Characterisation of polymers and particles by asymmetrical flow field-flow fractionation
van Bruijnsvoort, M.

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CHAPTER 3

Hollow-fiber flow field-flow fractionation of synthetic polymers in organic solvents

ABSTRACT

A modified polyacrylonitrile (PAN) hollow-fiber membrane from a commercial source has been applied as the separation channel in flow field-flow fractionation (FFF). With the PAN membrane fiber the application range of flow FFF could be extended to synthetic polymers soluble in a variety of organic solvents. The PAN membrane was shown to be resistant to hydrophobic solvents such as dichloromethane (DCM), tetrahydrofuran (THF), ethyl acetate and methyl ethylketone (MEK), as was illustrated by the successful fractionation of different polymer standards in these solvents. The system performance was assessed using polystyrene (PS) standards with ethyl acetate as the solvent. For a 100 kDa PS standard the average recovery was 57%, while for standards with a molar mass of 400 kDa and higher 100% recovery was obtained. A linear relationship between peak area and injected mass was found. The run-to-run and fiber-to-fiber repeatability was determined using 100 and 400 kDa PS standards. The repeatability appeared to be satisfactory with relative standard deviations < 2% for the retention times and < 5% for the recoveries of the standards. Plate numbers for the 400 kDa standard on different fibers were in the order of 110. From measurements on the fractionation of ferritin aggregates it is concluded that the instrumental band broadening is negligible. For an accurate determination of diffusion coefficients and molecular sizes based on retention times, calibration of the channel with standards appeared to be necessary. However, it was shown that the FFF system could be coupled to a multi-angle light scattering (MALS) detector, providing an alternative on-line method for calibration. Expressions for the maximum attainable plate number per unit of time have been derived for a hollow-fiber flow FFF system. It is shown that an increase in the system performance can be expected from a scaling down of the fiber diameter.
INTRODUCTION

Flow field-flow fractionation (FFF) is one of today's most versatile and universal separation methods for polymers and particles. In flow FFF, solutes are retained according to their diffusion against a field induced by a flow through a membrane. Owing to the open channel structure and the ability to vary the magnitude of the field, a size range from approximately 5 nm up to 100 μm can be covered with flow FFF. Flow FFF is particularly valuable for the analysis of high-molar mass compounds and particles. For such analytes, flow FFF can offer advantages in comparison with the more established technique of Size Exclusion Chromatography, where secondary interactions with the packing material can disturb the separation.

However, the application range of flow FFF is severely limited by the poor resistance of polymeric membranes to organic solvents. Up till recently, flow FFF could only be used reliably for the analysis of water-soluble polymers or aqueous suspensions of particles. With a solvent-resistant membrane it should be possible to utilize the advantages of flow FFF for high-molar mass compounds in organic solvents. In search of a suitable membrane, researchers have made use of cellulose nitrile, modified cellulose and polyaramide materials. Wijnhoven et al. have tested several other membrane types and summarised the main practical bottlenecks of organic solvent flow FFF. Although it was found that, in principle, organic solvents can be used, the long-term stability of the membranes was generally quite poor and adsorption of solutes on the membranes frequently occurred. The latter phenomenon has an adverse effect on the separation efficiency.

Since hollow-fiber membranes have a large surface-to-volume-ratio and since they can be easily incorporated in flow streams, they are an elegant alternative to flat membranes in many analytical applications. Hollow-fiber flow FFF (HF5) has been pioneered by Lee et al. and later matured at the hands of Carlshaf and Jönsson. HF5 has been applied to the analysis of polystyrene sulfonates and employed as a tool to study peak overloading. Despite the great promise of the technique, the full possibilities of HF5 have yet to be exploited. The main practical obstacle for HF5 is that it requires a highly consistent membrane quality. Inhomogeneities of the porosity of the membrane may lead to a poor fiber-to-fiber repeatability and to a loss of performance. However, recently Lee et al. have accomplished an impressive separation of latex particles. The relatively small dimensions of the hollow-fiber set-up facilitate temperature control of the system.

