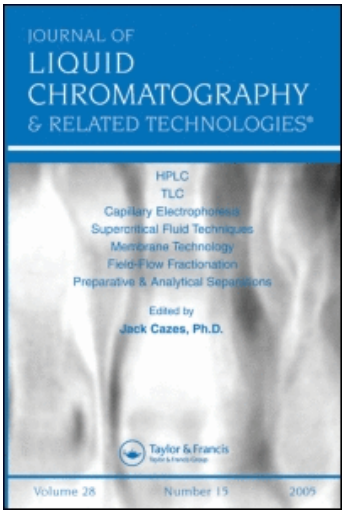

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Alternatives to Size Exclusion Chromatography

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Review

ALTERNATIVES TO SIZE EXCLUSION CHROMATOGRAPHY

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ABSTRACT

Size Exclusion Chromatography (SEC) is a convenient and powerful method for polymer characterization, but alternatives have appeared to overcome its limitations or improve its responses. These methods are examined, comparing their advantages and disadvantages, with a special attention to Hydrodynamic Chromatography (HDC).

INTRODUCTION

Basic questions in a polymer laboratory are molecular and macromolecular characterization in relation with synthesis and expected properties. A favourite method for molecular weight determination is size exclusion chromatography (SEC), the advantages of which are well-known. But some polymer characteristics may depend not only on molecular weight, but also of particle size, so that analysts are faced to a more general problem, the solutions of which are numerous. Nearly 3 thousand papers have been published on particle size

measurements, as reviewed regularly by Barth et al (1-4) for actualization of a basic book (5). We first examine limitations of SEC, then give an evaluation of other methods and report comparative results in HDC.

SEC AND PERSISTING PROBLEMS

Separation by SEC suffers practical difficulties for : i) very high molecular weights, ii) for complex mixtures, iii) for unsoluble polymers. It suffers also theoretical problems for getting the accurate molecular weight values. Thirty years of practice have lead to improvements of column packings and to appearance of new types of detectors, allowing simultaneously i) rapid analysis, ii) rather high resolution and iii) absolute results, so that this analysis is routinely used in polymer laboratory and has appeared in process control.

Actuality of S.E.C. is continuously proved by its mention in every polymer publication and by a series of papers which appeared at the Fall ACS Meeting 1993 (6), as well as in the 1993 ISPAC Summer Meeting (7). These recent papers are essentially devoted to calibration problems, which may be partially solved with the help of on-line multidetection. Absolute weight average molecular weight (M_W) was obtained first by coupling a concentration detector with a low-angle laser light scattering detector. Moreover, this procedure allows to derive true values, whatever is the separation power of the columns. Radius of gyration or hydrodynamic radius may be measured by using multi-angle or dynamic light scaterring detector, respectively. Complementary information on the macromolecular chain (branching, solvent thermodynamic quality) is obtained by adding a viscometer.

These papers indicate that, despite these improvements, some limitations are remaining : e.g. the difficulty to determine volume correspondance and coupling between detectors signals

of different width and sensitivity (to c, cM, η). Other problems are the absence of ultra-porous packing (with sufficient mechanical properties) for very large molecules, the low amplitude of the working volume Vp of the packing (between V_0 and $2V_0$, what means a capacity factor of 2 only), the decrease of resolution when molecular weight increases, the presence of secondary effects (from enthalpic origine), the high shear rate in pores, the evident necessity to have soluble samples. The aim of this paper is examination of other methods for solving some problems in polymer characterization : molecular weight, composition, number of components, particle diameter, effect of detector type on signal intensity, phase volume effectively available for separation, diffusion effects altering an effective use for samples with a large size distribution.

ADDITIONAL SEPARATION METHODS

To the question, "why and when change a technique?" answer may be : to improve it or overcome its limitations, either without large changes in equipment, keeping chromatography in mind, or using other separation principles. These attempts may be summarized as :

Source of limitations : proposed solution

-high shear rate : greatly increase channel dimensions

-lack of large internal diameter support (limiting sample size or molecular weight) : use of "infinite" one

-limited porosity : work in unlimited domain

-interaction with stationary phase : suppress this phase or use a liquid one

-problem with mobile phase : suppress it and use electric motion

-constant flow-rate : variable with time

-pore size is governing parameter : changing the origin of the driving force

-constant composition of eluent : gradient of elution
-constant temperature : programmed T
-no internal field in the column : add one
-no external field : add one
-resolution decreases when elution time increases : reverse elution order
-sample in solution : sample in dispersion.

Some of these difficulties and some of the above suggestions may be solved by using other types of chromatography : liquid (LC), field-flow fractionation (FFF) normal and steric (SFFF) (8, 9), hydrodynamic (HDC) (10), respectively packed or capillary (CHDC) column, supercritical fluid (SFC) (11, 12), capillary electrophoresis. Countercurrent chromatography (CCC or Centrifugal Partition Chromatography, CPC) is a potential tool to separate copolymers of different structure or nature, but at this time, is poor in exemples (13, 14). These methods correspond to a large variety of separation mechanisms based on kinetics or thermodynamics, where surface, volume, active specific sites of materials, or thermal, gravity, electric, magnetic applied fields, bring their contribution. They are called : "fractionation" and have some similar features with chromatography. Basis may be found in ref. 15 and 16. Their progress is presented in Symposium and accounted recently for instance in ref. 17 and 18. One of the characteristics is the absence of stationary phase, which solves the problems of side phenomena (adsorption, no active and sometimes disruptive interface across which the sample must partition, mass transport, column channeling, degradation, shear rate) and allows a higher variety for eluent choice.

Some other solutions are non-chromatographic methods, working with or without separation, such as mass spectrometry, NMR spectrometry, dynamic light scattering (photocorrelation spectroscopy), centrifugation.

