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Practical Experience with Organic Solvent Flow Field-Flow Fractionation

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Key Words

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Particle size determination

Polystyrene

Polystyrene latexes

Summary

Several types of membrane have been tested for use in organic solvent flow field-flow fractionation in an asymmetric channel. The practical problems most commonly encountered were leakage of air and solvent through the support layer on which the membranes are cast, and unequal swelling of the membrane and the support layer in the organic solvent, leading to ridging of the membrane in the channel. Three types of membrane were found suitable for the separation of polystyrene standards with tetrahydrofuran as solvent. The best results were obtained with a fluoropolymer membrane.

Fair agreement was found between theory and practice for the dependence of retention times on the relative molecular mass of the standards and on the flow regime. Use of scanning electron microscopy revealed that for a number of the membrane materials some pores were much larger than expected on the basis of the indicated molecular weight cut-off. Whereas these materials could not be used for the fractionation of soluble polymers, they could be applied with some success to the separation of solid latex and silica particles. A PTFE membrane could be used for the separation of latexes and silica particles suspended in acetonitrile as carrier liquid. In general, however, the retention times of these particles were shorter than theoretically predicted.

Introduction

Field-flow fractionation (FFF) is a family of separation techniques that can be applied to a very wide size-range

of molecules and particles [1]. The range of application starts with soluble molecules with a relative molecular mass of approximately 1000 and ends with solid particles with diameters up to 100 μm . Thermal and flow FFF have been successfully employed for the fractionation and characterization of polymers. If they could be used for the same samples, flow FFF and thermal FFF would complement each other very well, the former yielding size-related data only and the latter also yielding data on the chemical composition of the polymer molecules [2]. Although thermal and flow FFF have many similarities, their range of application is, however, different. Whereas solid particles can be separated in aqueous solutions with thermal FFF [3], its application to dissolved polymers is mostly limited to use with organic solvents. Thermal diffusion, the force driving the separation in thermal FFF, is generally very weak in water for polymer molecules. Flow FFF, on the other hand, is mainly used in an aqueous environment. FFF in organic solvents is difficult to implement because of the lack of suitable membranes. In the literature on flow FFF few articles report on the use of organic solvents [4–7].

A membrane for flow FFF should have: a smooth surface, uniform porosity, a suitable pore size, a low flow resistance for the solvent, and inertness to carrier liquids and samples. The last requirement is the most difficult to meet when using organic solvents – the literature reports the use of only two types of membrane in organic solvent flow FFF. Brimhall et al. [4] and Caldwell et al. [5] used cellulose nitrate membranes and ethylbenzene as a solvent for the fractionation of polystyrene samples. Kirkland and Dilks [6] used modified regenerated cellulose membranes with various solvents and samples. They found that some samples were strongly adsorbed on unmodified membranes; modification prevented this adsorption, but after three weeks of continuous use the membrane was dissolved by the mobile phase. Miller et al. [7] did not mention the membrane used. The solvent used was hexane and copolymers were fractionated.

In general, three different basic types of membrane can be distinguished: porous membranes (used for micro- and ultra-filtration), non-porous membranes (for gas separation and pervaporation) and liquid membranes

Figure 1
SEM image of the cross-section through an asymmetric polysulphone membrane; magnification $\times 1128$.

Figure 2
Cross section of the asymmetric FFF channel (not to scale).

(with carrier-mediated transport). Of these, only porous membranes are suitable for use in flow FFF. The best membranes for this purpose are of the asymmetric type, i.e. those with a very thin ($0.5\ \mu\text{m}$), dense upper layer, which determines the transport rate through the membrane, supported by a macroporous sub-layer of the same material, usually $50\text{--}200\ \mu\text{m}$ thick, the sole purpose of which is to provide mechanical stability. An asymmetric membrane is robust enough to be handled and the flow resistance for the solvent is low enough. Figure 1 shows a scanning electron microscopy (SEM) image of an asymmetric polysulphone membrane.

Lee et al. [8] and later Jönsson and Carlshaf [9] showed that porous hollow fibres can be used as membranes for flow FFF. In previous work in our laboratory [10, 11], we

demonstrated the practical advantages of hollow fibre flow FFF for the separation of water-soluble polymers. The polysulphone fibres used in these studies are not resistant to most organic solvents, however, and other suitable fibres are not yet available.

An asymmetric flat channel was used for the experiments described in this paper. Several membrane materials were tested for their suitability in flow FFF with organic solvents. The retention behaviour of polystyrene standards, latexes and silica particles was studied.

