C. Quivoron, "Use for Polymer Analysis", p. 213 in "Steric Exclusion Liquid Chromatography of Polymers", ed. J. Janca, *Chromatographic Science Series*, vol. 25, Marcel Dekker, Inc., New York (1984).
48. S.T. Balke and R.D. Patel, *J. Polym. Sci., Polym. Lett. Ed.*, **18**, 453 (1980).
49. J. Porath and P. Flodin, *Nature*, **183**, 1659 (1959).
50. A.R. Cooper and D.S. VanDerveer, *J. Liq. Chrom.*, **1**(5), 693 (1978).
51. H.G. Barth, *J. Chrom. Sci.*, **18**, 409 (1980).
52. P.L. Dubin, *Sepr. Purifn. Meths.*, **10**(2), 287 (1981).
53. *ibid.*, *Am. Lab.*, **15**(1), 62, 64-7, 70-3 (1983).
54. J.E. Rollings, A. Bose, J.M. Caruthers, G.T. Tsao and M.R. Okos, *Adv. Chem. Ser.*, **203** (Polym. Charact.), 345-360 (1983).
55. F.E. Bailey and J.V. Koleske, "Poly(ethylene oxide)", Academic Press, New York (1976).

56. K.F. Krebs and W. Wunderlich, Angew. Makromol. Chem., **20**, 203 (1971).
57. H. Small, J. Coll. Interface Sci., **48(1)**, 147 (1974).
58. R.J. Noel, K.M. Gooding, F.E. Regnier, D.M. Ball, C. Orr and M.E. Mullins, J. Chromatogr., **16**, 373 (1978).
59. J.C. Giddings and M.N. Myers, Sepn. Sci. Tech., **13**, 637 (1978).
60. W.W. Yau and J.J. Kirkland, J. Chromatogr., **218**, 217 (1981).
61. A. Penlidis, A.E. Hamielec and J.F. MacGregor, J. Liq. Chrom., **6(S-2)**, 179 (1983).
62. A. J. McHugh, C.A. Silebi, G.W. Poehlein and J.W. Vanderhoff, "Colloid and Surface Science", vol. 4, ed. M. Kerker, Academic Press, New York, 549 (1976).
63. H. Small, Adv. in Chromatogr., **15**, 113 (1977).
64. H. Coll and G.R. Fague, J. Coll. Interfac. Sci., **76(1)**, 116 (1980).
65. M.G. Styring, C.J. Davison, C. Price and C. Booth, J. Chem. Soc. Faraday Trans. I, **80**, 3051 (1984).
66. M.G. Styring, Ph.D. Thesis, University of Manchester (1984).
67. H. Small and M. Langhorst, Anal. Chem., **54(8)**, 892A (1982).
68. H. Small, U.S. Patent 3,865,717 (1975).
69. D.J. Nagy, C.A. Silebi and A.J. McHugh, J. Coll. Interfac. Sci., **79**, 264 (1981).
70. D.J. Nagy, J. Coll. Interfac. Sci. Lett., **93(2)**, 590 (1983).
71. C.A. Silebi and A.J. McHugh, "Emulsions, Latices and Dispersions", eds. P. Becker and M.N. Yudenfrund, p. 155, Dekker, New York (1978).
72. C.A. Silebi and A.J. McHugh, J. Appl. Pol. Sci., **26**, 1555 (1979).
73. G.R. McGowan and M.A. Longhorst, J. Coll. Interfac. Sci., **89**, 94 (1982).
74. E.A. Di Marzio and C.M. Guttman, Polym. Lett., **7**, 267 (1969); Macromols., **3**, 131 (1970).
75. R.K. Prud'Homme, G. Froiman and D.A. Hoagland, Carbohydrate Res., **106**, 225 (1982).
76. R.K. Prud'Homme and D.A. Hoagland, Sepn. Sci. Technol., **18**, 121 (1983).
77. D.A. Hoagland, K.A. Larson and R.K. Prud'Homme, Chem. Anal. Ser. **73**, "Modern Methods of Particle Size Analysis", Chapter 9, p. 277, ed. H.G. Barth, Wiley, New York (1984).
78. J. Lecourtier and G. Chauveteau, Macromols., **17**, 1340 (1984).
79. T. Shiibashi, K. Ito and T. Homma, Kobunshi Robunshu, **41(6)**, 345 (1984).
80. K. Ito, T. Shiibashi, E. Maekawa and T. Homma, ibid., p. 361.
81. ibid., p. 365.
82. C.A. Silebi, "Colloidal Particle Chromatography" in "Advances in Emulsion Polymerisation and Latex Technology: 15th Annual Short Course", Lecture notes, Vol. 2, Lehigh University, June 1984.