RESULTS

Elution

Figure 1 presents schematically the different elution characteristics of several separation modes, in a semi-log plot. Three observations may be made. i) We first consider the order of elution : increasing volume for decreasing size in classical SEC, also in HDC (with packed or capillary columns) and steric FFF, and the reverse for LC, FFF and SFC. These curves indicate only the tendency for the relation : size-V, and are not true calibration curves. ii) Second, independently of elution order, we see that LC, SFC, FFF (normal and steric SFFF) may have very large elution domain (theoretically unlimited), on the contrary of SEC and HDC, which have limited ones (respectively 2 and about 1.3, as measured by the capacity factor). Wide domain is obtained when enthalpic process makes difference between molecules and when a gradient can increase the effects, either of solvent strength or temperature. iii) Third, depending on the mechanism, the sample size domain varies from nanometer to several microns (or MW from about 10^2 to more than 10^{10} mol/g). LC is concerned by the smaller sizes, whereas SEC is convenient for polymers, but also for oligomers and some low molecular weight compounds, while SFC may also cover this domain. Polymers and larger species, soluble or not, may be analyzed by FFF under different forms. Finally, the number in italic figures indicates approximate ratio of maximum to minimum sizes measured by the considered technique. The numerous possibilities of FFF allow to cover the wider range of sizes.

After this first approach, we may try to better determine the shape of the calibration curve (diameter D or mass M as a function of V), which is a semi-log plot in SEC and HDC, whereas this relation does not hold for certain other modes. Log plot of either M or M square root versus V does not lead to a straight

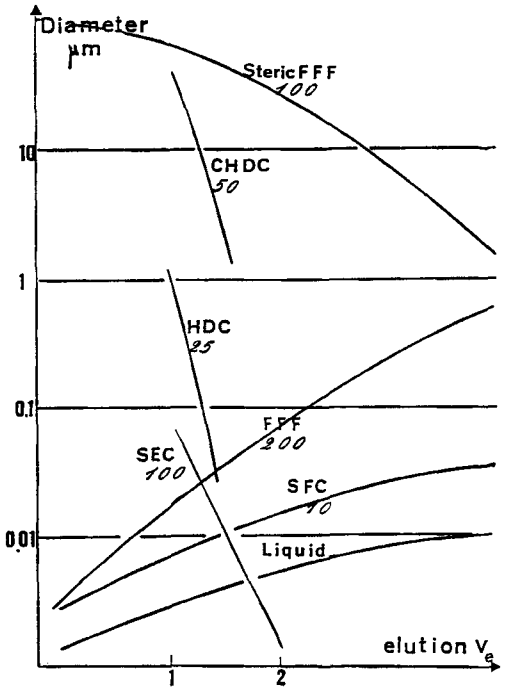

Figure 1. Semi-log plot of sample diameter versus elution volume for different liquid chromatographic modes (italic figures are the ratio of maximum to minimum diameters).

line. Small molecules are eluted on the effect of chemical or physical forces, roughly in increasing order of size, but this order is strongly affected by the choice of the two antagonist phases. In SFC, M is proportional to $V^{0.5}$ (low values), then to V (higher values) (19). In thermal FFF with programmed temperature (TDE), log MW may be proportional to retention time (20), at least in a limited zone (21), but size may vary approximately as the square of V. The calibration difficulty may be partly solved by using a continuous viscosity detector (20). Log of MW increases with retention time, so as the Log of the product "viscosity * MW", but different plots are obtained

for polymers of different nature, instead of the unique calibration curve, which is observed in SEC.

When conditions are drastically changed (high flow-rate and high temperature gradient between the two walls) the order is the reverse : the process is called T hyperlayer FFF (22). Size rather than MW is the fundamental factor governing the retention, as demonstrate by elution of linear and star polymers (23). In (sedimentation) SedFFF, most of the papers show exemples of separation, but do not indicate a calibration curve (24). "The trend of increasing retention with increasing particle size is apparent" (25). In (steric) SFFF, elution time is approximately proportional to particle mass (26).

Resolution

We are now tourning to comparison of resolution (R) in the different processes : we shall examine R value and its variation with elution volume. We suggest to define first, a specific resolution (Rs) = R/ratio of diameter of considered species and second, resolution per unit of time (Rt) = Rs/time of measurement, in order to compare respectively different samples and different elution conditions. To obtain polymer dimensions in solution, molecular mass is converted to diameter, applying the equation : diameter proportional to $M^{0.6}$.

R depends on chemical or physical factors, packing size being one of them. As a general rule in chromatography, resolution increases when packing size decreases (N is inversely proportional to the square of particle diameter). Generally LC has a very high and constant resolution (corresponding to about 100 000 theoretical plates per meter), which is interesting to separate compounds of very similar structure. Polymers are considered to present continuous distribution of species, fitting known theoretical equations and their

representative curves. In spite of its rather low resolution, even with new small sizes packings (particle diameter lower than 5 μm, almost same number of theoretical plates per meter as in LC), SEC is sufficient to obtain this molecular weight distribution. Yet, "high" resolution is specially interesting for separation of oligomers (up to 30-40 mers). This gives information on polymerization mechanism and allows to follow step-condensation process kinetics. In SEC, calibration is not affected by particle size : ratio of elution volumes of two compounds is unchanged; the resolution is increased since peaks are not so broad. Whereas in HDC with packed column, this R_f ratio is increased by using fine packings and resolution is increased (27). Figure 2 illustrates such effects. Pore size and pore volume being the origin of molecules separation, strongly affect elution in SEC, whereas effect of porosity is controversial in HDC, where the main phenomenon takes place essentially between (and not in the porous part of) particles. Combined HDC+SEC action increases separation domain, in terms of sample mass and elution volume (28).

For other separation modes, resolution depends on the applied field. In SFC, peak width decreases when V increases, so that N is greatly increased with V. The resolution is good for polymer standards, but commercial polymers may cover a very large elution domain, so that effective separation seems difficult (19). Excellent resolution for oligostyrenes (29, 30) has been obtained with packed columns (silica, 5 μm, pressure, flow rate and composition gradients) and 200 000 plates have been claimed, but exemples concern only small species (31).