In this work, results are presented of organic solvent flow FFF in a commercially available hollow-fiber ultrafiltration membrane made of modified polyacrylonitrile (PAN). Non-solvents of PAN, to which the membrane can be assumed resistant, include: water, alcohols, ketones, hydrocarbons and chlorinated hydrocarbons. PAN does, however, dissolve in polar organic solvents such as dimethyl sulfoxide and dimethyl formamide. The hollow-fiber geometry of the membrane offers some specific advantages for the work with organic solvents. Solvents are contained in a relatively small volume, so that membranes can be replaced easily with a low risk of exposure to hazardous fluids and vapours. Moreover, the set-up of HF5 is such that there is room for the fiber to expand under the influence of the solvent.
In flat-channel asymmetrical flow FFF, where the membrane is clamped between two plates, swelling of the support of the membrane can lead to deformation or rupturing of the membrane.\footnote{7}

A module has been developed to contain the hollow-fiber membrane, which was inspired by a set-up developed by Jönsson and Carlshaf,\footnote{11} but has the advantage that it is constructed completely from standard liquid chromatography equipment. All connections to the fiber consist of teflon and stainless steel, which are resistant to all but the most aggressive solvents.

Results are presented in two parts. The first part covers the quality of the system and its suitability for quantitative analysis. The repeatability of calculated diffusion coefficients, plate numbers and the recovery of two polystyrene standards, fractionated in ethyl acetate, are reported. The instrumental band broadening was investigated by the fractionation of a (monodisperse) protein, ferritin.

The second part covers the application of HF5 with PAN hollow fibers in a wide variety of solvents to investigate the universality and versatility of the method. A broad polystyrene standard was analysed with on-line multi-angle light scattering (MALS) and UV detection. Expressions for the maximum attainable number of theoretical plates have been derived, a subject discussed in general terms by Giddings\footnote{21,22} and for flat-channel asymmetrical flow FFF by Litzén and Wahlund.\footnote{23} Suggestions are made for the optimum dimensions of a hollow-fiber membrane for flow FFF.

\section*{THEORY}

The theory and principles of hollow-fiber flow FFF have been described extensively in the literature.\footnote{11,15,24} Here, we limit ourselves to the theory of normal-mode flow FFF, neglecting the deviations that may occur due to steric effects.\footnote{25} The cross-flow induces an exponential concentration profile of the analyte as a function of the distance to the accumulation wall (z):

\begin{equation}
    c(z) = c(0) \exp\left(-\frac{z u_{cr}}{D}\right)
\end{equation}

where $u_{cr}$ is the velocity of the cross-flow and D the diffusion coefficient. The ratio of D and $u_{cr}$ represents the average layer thickness occupied by the species near the wall, which determines its axial velocity. Integrating Equation 1 with the parabolic velocity profile in the fiber, gives an expression for the retention time ($t_r$) at sufficiently high retention:\footnote{11}

\begin{equation}
    t_r = \frac{R^2}{8D} \ln\left(\frac{F_{in} - \zeta F_{cr}}{F_{in} - F_{cr}}\right)
\end{equation}

where $R$ is the inner radius of the fiber, $F_{in}$ the inlet flow, $F_{cr}$ the cross-flow. $\zeta$ denotes the distance of the focusing point from the entrance of the fiber relative to the length of the fiber (after injection, samples are focused into a narrow zone inside the channel).
The peak variance in time units ($\sigma^2$) due to non-equilibrium effects in HF5 has been evaluated by Doshi:

$$\sigma^2 = \frac{16\pi R^2 L^2 D}{F_c^2} t_c$$  \hspace{1cm} (3)

where $L$ is the length of the fiber. Rewriting this equation and substituting:

$$F_c = 2\pi RL u_c$$  \hspace{1cm} (4)

yields a simple expression for the theoretical number of plates ($N$) per unit of time:\n
$$\frac{N}{t_r} = \frac{u_c^2}{4 D}$$  \hspace{1cm} (5)

Equations 2 and 5 reflect the versatility of flow FFF. The retention time can be optimised for a certain size range by tuning the inlet- and cross-flows (Equation 2). An increase in the cross-flow strongly enhances the efficiency. By lowering the inlet-velocity, and hence increasing the retention time, separation speed can be sacrificed for theoretical plates (Equation 5).