Experimental

Apparatus

The flow FFF system was similar to that described earlier [10], except that the hollow fibre was replaced by an asymmetric channel. The system consisted of two pumps (P-6000; Pharmacia, Uppsala, Sweden) providing the axial and cross flows. The pumps were connected to a LCC-500-plus controller (Pharmacia) to regulate the flow rates. Injection was performed with a $5\ \mu\text{L}$ injection valve (Rheodyne, Berkeley, CA, USA). The cross section of the home-made separation channel is shown in Figure 2. The channel was formed between two 16-cm-long Duran 50 (Schott, Mainz, Germany) glass plates clamped together with ten bolts. Stainless steel tubing was mounted in inlet and outlet apertures drilled in the upper plate. An aperture for the cross flow and a room for the ceramic frit were drilled in the lower glass plate. The ceramic frit was used as a support for the membranes. A membrane and a 254-mm thick Mylar spacer were sandwiched between the glass plates. The rectangular separation channel cut out of the Mylar spacer was $10.5\ \text{cm}$ long and $1\ \text{cm}$ wide. Detection was performed with an ABI 575 UV detector (Applied Biosystems, Ramsey, NJ, USA). The wavelength used was $254\ \text{nm}$ for polystyrene and polystyrene latex particles and $210\ \text{nm}$ for silica particles. A Varex (Burtonsville, MD, USA) evaporative light scattering detector was used for detection of polyethylene oxide samples.

Chemicals and Solutions

Polystyrene sulphonate standards with relative molecular masses (M_r) of 100 000 and 200 000 were obtained from Polymer Laboratories (Church, Stretton, UK). Polystyrene standards with M_r values from 34 500 to 4 000 000 were obtained from Machery-Nagel (Düren, Germany), Merck (Darmstadt, Germany) and Pressure Chemical Company (Pittsburgh, PA, USA). Polystyrene latex particles were from Duke Scientific Corporation (San Antonio, CA, USA; $40\ \text{nm}$) and from Polysciences (Warrington, PA, USA; $110\text{--}930\ \text{nm}$). Silica particles of 59 , 155 and $240\ \text{nm}$ diameter were home-made. The membranes tested are listed in Table I. For each type at least three membranes were tested. Solvents (p.a.) were from Janssen (Geel, Belgium).

Table I. Characteristics of the types of membrane tested.

Membrane type	Supplier	Material	Solvent	Sample	Remarks
PM 30	Amicon (Danvers, MA, USA)	Polysulphone MWCO 20 000	Water	Polystyrene sulphonates	Only a few organic solvents possible
Thomapor dialysis foil	Reichelt (Heidelberg, Germany)	Regenerated cellulose MWCO 20 000	THF	–	Very high flow resistance
TE 30 membrane filter	Schleicher & Schuell (Dassel, Germany)	PTFE pore size 0.02 μm	THF Acetonitrile	Polystyrene Polyethylene oxide; latex and silica particles	Support layer removable; some pores much larger than 0.02 μm
UF-PA-50H	Hoechst High Chem (Wiesbaden, Germany)	Polyaramide MWCO 50 000	THF	Polystyrene	Support or membrane swells in THF
AMT	Advanced Membrane Technology (Berkeley, CA, USA)	Polyvinylidene fluoride MWCO 50 000	THF	Polystyrene	Some pores too large
MPF-u20-s	Kirgat Weizman (Rehovot, Israel)	Polyphenylene oxide MWCO 20.000	THF	Polystyrene	Support or membrane swells in THF
DDS	De Danske Suckerfabrikker (Nakskov, Denmark)	Fluoro polymer MWCO 30 000	THF	Polystyrene	Membrane thickness a few μm

Procedures

Before an experiment was started the channel was always flushed with eluent at an axial flow rate of 0.5 mL min⁻¹ to remove possible contamination from a previous run. After one minute of cleaning the cross flow was set at the relaxation value of 0.1–0.35 mL min⁻¹, and the axial flow was set at the appropriate value. During relaxation 10 % of the flow enters the channel at the inlet and 90 % at the outlet, resulting in a relaxation point 1 cm from the inlet. After 1 min the sample was injected; after 10 min relaxation, the axial flow was set to the elution value of 0.5 mL min⁻¹. For the fractionation of polystyrene samples gradient elution was performed. The axial flow was 0.5 mL min⁻¹ and after the relaxation the cross flow was linearly reduced from 0.35 to 0.10 mL min⁻¹ in 25 min.