In thermal FFF, peak width increases (factor 1.5) with V, but N may stay constant or even increases (from 18 to 70), since V is greatly increased : three times in this separation of three polystyrene standards (21, 32). Even for these narrow molecular weight distribution samples, plate number is low (about 70), so as Rt : 0.009 (21). Yet, separation of 3 PS may be obtained in 2 min (by high speed thermal FFF) as well as in 2

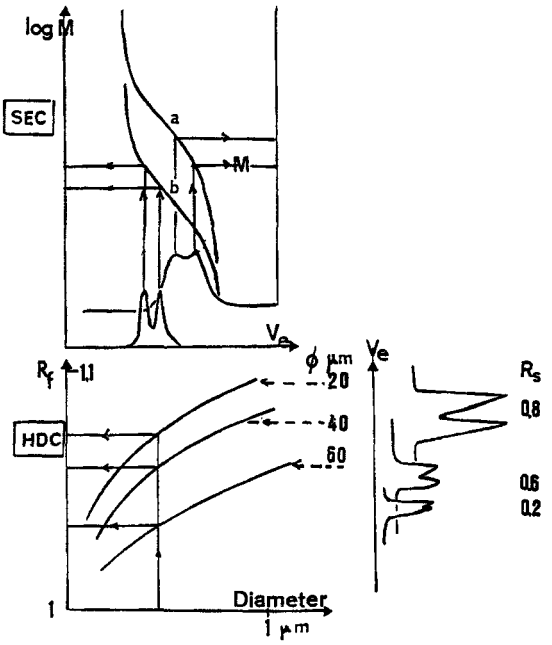

Figure 2. Particle size effects on elution volume and resolution in SEC (same calibration curves for a and b, b being fine particles) and HDC (particle size, R_S and R_f as indicated).

hrs (33). Plate numbers in both cases are of the same order (about 50), so as resolution : 0.8, but Rt is equal respectively to 0.21 and 0.013. For SEC, HDC, SFC, TFFF, theoretical plate height generally increases with flow rate according to van Demter law, so that resolution decreases, but sometimes Rt may increase, particularly by a proper use of gradient or when mass transfer does not play a role.

For all separations, it is possible to obtain an accurate molecular weight by using deconvolution to remove system dispersion (34).

Table 1 indicates meaningful values of R, Rs, Rt and analysis time.

TABLE 1
Resolution of Various Fractionation Modes

mode / Param.	CHDC	HDC	DCP	TFFF	SFFF	SFC
Rs	0.15	0.15	1.5	0.7-2	2.2	1.2
Rt	0.025	0.025	0.15	0.2-0.02	0.07	0.03
t (min)	6	6	10	45-120	30	20-60
ϕ µm	>1	<1	<60	<1	<2	0.05

Separation mode

Classical chromatographic separation occurs from the difference in partition of a soluble compound between mobile and stationary phases. More generally, separation is the result of local differences in distribution of the sample compounds in the mobile phase. The partition coefficient is related to the thermodynamic process : $LnK = \Delta G°/RT$, relation which indicates the possible effects of three factors : enthalpy (ΔH) or entropy (ΔS) changes and temperature T. Practically, separation is achieved under the effect of two forces (or fields) in one phase or two phases. One phase is necessary for transport and may have a physical or chemical role in the separation. It is to be noted that in capillary electrophoresis, motion is due to an internal electric field, and not to the liquid phase.

LC is governed by an enthalpic process between two active phases, the internal field having only a transport role. SEC is governed by an entropic process between two phases : a stationary inert one, and a mobile one, which creates an hydrodynamic field. Nature of phases is chemically and physically indifferent. HDC is acting under the effect of one hydrodynamic field moving one mobile phase; even if a packing is present, its only role is to decrease the capillary size. The separation is due to the existence of a flow velocity profile

in the channel, in which small particles tend to be closer of the external wall, where the flow is stagnant. The nature of mobile phase is theoretically indifferent, but differences between solvents have been observed.

In FFF, separation is result of one phase under two fields : one hydrodynamic internal field, one other external, perpendicular to the flow. Examination of the various subtechniques is out of the scope of this presentation and may be found in 15, 16, 35, but two exemples will be cited. This field may be a thermal one (TFFF), which drives species to one wall (accumulation wall). Brownian diffusion acts slightly in the opposite direction. Larger species are located in a region of low velocity, so that retention increases with size. This soft process has been shown to be valuable with ultrahigh molecular weight polymers, since there is no shear degradation. Moreover band broadening is low, so that true molecular weight distribution may be obtained (36).

At high flow rates, deformation of long polymer chains leads to entropy decrease, so that an entropy gradient gives rise to a driving force, opposite to the field-driven motion. The result is an equilibrium of species at some distance from the wall. This distance increases with MW, so that large molecules are in a high velocity domain and elute first. This is called hyperlayer TFFF (22) or focusing FFF (36, 37). Elution time is greatly reduced : some minutes are sufficient for elution of 3 to 20 10^6 mol/g polystyrenes. Peak width is rather broad and increases for lower MW (22).

A second source of field is provided by centrifugation. Larger species tend to accumulate at the external wall, where flow velocity is low : again, elution is in the order of increasing size. Yet, for particles larger than 1 μm, Brownian motion becomes negligible and their bulk becomes the efficient parameter, so that largest particles, far from the wall, are carried faster than the smaller ones : consequently, they are eluted first. A mixture of glass beads is nicely separated in

components (10 to 32 μm diameter) in a few minutes (26), the same for a mixture of polystyrene lattices (2 to 45 μm diameter) (32).

The existence of an external field allows the possibility to monitor the elution, by changing it with time, for instance programming the temperature gradient. This can be done rapidly and easily. Three effects may be obtained : i) increasing rapidity of analysis, ii) decreasing peak width, leading to better detection sensitivity and constant resolution with retention volume and iii) changing retention mechanism, from normal to inverse mode. For instance, first and second features are obtained by decreasing the temperature according to an exponential decay program (constant τ) after a given time, τ (21) : "time-delayed exponential-decay (TDE)". Number of plates is increased, capacity factor is decreased from 9 to 5, resolution is maintained and Rt is increased from 0.009 to 0.025. The peak capacity is increased from 3 to 6 in the same analysis time. The same profile is applied for centrifugational field decrease (39). It appears to be more beneficial than simple linear or parabolic or simple exponential field-programmed.