In order to obtain maximum separation efficiency, the cross-flow velocity should be as large as possible. However, there are several limitations to the cross-flow. A physical limitation is the maximum permeability of the membrane. In addition, an increased cross-flow may induce peak overloading. When the concentration of the analyte at the accumulation wall becomes too high, repulsive interactions or viscosity effects can affect the peak shape or induce a change of the retention time.\textsuperscript{4,15,16} It has been shown that such overloading occurs when the concentration of the analyte at the accumulation wall exceeds a certain critical value $c^*$ (which depends on the polymer-solvent system and on the molar mass of the polymers to be analysed). Hence, in order to apply a high cross-flow the injected mass should be kept low. On the other hand, the detectability of the analyte sets a limit to the minimum amount of sample that can be introduced into the separation system: the concentration of an analyte after elution from the separation channel should at least exceed the detection limit ($c_{\text{LOD}}$). Since the concentration of an eluting compound in the detector ($c_{\text{det}}$) is directly related to its concentration at the membrane wall ($c_0$) by:\textsuperscript{15}

$$c_{\text{det}} = \frac{8 D^2}{u_c^2 R^2} c_0$$  \hspace{1cm} (6)

limits are also set to the cross-flow velocity. Combining Equations 5 and 6, with the concentration limits set by overloading ($c^*$) and detectability ($c_{\text{LOD}}$), yields an expression for the maximum number of plates per time unit ($N/t_r$)\textsubscript{max}:

$$\left(\frac{N}{t_r}\right)_{\text{max}} = 2 \frac{D}{R^2} \frac{c^*}{c_{\text{LOD}}}$$  \hspace{1cm} (7)

Equation 7 summarises the factors that determine the optimal performance of an HF5 system. In first instance, the system performance is determined by the physical and chemical characteristics of the compounds to be separated, in particular their detectability and susceptibility to overloading.
In general, the system performance is expected to decrease with increasing molar mass, not only because of the occurrence of D in Equation 7, but also because $c^e$ decreases with increasing molar mass. Equation 7 also shows that, as with many other separation methods, miniaturisation can boost the performance of the HF5 system. With narrower fibers, less dilution of the sample occurs so that higher cross-flows can be applied and smaller amounts of sample can be injected. In theory there is no effect of the length of the fiber. However, an increased length may reduce band-broadening effects. Inhomogeneities of the fiber porosity are averaged over a longer length and the contributions of the entrance and exit of the fiber to band broadening become relatively smaller.

**EXPERIMENTAL**

**Instrumental**

A module, containing the hollow-fiber membrane, was installed in the set-up depicted in Figure 1. All connections were made using standard PEEK and teflon tubing. A model 400 HPLC pump (Gynkotek, Germering, Germany) delivered the inlet-flow. Two 1/16” needle valves (Hoke, Creskill NJ, USA) controlled the position of the relaxation point and the magnitude of the cross-flow, respectively. Samples were injected from a 5 µl injection loop on a model 7010 injection valve (Rheodyne, Berkeley, CA, USA). Valve V (Rheodyne) enabled switching between the so-called relaxation (or focussing) mode and fractionation mode.

In order to improve the signal-to-noise ratio, with multi-angle laser light scattering (MALS) detection, a 0.2 µm PTFE in-line filter (Millipore, Bedford MA, USA) was installed between valve V and the detector in an A315 small-volume precolumn filter holder with an A 101x stainless steel frit, pore size 2 µm (Upchurch Scientific, Oakharbor WA, USA).