Results and Discussion

Testing of Membranes

The home-made channel was first tested with a polysulphone PM 30 membrane and aqueous carrier solutions. The channel design seemed to be satisfactory. In the fractionation of sodium polystyrene sulphonates (PSS), the results obtained were similar to those in previous experiments in which polysulphone hollow fibres were used for flow FFF [10, 11]. The recovery of the PSS samples from the channel after relaxation was complete, and their retention behaviour was in accordance with

expectations based on the experiments with hollow fibres. The polysulphone membranes could not, however, be used for organic solvent flow FFF because they are known to dissolve in most organic solvents. Several other commercially available types of membrane were, therefore, tested for this purpose. The membranes tested are listed in Table I.

During the testing of the different types of membrane three main problems became apparent. The first is related to the support layer on which most membranes are cast. This support is necessary because the membrane material is too thin and vulnerable to be handled on its own. Often, however, the support layer is made from a material different from that used for the membrane itself and, therefore, the two layers may swell to a different extent in the organic solvent used, resulting in ridges in the membrane. These ridges could not be completely smoothed when the membrane was mounted into the channel, which resulted in an irreproducible void volume in the channel (see below), uneven flow patterns inside the channel and thereby irreproducible retention times and inaccurate results. Severe ridging was, for example, observed with the polyphenylene oxide MPF membranes, and to a lesser degree with the membranes made of polyaramide (UF-PA) or a fluoropolymer (DDS). The ridging effects could be reduced to some extent by leaving the membranes to swell for a long period (e.g., a week) in the solvent before mounting them in the channel. With the TE 30 membrane filter, made from PTFE, the support layer could be removed to prevent ridging.

A second problem frequently encountered is related to the low permeability of the membranes for the carrier solution in combination with the low vapour pressure of the organic solvents used. Because in our arrangement the cross-flow pump is used in the counter-direction (and is, therefore, sometimes called an unpump), an underpressure is created below the membrane. Because of the low vapour pressure of the solvent, the desired cross-flow rate through the membrane for flow FFF could not be realized. For this reason we could not use the Thomapor regenerated cellulose dialysis foil, the only membrane without a support layer which was tested. A related problem was found with membranes with a support layer. Because the permeability of the support is usually much higher than that of the actual membrane, the underpressure causes air to leak into the channel compartment under the membrane through the sides of the support layer. In extreme cases up to 50% of the volume displaced by the cross-flow pump consisted of air. With the polyamide and the polyphenylene oxide membranes the leakage of air decreased during use for several hours, probably because washing out of the viscous protection liquid inside the pores of the membrane with the cross flow, increased the permeability of the material. Leakage of solvent through the support layer out of the channel was also a problem with some types of membrane. When the pumps were left working continuously, this resulted in an appreciable loss of solvent (up to 150 mL of THF per 24 h). When, on the other hand, the pumps were stopped overnight, the (swollen) membranes dried out and frequently cracked. Loss of solvent through the sides of the membrane also prevented the use of flow restrictors to increase the overall pressure in the system in an attempt to solve the problem of leakage of air into the channel.

A third problem often encountered is non-uniformity of the pores in the membrane. There may be pores present which are much larger than the average pore size or as indicated as the molecular-weight cut off (MWCO) given by the manufacturer. An example is given in Figure 3, which shows an SEM image of a TE 30 PTFE membrane. Although the pore size is stated by the manufacturer to be 20 nm, some holes visible at the surface of the membrane have a size of the order of 50–100 nm. Large pores were also seen in SEM images of PVDF membranes. The larger holes probably arise from the casting of the polymer on the support layer. The presence of the holes makes the membrane less suitable for flow FFF. Firstly, because the local permeability of the membrane at the position of a hole may be much higher than average, a considerable part of the cross flow will be focused at these places and the cross flow in the remainder of the channel will be less than expected. Secondly, the sample may disappear through the wide holes with the cross flow during relaxation. This is probably what happened when the TE 30 membrane was tested for the fractionation of polystyrene (PS) or polyethylene oxide samples with THF or acetonitrile, respectively, as carrier solution. It was found that the

Figure 3

SEM image of the surface of the TE 30 membrane filter; magnification $\times 11\,700$.

recovery from the channel of a PS standard with an M_r of 675 000 decreased substantially with relaxation time, even though this polymer has a radius of gyration of 37 nm [12] and the average pore size is only 20 nm. Similar observations were made with the polyethylene oxide standards in acetonitrile.