HYDRODYNAMIC CHROMATOGRAPHY

Hydrodynamic chromatography (HDC) is a technique for separating solutes or particulates, at high dilution, in the micron range and according to decreasing sizes (10). Separation mechanisms are taking into account hydrodynamic and electrostatic effects. Larger particles are located preferentially in the axis of capillaries where the flow-rate is maximum (parabolic profile), whereas smaller ones are close to the walls, where the rate is minimum. Ratio of their respective elution volume is called separation factor $R_f>1$. Classical capillary

(CHDC) columns are operating in the range from 1 to 30 μm. Voids between beads in packed columns play the role of small channels - of continuously changing diameter - similar to a capillary. Packed columns are effective for diameter 30 to 1000 nm. New capillary columns allow also separation in the sub-micron range (40). It is a rapid and convenient method to obtain a finger-print of size distribution with an instrument similar to those of liquid chromatography, easy to operate. There is no limitation of solvent nature. Difficulties are low plate number N, so that generally the number of peaks (peak capacity n) in a chromatogram is low. Quantitative interpretation needs calibration (in elution volume and signal intensity).

Since its introduction by H. Small (10), studies have been mainly devoted to packed columns. It has been shown (10) that resolution is increased by packing with small monodisperse spheres. Nevertheless, the elution domain is very limited, defined by a maximum ratio R_f of 1.15 in most papers. By using 2 μm non-porous silica gel packings, a value of 1.21 is obtained for R_f, but the chromatogram has only 5 PS peaks (41). Additives, either surfactants or electrolytes may vary this limiting value. A recent work indicates that at high ionic strength, elution is opposite of "normal" mode. The raison could be that very strong van der Waals interactions dominate over the hydrodynamic effect (42). Moreover, the chemical composition could play a role, so that mixture of latex (PS and PMMA) of identical size can be separated.

Large porous particles are still used (at low flow rate) to take profit of the pores as capillaries, with a R_f value of 1.16 (43). But, combining HDC and SEC, by using porous particles, the R_f ratio may be increased to 2.11 (44). Moreover small size packing allows to obtain a high plate number, so that expected peak number is 66. Considering resolution is unity and constant with V, it is easy to express this capacity as :

$$n = 1 + (R_f\text{-}1)\ N^{0.5}\ /4\ R_f.$$

This corresponds to 37 in this case, and the obtained number is approximatively 15, which is an excellent value. These autors are also using non-porous monodisperse particles, leading to the theoretical plate height minimum value, with a low dependence of flow rate (45, 46).

With normal open capillary tube, the resolution is poorer, but the R_f may be as high as 1.45, so that the peak capacity is the same that with packed columns. (Figure 3). The choice of tube or beads diameter corresponds to the different ranges of sizes to be separated (47). Table 2 (40, 48-62) summarizes conditions of separation by CHDC and typical results (N, R_f). It can be seen that capillary diameter varies considerably, from 1 to 1000 (or even 15000) μm. Most of the initial work has been done with 250-500 μm diameter. To reduce extra-column band broadening, an optimization of injection-detection system has been presented with 50-100 μm capillaries with exemples of results by capillary electrophoresis (63). With microcolumns, efficient separation has been obtained for PS 10^3 to 10^6 daltons. The chromatogram is similar to that of SEC, but with a limited R_f of 1.1 (instead of 2) and a low number of plates N = 50 (53). Another work (54, 55) shows a very high number of plates : 10^5/m, but a more limited R_f : 1.05. Increase of N is not accompanied with that of R_f, so that the peak capacity remains low (less than 10). More recent work indicates higher values of R_f : 1.63 and rather good resolution between latex samples (62). Even with 4000 plates, the peak capacity is about 7.

Our effort was mainly devoted to optimize parameters in capillary HDC and determine exact diameter values.

EXPERIMENTAL

The instrument we used is similar to a liquid chromatograph : Waters pump 510 (Milford, Mass.), six-port injection valve

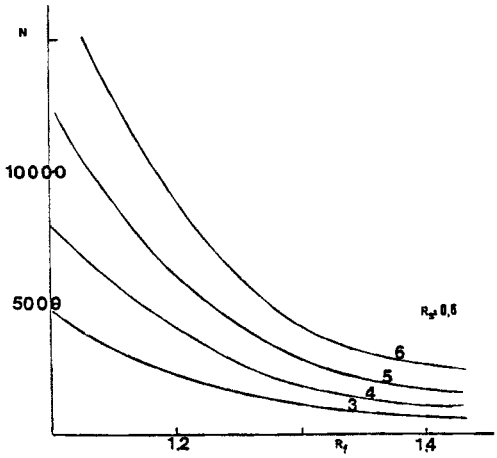

Figure 3. Peak capacity versus N and Rf (resolution assumed to be 0.6).

TABLE 2
Characteristics of Capillary Hydrodynamic Chromatography

Length, m	Int.diam., mm	N/m	Rf max	Ref.
88-201	250-500	?	1.3	48
50	180-450	25	1.5	49
60-200	250-500	10	1.55	50
15	100	25-200	> 1.2	51
0.45-7	1000	6	1.5	52
0.15-0.2	1	250	1.1	53
0.7-3.3	1.2-10	105	1.05	54, 55
91-168	250-500	16-100	1.4	56
12	15000	?	1.15	57
30-120	250-500	16-250	1.5	58
2	4	2000	>1.15	59, 60
2	6.5	?	1.42	61
5	7	600	1.63	62
2.5	10	580	>1.46	40

Rheodyne 712 (Cotati, Cal.) with external loop 20 µl. It may be replaced by a 12-port valve with two injection loops (Valco Europe, Vici, Switz.). This allows the injection of two (identical or different) samples on one or two consecutive columns. One of the advantages is the Rf accurate determination by the help of an internal standard and this avoids peak interfernce. Detector is a UV spectrometer Waters 455. A microcomputer IBM PCXT Model 286 (RAM 640 Ko), equipped with an acquisition card (analogic/digital converter) is directly connected to the output of the spectrometer. An IBM Proprinter x24, with a buffer memory (16 Ko) allows accurate drawing and the rapid transfer of values in a few seconds. Software is written in Turbo Pascal 4.0.