Hollow-fiber membranes were cut out of a MOLSEP hollow-fiber module type FS-03-FC FUY 03A1 (Hoechst Celgard, Wiesbaden, Germany). The membranes were reported by the manufacturer to consist of modified polyacrylonitrile, with a nominal molecular weight cut-off of 30 kDa. The fibers have a length of ca. 30 cm. An inner diameter of 1 mm and an outer diameter of 1.6 mm are reported by the manufacturer. A blow-up of the hollow-fiber module is shown in Figure 1. The module, with a length of 20 cm, was constructed from two 6.2 mm stainless steel columns with an inner diameter of 4.5 mm that were coupled by a ½” T-piece to provide an outlet for the cross-flow. Fittings at the ends of the columns transferred from ½” to 1/16”. The fiber was inserted into the module. Into both ends of the fiber, a steel capillary (length 5 cm, inner diameter 0.6 mm, outer diameter 0.9 mm) cut from a Microlance 3 injection needle (Becton Dickinson, Fraga, Spain) was inserted. 1/16” teflon ferrules were shoved over the fiber and, after tightening by a nut, sealed the module by clamping the fiber onto the needles. Standard connections were made from the needles to 1/16” tubing.
Figure 1. Scheme of the HF5 set-up. The enlargement shows the construction of the hollow fiber module (for easy reference, connections to the ¼" T-piece and column are left out and only one end of the fiber is shown). Arrows show the directions of the flows during relaxation (r) and fractionation (f). Legend: needle valves (NV1 and NV2); injector (I); valve (V); detector (D); fiber (a); ¼" column (b); teflon ferrule (c); 1/16" nut (d); steel needle (e).

A Spectroflow 757 UV detector (Applied Biosystems, Ramsey, NJ, USA) SEDEX 55 (S.E.D.E.R.E., Alfortville, France) an evaporative light scattering detector (ELSD) or a DAWN-DSP MALS detector (Wyatt Technology, Santa Barbara, CA, USA) in combination with a UV detector were used. The MALS detector was equipped with a 30 mW argon laser, generating light with a wavelength of 488 nm. The settings of the ELSD were optimised for the respective solvents used. ELSD and UV signals were amplified by a model 113 pre-amplifier (Princeton Applied Research, Princeton, NJ, USA) and processed by a personal computer after conversion by a Smartlink DS-12 A/D converter (Keithley, Cleveland OH, USA). In case of MALS detection, all signals were processed by ASTRA 4.5 software (Wyatt).
Chemicals and solutions

All chemicals and solutions were purchased from standard suppliers and were of at least 99% purity. Non-stabilised THF (Acros, Geel, Belgium) was used. A comprehensive list of the polymer standards that were used and their suppliers is provided in Table 1. Standards were diluted in the carrier liquid to the required concentration. All experiments were carried out at room temperature (20 ± 1°C). Membranes were stored in an aqueous 0.02% (m/v) sodium azide solution in a dark place.

Procedure

The principle of the operation procedure was similar to that employed by Lee et al. After installation of the fiber, the system was flushed with solvent for 30 minutes to remove air bubbles and to equilibrate the membrane. Injection was performed in the relaxation mode with the inlet-flow being split in a part that enters the front end and a part that enters the back end of the fiber, creating a focusing of the sample in a narrow zone. The ratio of the forward and backward flows determines the distance of the focussing point from the inlet relative to the length of the fiber (ζ in Equation 2), which was adjusted to 0.15 with valve NV1. The sample was focussed with an inlet-flow of 0.2 ml min⁻¹ during 5 minutes. Valve V was switched to start the fractionation, during which the cross-flow was regulated by valve NV2. Flows were determined gravimetrically.

Table 1. Polymer standards and suppliers. M_p: molar mass at concentration maximum; μ: polydispersity (specified by the manufacturer).