The same membrane could, however, be used successfully for the separation of solid particles. Polystyrene latex and silica particles in the size range 40–930 nm could be fractionated with acetonitrile as carrier solution. Even when the smallest latexes were considerably smaller than the PS 675 000 molecules, they did not disappear through the membrane. Different explanations can be given for this inconsistency. Firstly, it may be possible that the PS molecules, which behave as random coils in THF, enter the pores by a creeping process. When the large pores do not penetrate the membrane completely, the PS molecules may still get stuck inside the membrane. A second explanation may be that electrostatic repulsion of the slightly charged latex particles by the membrane material in the pure acetonitrile solvent prevents them from entering the larger pores.

Of all the membranes tested, the fewest practical problems were encountered with the DDS fluoropolymer membranes. When these membranes were left to swell in the organic solvent before being mounted in the channel, ridging was limited. Their permeability to the carrier was high enough to prevent leakage of air into the channel. The PTFE membrane filters (for particle separations), the polyamide membrane and the polyphenylene oxide membranes could, however, also be used for organic solvent flow FFF, albeit with more practical difficulties and a higher failure rate.

Figure 4
Influence of the flow regime on the retention times of polystyrene standards with $M_r = 675\,000$ (■) and $4\,000\,000$ (○). Carrier: THF; membrane material: polyaramide.

Applications

Despite the practical problems mentioned in the preceding paragraphs, a number of different membrane materials could be used for the fractionation of polystyrene samples with tetrahydrofuran (THF) as carrier liquid. Initially we tried, using the method described by Giddings et al. [13], to establish the void volume of the channel for various types of membrane. A sample containing a high-molecular-weight component (PS 675 000) was injected into the channel, with zero cross flow and without relaxation. The break-through volume of the system, calculated from the front of the observed peak, was multiplied by 1.5 to give an estimate of the channel volume after correction for the volume of the connecting capillaries. The void volume calculated from the geometry of the spacer was 0.22 mL. It was found that the void volume determined experimentally varied considerably with the type of membrane, the particular membrane mounted in the channel and even from day-to-day with the same membrane. With polyaramide membranes, for instance, values between 0.26 and 0.32 mL were found experimentally; with polyphenylene oxide membranes the values were between 0.23 and 0.27 mL. The systematic bias observed with most types of membrane may be related to the construction of the channel. It is possible that the ceramic support frit below the membrane is positioned slightly too low in the lower glass plate, and that this results in increased channel thickness. An explanation of the experimental irreproducibility is the varying degree of swelling and ridging of different membranes. Unfortunately, the flow and pressure conditions with these void volume determinations have to be different from those during the actual measurements, when a cross flow is applied. The channel thickness as calculated from the void volume may, therefore, not represent the real

value during fractionation experiments. Because of this the nominal value for the spacer thickness was used in further calculations.

The next test was to determine whether the retention behaviour of polystyrene standards follows the theory for asymmetric flow FFF. When the focusing point of relaxation is close to the inlet of the channel, the retention time in asymmetric FFF for compounds with a moderate to high retention ($t_R > 10t_0$) is approximately given by [14]:

$$t_R = \frac{w^2}{6D} \ln \frac{F_{in}}{F_{out}} \quad (1)$$

where w is the thickness of the channel, D the diffusion coefficient of the compound and F_{in} and F_{out} are, respectively, the flow rates at the inlet and the outlet of the channel. Polystyrene standards with molecular masses of 675 000 and 4 000 000 were injected in a channel with a polyaramide membrane under various flow regimes; the observed retention times as a function of $\ln(F_{in}/F_{out})$ are plotted in Figure 4. The lines in the figure represent the theoretical values predicted using Eq. (1); literature values for the diffusion coefficients of 1.78 and $0.65 \times 10^{-11} \text{ m}^2 \text{ s}^{-1}$ [15] were used to calculate these lines. It is apparent that there is reasonable agreement between the observed retention times and the theory. Deviations at the lower end can only partly be explained by the fact that Eq. (1) is not accurate in this region. These data points were, however, obtained with very low cross flows, outside the specification range of the pump. The data points given in Figure 4 were obtained on different days with different membranes. The deviation of the experimental data from the theoretical expectation may also be the result of variations in the actual void volume of the channel caused by the varying extent of 'ridging' of the membranes.