Analysis has been performed either with a packed column (crosslinked polystyrene spheres 20 µm : 50 cm length, 0.78 cm internal diameter), water being the eluent at a flow-rate 1.5 mL/min or with capillary stainless steel columns tested separately (L=30-60-120 m, internal diameters 2R = 0.25 and 0.5 mm). An additional fine metering valve (Vernier Handle, 18/21Y, Hoke, Creskill, N.J.) was placed before the packed column, to divide the main flow into two parts, one entering directly into the column and the other through the injection valve. It plays an important role on peak symetry. Three different eluents were used : H_2O, CH_3OH and THF, in presence of various additives. The reference sample, used as "marker", was $Cr_2O_7K_2$. Other reference materials for calibration were monodisperse polystyrene latexes (0.84 and 4 µm), prepared by emulsion polymerization in our laboratory. A larger (crosslinked) polystyrene was a 10 µm Microstyragel phase for SEC. Other samples were unknown materials (polymers and silica beads). Quantitative measurements on peaks were : height (h), base width (w), half-height width ($w_{1/2}$), skewing factor (sk=a/b, ratio of second to first part of the peak at h/10), absolute (V_e) and relative (R_f) elution volumes. Samples were injected separately and in mixtures.

Diameters were determined also by TEM (An Hitachi HU 12-A transmission electron microscope was used for dried samples deposited on a 200 mesh grid.) and quasi-elastic light scattering (QELS-PCS, photon correlation spectroscopy Brookhaven goniometer and software, a ionized argon laser, Spectraphysics 2020, being the light source). In PCS, diameter is derived from the instantaneous changes in scattered intensity due to Brownian motion. The correlation curve of intensity (correlation time τ) gives access to the translational diffusion constant $D = \tau / q^2$, related to equivalent radius a by the Stokes-Einstein law :

$$D = k_B T / 6 \Pi \eta a$$

For sizes larger than one micron, light diffraction (LD) was achieved with a Malvern Master Particle Sizer (Malvern, UK).

Sedimentation measurements were performed with the Brookhaven (Holtsville, NY) BI-DCP Particle Sizer (Disc Centrifuge Photosedimentometer).

RESULTS

Effect of Additives in Water (Ionic Strength, Surfactant and Viscosifying Agent)

With packed columns, several authors (10, 47) have observed a change of R_f with ionic strength, due to competition mainly between van der Waals (attractive) and double layer (repulsive) strengths. Electrolytes affect the critical micellar concentration (CMC) of the surfactant, the role of which appearing to be more essential. In capillary columns, we did not observed any effect of either $NaNO_3$ (0 to 28.6 g/l) or NaCl (0 to 48.7 g/l) on the shape of peak and elution volume of samples and marker.

Sodium dodecyl sulfate (SDS) from 0 to 4.8 g/l has been added to the water eluent. Its CMC is 2.5 g/l. This agent provides wettability of beads and ensures a low ionic strength (0.01) which favors higher R_f and better elution and resolution. Another work found that non-ionic surfactants lead to higher R_f than ionic ones (61).

A small amount of ethylene glycol (EG) may prevent aggregation. Theoretically, transversal motion must be decreased and R_f increased, when the viscosity is increased. In this respect, polymers have been used with more or less success (64). We did not observed an increase of R_f or a better shape of peaks in the range 0 to 6% EG. The pressure is markedly increased, according to Darcy's law and a permeability K_o coefficient may be derived. For L = 60 m, the value of 2.4 10^{-8} m^2 is far lower than that of filtration membranes (10^{-12} m^2), which indicates the low separating power of a capillary column.

In consequence of these observations, only surfactant - SDS : 3 g/l -was used.

Flow Rate Q and Nature of the Eluent

Different variations of N and R_f with flow-rate Q have been observed. They depend upon the sample size. Some deformation of peaks was observed at low flow-rates. The resolution between 0.84 µm and marker or 4 µm was unchanged, but was decreased for 4 µm and marker. The interpretation may be found in the tubular pinch effect, taking account of the Reynolds number of the particle $R_{e\,p}$. Effectively, the limiting value is 10^{-3}, which corresponds to very different flow-rates : 0.6 and 74 ml/min for 4 and 0.84 µm sample, respectively. For this latter latex, a change in mechanism occurs in the investigated domain - $R_{e\,p}$ from 1.74 to 41.7 10^{-4}- whereas the smaller latex is not concerned by this effect : $R_{e\,p}$ varies from 1.6 to 39 10^{-6}.

It is known that generally, low flow-rates v favor minimum plate height, according to van Demter law ($H = L/N = a + b/v + cv$). Here, this general expression holds with zero as c value, since it represents the mass transfert term. Diffusion is $b = 7$ cm^4/min and $a = 10$ cm for longitudinal and eddy terms, respectively. These values, far higher than in liquid chromatography, mean a high sinuosity coefficient and large equivalent beads size. Number of plates increased with flow rate, but R_f decreases for the 4 μm sample and the resolution is slightly affected, which is shown on Figure 4.

These different results for R_s, N and R_f show that operating conditions must be chosen in function of the analysis and not in an absolute way. It may be interesting to obtain either rapid results in 1.5 min with a medium resolution or higher number of plates, but in 36 min. It is also interesting to note that the highest number of plates is obtained not only for the marker, but for a largest sample.