83. T.R. Paxton, J. Coll. Interfac. Sci., **31**, 19 (1969).
84. I. Piirma and S.R. Chen, ibid., **74**, 90 (1980).
85. V.F. Gaylor, H.L. James and J.P. Herdering, J. Pol. Sci., Pol. Chem. Ed., **13**, 1575 (1975).
86. T.J. Williamson, V.F. Gaylor and I. Piirma, ACS Symp. Ser., **138**, 77 (1980).
87. M.Y. Hellman, T. Bowmer and G.N. Taylor, ACS Org. Coat. Plast. Chem. Prepr., **45**, 126 (1981).
88. F.B. Malihi, C.-Y. Kuo and T. Provder, J. Liq. Chrom., **6(4)**, 667 (1983).
89. L. Letot, J. Lesec and C. Quivoron, J. Liq. Chrom., **4(8)**, 1311 (1981).
90. T. Kato, T. Tokuya and A. Takahashi, J. Chromatogr., **256**, 61 (1983).

91. Z. Grubisic, P. Rempp and H. Benoit, J. Pol. Sci. Part B, **5**, 753 (1967).
92. E.G. Malawer, J.K. DeVasto, S.P. Frankoski and A.J. Montana, J. Liq. Chrom., **7**(3), 441 (1984).
93. A.M. Abdel-Alim and A.E. Hamielec, J. Appl. Pol. Sci., **18**, 297 (1974).
94. S.N.E. Omorodion, A.E. Hamielec and J.L. Brash, A.C.S. Symp. Ser. 138 (SEC), 267 (1980).
95. J. Klein and A. Westerkamp, J. Pol. Sci. Pol. Chem. Ed., **19**, 707 (1981).
96. R. Biran and J.V. Dawkins, Eur. Pol. J., **20**(2), 129 (1984).
97. V.V. Nesterov, E.V. Chubarova and B.G. Belen'kii, Vyskomol. Soed., Ser. A, **26**(4), 864 (1984).
98. S.A. Rice and M. Nagasawa, "Polyelectrolyte Solutions", Academic Press, New York (1961).
99. F. Oosawa, "Polyelectrolytes", Dekker, New York (1971).
100. A. Eisenberg and M. King, "Ion Containing Polymers, Volume 2. Physical Properties and Structure", Chapter 5, Acad. Press, New York (1977).
101. A.L. Spatorico and G.L. Beyer, J. Appl. Pol. Sci., **19**(11), 2933 (1975).
102. A.R. Cooper and D.P. Matzinger, J. Appl. Pol. Sci., **23**, 419 (1979).
103. C. Rochas, A. Domard and M. Rinaudo, Eur. Pol. J., **16**, 135 (1980).
104. J. Desbrieres, J. Mazet and M. Rinaudo, ibid., **18**, 269 (1982).
105. A. Bose, J.E. Rollings, J.M. Caruthers, M.R. Okos and G.T. Tsao, J. Appl. Pol. Sci., **27**(3), 795 (1982).
106. O.B. Ptitsyn and Y.E. Eizner, Soviet Phys. Tech. Phys. (Engl. Transl.), **4**, 1020 (1960).
107. G. Callec, A.W. Anderson, G.T. Tsao and J.E. Rollings, J. Pol. Sci. Pol. Chem. Ed., **22**, 287 (1984).
108. S.N.E. Omorodion, A.E. Hamielec and J.L. Brash, J. Liq. Chrom., **4**, 1903 (1981).
109. ibid., p. 41 (1981).
110. M.G. Styring, C. Booth and C. Price; to be published.
111. I. Noda, T. Tsuge and M. Nagasawa, J. Phys. Chem., **74**, 710 (1970).
112. M. Rinaudo and J. Desbrieres, Eur. Pol. J., **16**, 849 (1980).
113. S. Kadokura, T. Miyamoto and H. Inagaki, Makromol. Chem., **184**, 2593 (1983).
114. F.A. Buijtenhuis and F.P.B. van der Maeden, J. Chromatog., **149**, 489 (1978).
115. C.P. Talley et al., U.S Patent 4,118,316, October 3 (1978).
116. C.P. Talley and L.M. Bowman, Anal. Chem., **51**(13), 2239 (1979).
117. A. Domard and M. Rinaudo, Polym. Commun., **25**, 55 (1984).
118. D.L. Gooding, M.N. Schmuck and K.M. Gooding, J. Liq. Chrom., **5**(12), 2259 (1982).
119. G.B. Guise and G.C. Smith, J. Chromatogr., **235**, 365 (1982).
120. M. Stickler and F. Eisenbeiss, Eur. Pol. J., **20**(9), 849 (1984).
121. W.W. Yau and C.P. Malone, J. Pol. Sci. Pol. Lett. Ed., **5**, 663 (1967).
122. P.L. Dubin and I.J. Levy, Pol. Prepr. Am. Chem. Soc. Div. Pol. Chem., **22**(1), 132 (1981).
123. I.J. Levy and P.L. Dubin, Ind. Eng. Chem. Proc. Res. Dev., **21**, 59 (1982).
124. P.L. Dubin and I.J. Levy, J. Chromatogr., **235**, 377 (1982).
125. P.L. Dubin, I.J. Levy and R. Oten, Org. Coat. Appl. Pol. Sci. Proc., **48**, 627 (1983).
126. ibid., J. Chrom. Sci., **22**, 432 (1984).
127. Y. Kato and T. Hashimoto, J. Chromatogr., **235**, 539 (1982).