The relationship between the apparent diffusion coefficient and the molecular mass of polystyrene standards was investigated using three different types of membrane. The diffusion coefficient of a polymer molecule can be expressed as a function of its relative molecular mass M_r by [16]:

$$D = A \cdot M_r^{-b} \quad (2)$$

where A and b are empirical constants. For randomly coiled molecules the value of b is generally between 0.50 for a theta solvent and 0.59 for a good solvent. Because the retention time of a compound in flow FFF is theoretically inversely proportional to its diffusion coefficient Eq. (1), a plot of $\log t_R$ against $\log M_r$ is expected to have a slope equal to the value of b in Eq. (2).

The results of the experiments are shown in Figure 5. Each point represents the mean of at least three (consecutive) measurements. The relative standard error in the retention times was between 3 and 12%, or between 0.01 and 0.05 units in the logarithmic plot of Figure 5. Although these deviations between individual experiments were relatively large, significant differences be-

Figure 5

Dependence of the retention time of polystyrene standards on the molecular mass. Carrier: THF; axial flow 0.5 mL min^{-1} ; cross flow 0.2 mL min^{-1} . Membrane materials: polyamide (■); polyethyleneoxide (Δ); fluoropolymer (○).

Figure 6

Fractionation of a mixture of polystyrene standards with a cross flow gradient. Axial flow: 0.5 mL min^{-1} ; cross flow $0.35\text{--}0.10 \text{ mL min}^{-1}$ in 25 min. Carrier: THF; membrane material: polyamide.

tween the membrane materials were not observed. The slope of the linear regression line based on all data points in Figure 5 is 0.60 ± 0.04 . This is in agreement with the theoretically predicted value of 0.59 for polystyrene in THF.

In terms of separation efficiency the best results were obtained with the DDS fluoropolymer membrane. For PS 675 000 plate numbers of the order of 30–40 were obtained with an axial flow of 0.5 mL min^{-1} and a cross flow of 0.2 mL min^{-1} ; under these conditions the theoretically predicted plate number is approximately 50. With the other types of membrane, which showed more

Figure 7

Dependence of retention time on the diameter of solid particles. Carrier: acetonitrile; axial flow: 0.5 mL min^{-1} ; cross flow: 0.2 mL min^{-1} ; membrane material: PTFE. Samples: polystyrene latexes (○) and silica (□). The dashed line shows the theoretical dependence.

ridging, the separation efficiency was considerably lower.

With a polyamide membrane polystyrene standards were separated by using a cross-flow gradient with THF as carrier liquid. After relaxation, the cross-flow was linearly decreased from $0.35\text{--}0.10 \text{ mL min}^{-1}$ in 25 min. The axial flow rate was 0.50 mL min^{-1} . Figure 6 shows the fractogram obtained.

In the previous section it was mentioned that the TE 30 membrane filters could not be used for the fractionation of polymers ??dissolved?? in organic liquids. Solutes were not recovered from the channel either because they were irreversibly adsorbed on the membrane material or because they could penetrate the large pores of the filter. These filters could, however, be used with some success for the separation of solid particles. Suspensions of polystyrene latexes and spherical silica particles in the size range 40–930 nm were injected using pure acetonitrile as the carrier liquid. The observed retention times are plotted as a function of the particle diameter in Figure 7. The diffusion coefficient of a spherical particle can be calculated with the well-known Stokes equation:

$$D = \frac{kT}{3\pi\eta d} \quad (3)$$

where η is the viscosity of the solvent and d the particle diameter. Combining Eqs. (1) and (3) shows that the retention time of a particle is expected to be proportional to its diameter. The line in Figure 7 gives the theoretical prediction. It can be seen that most particles elute earlier than expected. For the largest latex particles this can be explained by steric effects. For the 570 nm particles, for instance, a theoretical layer thickness of 550 nm can be calculated under the flow conditions employed. Since steric FFF starts to predominate when the particle size and the layer thickness are of the same

order of magnitude [17, 18], the inversion of the elution mode from normal to steric is, apparently, in this size region. For the smaller particles also, however, the retention times are shorter than expected. This may be the result of electrostatic repulsion of the particles by the membrane in the pure acetonitrile solvent. Possibly also the thickness of the channel was smaller than assumed because of the compressibility of the PTFE membrane material. Unfortunately, as discussed above, the channel thickness could not be derived precisely from the void volume. The elution time of the unretained peak depended on the cross flow rate and was highly irreproducible.

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