Practical application must take account of Poiseuille law,

$$\Delta P = 8 \eta L D / \Pi R^4$$

Pressure increases with flow-rate, viscosity and decrease of capillary diameter (Tables 3 & 4).

In methanol, the number of plates was increased (Table 3). Its value was rather constant for 0.84, 4 μm and marker, but reached a maximum for the 10 μm sample, when Q increased. The resolution, so as R_f, decreased when Q is increased.

THF has a low viscosity and a good solvent power. Respectively, this may allow higher flow rates and the study of polymer in solution. In fact, although excellent base line, very high number of plates and fine chromatograms of crosslinked polystyrene were obtained, the upper limit of R_f was decreased (Table 3). With microequipment, separation has been reported with a good efficiency (53, 54).

Water being a more versatile eluent, and offering a good compromise between R_f and N values, will be the main solvent, at a flow-rate Q = 1 ml/min. This value also is a compromise

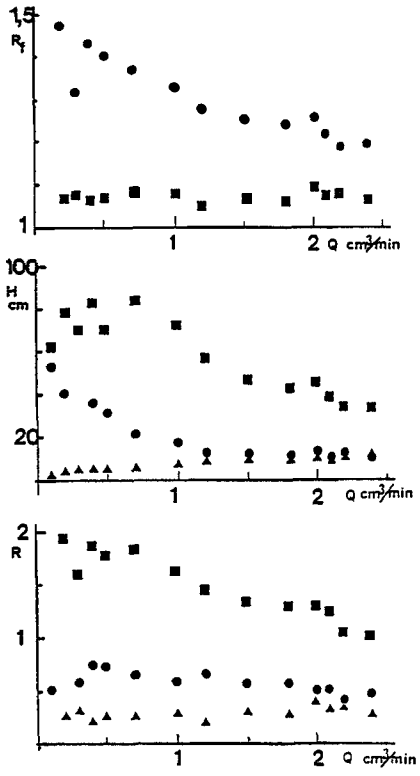

Figure 4. Effect of flow rate on R_f (for ● 4 µm, ■ 0.84 µm, marker M), H (for ● 4 µm, ■ 0.84 µm, ▲ M) and R (■ between 4 µm and M, ● 4 µm and 0.8 µm, ▲ 0.8 µm and M). Capillary column, L = 60 m, R = 125 µm.

between higher R_f and lower resolution at the higher flow rates.

Columns characteristics

Attempts realized with a 30 m column gave insufficient results, so that the essential of the work was done with 60 m

TABLE 3

Results in Hydrodynamic Chromatography

L m	30	60	120	120	60	120	0.5
R mm	0.125	0.125	0.125	0.125	0.250	0.125	3.7
Mode	capillary						packed 20 µm
Eluent	w	w	w	meth	w	THF	w
Pres. bar	45	90	175	120	12	60	35
u cm/s	34						0.1
Shear rate s^{-1}	10^4				1400	10^4	10^8
Rf max	1.4	1.45	1.45	1.45	1.6	1.26	1.15
N max	500	900	1900	7000	2300*	30000	7000
N max/m	16.7	15	15.8	58.3	38.3	250	14000
Range µm	0.8-20						0.05-0.8

TABLE 4

Effects of Operating Parameters on Peaks Characteristics

	L	R	Q	η	I	TA	C	λ
R_f	0	↘	0	0	0	↘	0	0
R_s	↗	↘	0	↘	↘	0	0	↗↘
N	↗	↘	↗	↘	↘	↘	0	
n	↗	↘	0	↘	↘	↘	0	
h	↘	↘	↘	0	↘	↗	↗	↗↘
w	↗	↗	↗↘	0	↗	↗↘	0	
t	↗	↗	↘	0	0	0	0	
ΔP	↗	↘	↗	↗		0	0	
R_e	0	↗	↗	↘	0	0	0	
$\dot{\gamma}$	0	↘	↗	0	0	0	0	

and finally optimized with 120 m length. A basic formula relating R_f and size of sample is :

$$R_f = 1 + 2\,a / R - (2\,g + 1)\,(a / R)^2$$

where g is a coefficient depending on flowing conditions. As expected, R_f must be - and was rather - independent of length L. The coefficients of the law are quite far from the theoretical values : that of a/R is ß =17 and g is 110 (instead of 2 and 2/3 respectively). This expresses that R_f is higher than expected and that particles move not far from the wall, according to ref. 47, 65.

The resolution was far better with 120 m, since w increased as the square root of L and V_e, proportionally to the length, as a general law in chromatography. By going from 60 to 120 m, H was found unchanged and N increased from 380 to 800, for the 4 µm sample. A way to illustrate that increase in resolution is to consider the calibration as represented in SEC. A lesser slope allows a better separation.

The second characteristics of the column is its diameter. The limit in R_f shows that over a certain sample size, no separation occurs. This limit of size may be related to the ratio : average radius of sample a to radius of tube R. A third order law, relating a to R may have a reasonable approximation in a linear one :

$$a = f + k\,R\ (\mu m)$$

with f = -7 and k = 0.1, R being in µm. A column of diameter 500 µm may have a medium domain of separation of 18 instead of 5 µm for the 250 µm one. Taking account on published results (44), ratio R to a is about 100, and for a given diameter of column, the usable a/R range varies roughly from 10^{-3} to 10^{-1}. The interest of large diameter is also the decrease of γ, rate of shear. It is to be noted that the resolution is higher for narrow tubes. The corresponding coefficients of the calibration curve R_f are ß = 10 and g = 25.

Table 3 summarizes the main effects of column and eluent variables on the chromatograms and separation possibilities.

These results are in general agreement with those published elsewhere.

Injection - Detection and Interpretation

Table 4 presents in a synthetic way the effects of all the parameters we varied in this study.

Signal increases linearly with concentration or injected volume (5 to 25 µl), which means that no interaction, adsorption or overloading were occuring. Sensitivity is so high than a few 25 particles in the detector cell give a noticeable signal. Peak characteristics V_e, w, N, R_f, R_s are not affected, but the slope of the corresponding curve varies with sample size. The result is that quantitative interpretation needs preliminary calibration. The reason is that signal depends upon absorption and scattering, which vary with sample diameter at a power a. Taking into account the number of particles in the samples, we found the exponent a being less than 3 for the 4 µm sample.

A general formula in liquid chromatography for the instantaneous detector response at each elution volume V is

$$H = \Sigma N_i (V) D_i^a (V) K_i (V)$$

where K_i is the extinction coefficient for particles of diameter D_i and a = 2 in the Mie scattering regime for a turbidity detector. In the Rayleigh regime, valid for $\lambda/D < 0.3$, a = 6 for turbidity and 3 for refractometry (66). This explain the effect of λ on signal shape and intensity. Moreover, the intensity decreases when there is no absorption at λ = 630 nm, in agreement with results in (67). Intermediate values of a may correspond to a combination of a and K (V); finally a = 1 holds for refractometry and spectrophotometry of polymers in SEC, so that the range 1 to 6 will be considered.

Assuming the formulas

$$\overline{D_n} = \Sigma ND/\Sigma N$$

$$\overline{D_w} = \Sigma ND^4/\Sigma ND^3$$

for number- and weight- average diameters and the simplified general relationship $H \propto ND^n$, (K and a are independent of V), then

$$\overline{D_n} = \Sigma(H/D^{n-1})/\Sigma(H/D^n) \text{ and}$$
$$\overline{D_w} = \Sigma(H/D^{n-4})/\Sigma(H/D^{n-3}).$$

For all the investigated polystyrene latexes, number and weight average diameters decrease deeply when n is increased (an exemple is given on Figure 5 for a PS standard of 106 nm diameter). The best agreement with TEM is obtained for n = 3-4, which is in agreement with theory since their diameters are in the range of the wavelength of detection (254 nm). Except for n = 2, the polydispersity index P is not affected by n, and is the same for all samples. Nevertheless distributions do not correspond to true ones. These high polydispersity values as compared to that determined by TEM analysis (1.02) clearly show the necessity of band broadening correction.

Dispersion Correction

There are many approaches for solving the dispersion problem. It has been shown (68) that skewed instrumental spreading functions derived from the plug-flow dispersion model (69) fit data for particle separations by HDC, where the spreading function is

$$G(V,y) = \{4(\pi Pe^{-1}(v/y))\}^{-0.5}\exp\{-(v-y)^2/4\ Pe^{-1}(v/y)\}$$

(with the dispersion term as the Peclet number Pe = UL/D'; Superficial velocity U, length of the packed bed L, an empiric dispersion coefficient D', elution volume v and at the maximum of peak y).

Pe = 100 corresponds to no correction of peak. The effect of larger Pe on diameter distribution (latex 106 nm) is shown on Figure 6. The results for the other samples are similar.

Whatever the value of n, Dn increases with Pe, the effect being stronger for higher n. On the contrary, Dw increases or

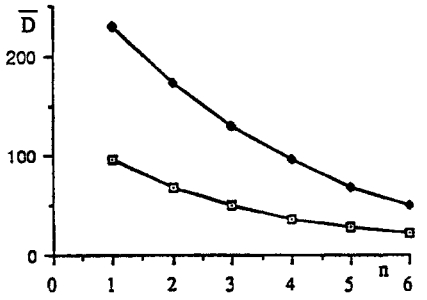

Figure 5. Average diameters versus exponent n for a PS latex standard, 106 nm (by TEM).

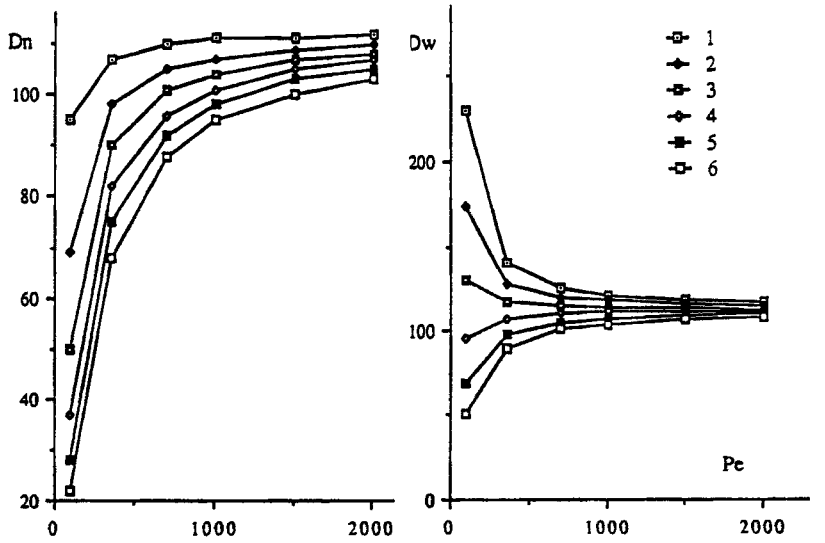

Figure 6. Average diameters versus Pe, for different values of exponent n for a PS latex standard, 106 nm (by TEM).

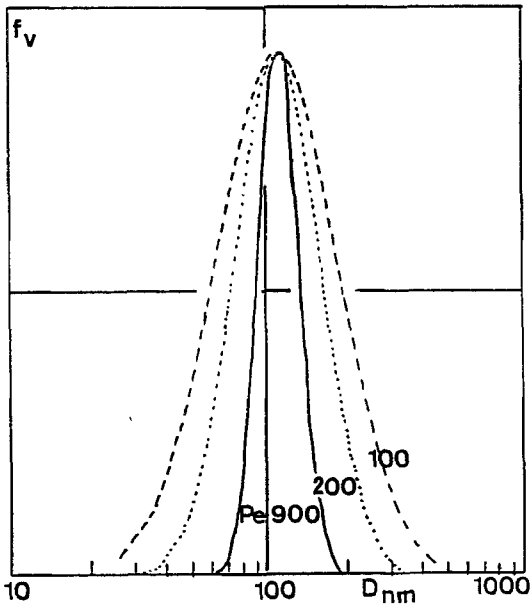

Figure 7. Effect of Pe on diameter frequence distribution for a PS latex standard, 106 nm (by TEM).

decreases with Pe, depending on n. Quasi-constant value corresponds to n = 3-4. More detailed results may be found in (70).

In another representation of results, Dw versus n, for different Pe values, curves for all samples have a common intercept between n = 3 and 4. A rapid change of D is noted up to Pe = 500, then Dn and Dw tend slowly to close values, independent of n.

Practically, considering the whole set of results, n must be chosen as 3-4 and Pe 500, for getting best average and distribution values, as well as narrow peaks, which means enhanced resolution. This effect on diameter distribution is shown on Figure 7.

It is necessary to have a high accuracy in data acquisition, since the useful elution domain is narrow. A frequent calibration procedure is also necessary, with possibility to correct small changes in elution volume (70.

To overcome calibration procedure and use of standards, on-line viscosity has been proposed, but this method "will await the development of improved pressure transducers" (71). Our approach was discontinuous measurements of sample size on eluted fractions. As mentionned earlier, they have been operated by TEM, PCS and sedimentometry. Exemple of these two last methods are given on Figure 8. It shows the high resolution of sedimentometry (time is inversely proportional to diameter squared) for a mixture of four latexes and a reasonable resolution for HDC. PCS, operated with a proper choice of parameters may give an excellent correlation with TEM values and an excellent resolution for a similar mixture of two latexes. For the three methods the analysis time is similar.

CONCLUSION

In capillary HDC, additives effect is low, surfactant being the most useful. The R_f is strongly affected by flow rate in methanol and is rather constant in water. The high number of plates in THF is not accompanied by a high maximum R_f value. This separation factor may be higher with 60 m than with 120 m, but the resolution is not so high. Even with microequipments (small diameter capillaries, short columns) of enhanced resolution, peak capacity is very limited. They are an interesting alternative for packed columns. HDC is rapid and very sensitive, but some conditions must be obeyed to obtain reliable results. First, the need of an accurate calibration, with frequent adjustment of parameters and choice of a column giving a low slope. Second, the need of changing the detector response factor (exponent n) and third, correcting for axial

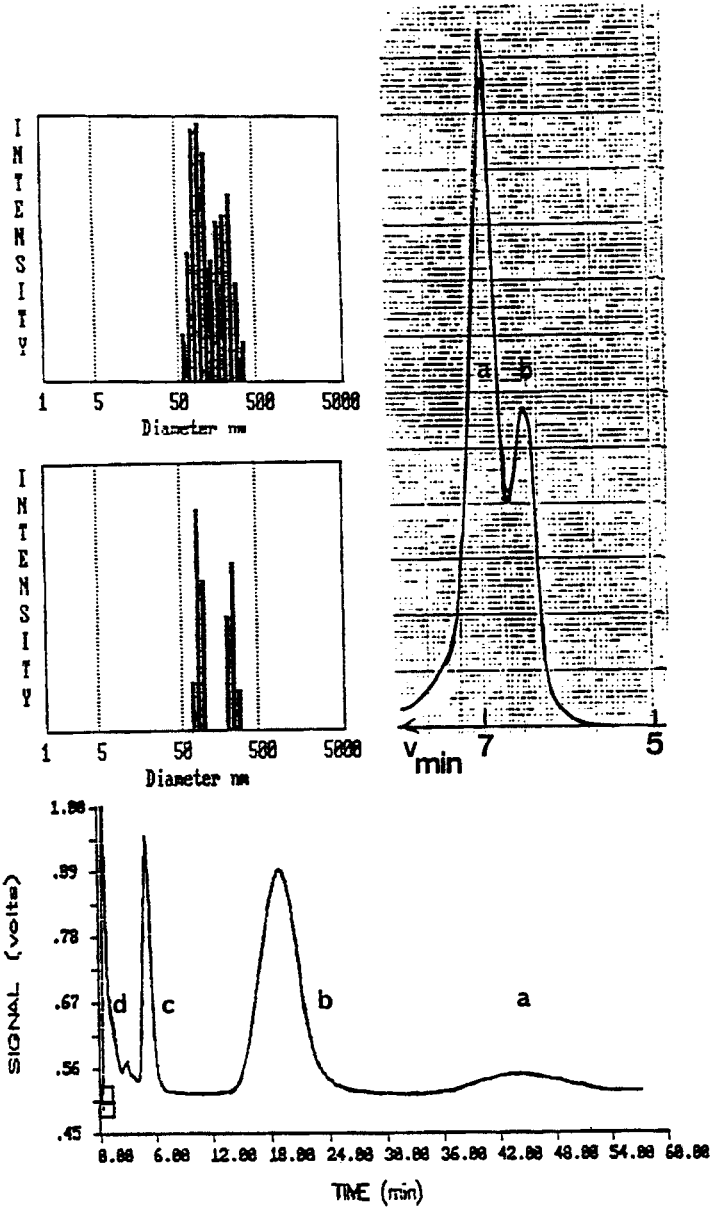

Figure 8. Mixture of PS latexes standards, 88 and 234 nm, by optimizing software parameters (below) in PCS analysis. HD Chromatogram on packed column (20 μm; 50x0.78 cm; water 1.5 ml/min) of PS latexes standards, a-50 and b-234 nm. Sedimentogram of a mixture of PS latexes standards, a-106, b-176, c-357 and d-1130 nm.

dispersion by use of a simple equation. As a consequence of the combined analysis of the effects of n and Pe, the proposed value for exponent n is 3.5 and a Peclet number of 500. Results are compared on eluted fractions with those of direct methods, for instance photon correlation spectroscopy and sedimentometry, this latter being of high resolution. By proper use of interpretation parameters, HDC leads to good results, in a short time, for a low cost and with a simple procedure.

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