<table>
<thead>
<tr>
<th>Polymer</th>
<th>M_p (kDa)</th>
<th>μ</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poly (methyl methacrylate)</td>
<td>88</td>
<td>1.04</td>
<td>Polymer Laboratories,</td>
</tr>
<tr>
<td></td>
<td>333</td>
<td>1.04</td>
<td>Church Stretton, UK</td>
</tr>
<tr>
<td></td>
<td>610</td>
<td>1.07</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1400</td>
<td>1.04</td>
<td></td>
</tr>
<tr>
<td>Cis-Polyisoprene</td>
<td>60</td>
<td>1.04</td>
<td><em>Idem</em></td>
</tr>
<tr>
<td></td>
<td>295</td>
<td>1.04</td>
<td></td>
</tr>
<tr>
<td></td>
<td>590</td>
<td>1.05</td>
<td></td>
</tr>
<tr>
<td>Polybutadiene</td>
<td>120</td>
<td>1.03</td>
<td><em>Idem</em></td>
</tr>
<tr>
<td></td>
<td>330</td>
<td>1.04</td>
<td></td>
</tr>
<tr>
<td>Poly(tert-butylvinylketone)</td>
<td>80</td>
<td>1.04</td>
<td>Polymer Standards Service,</td>
</tr>
<tr>
<td></td>
<td>380</td>
<td>1.15</td>
<td>Mainz, Germany</td>
</tr>
<tr>
<td>Polystyrene</td>
<td>100</td>
<td>1.06</td>
<td>Pressure Chemical Co,</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>1.06</td>
<td>Pittsburgh PA, USA</td>
</tr>
<tr>
<td></td>
<td>900</td>
<td>1.10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2000</td>
<td>1.30</td>
<td></td>
</tr>
<tr>
<td>Polystyrene SRM 706</td>
<td>271</td>
<td>2.1</td>
<td>BDH Chemicals, Poole, UK</td>
</tr>
<tr>
<td>Ferritin</td>
<td>440</td>
<td>1</td>
<td>Sigma, St. Louis MO, USA</td>
</tr>
</tbody>
</table>

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RESULTS AND DISCUSSION

System Performance

The width of a peak in HF5 is determined by the polydispersity of the sample, the band broadening by the (fundamental) non-equilibrium effects during elution and possibly by instrumental band broadening, caused by non-idealities such as extra-column effects or an inhomogeneous fiber porosity. It has been shown that with an ideal HF5 set-up, the reciprocal of the plate number for a polymer standard is given by the addition of non-equilibrium effects (Equation 5) and the contribution of the polydispersity

\[
\frac{1}{N} = \frac{4D}{u_{cr}^2 t_r} + b^2 (\mu - 1) \tag{8}
\]

where \( b \) is the Mark-Houwink constant of the polymer (the negative slope of the log \( D \) vs. log \( M \) relation, which is usually in the range of between 0.5 and 0.6) and \( \mu \) the polydispersity of the standard. Instrumental non-idealities will show up as an extra term on the right-hand side of Equation 8. Since the polydispersities of available synthetic polymer standards are usually not negligible (see Table 1) and not known accurately, it is impossible to obtain a reliable estimate of the instrumental band broadening contribution from the fractograms of synthetic polymer standards. Therefore, the instrumental band broadening was evaluated with a protein (ferritin), which is monodisperse by nature. Ferritin has a molar mass of 440 kDa and aggregates are formed in solution, providing an excellent test for the resolution that can be obtained with flow FFF.

Figure 2 shows an exemplary fractogram of ferritin, eluted with an aqueous buffer of 10 mmol L\(^{-1}\) 2-acetaminoethanesulfonic acid (ACES) with 10 mmol L\(^{-1}\) sodium chloride at pH 6.5. The aggregates were separated within 8 minutes, with approximately 180 plates for the main peak. Multiple fractionations were carried out with different inlet-flows and cross-flows, and the inverse of the plate numbers for the main ferritin peak was plotted against \( 1/(u_{cr}^2 t_r) \) (see Figure 3). In accordance with Equation 8 a linear relationship was found. From the slope of the regression line, the diffusion coefficient of ferritin was estimated as \( 3.50 \times 10^{-11} \text{ m}^2 \text{ s}^{-1} \), which is close to the literature value of \( 3.61 \times 10^{-11} \text{ m}^2 \text{ s}^{-1} \) at 20°C. From the intercept of the regression line with the Y-axis (and a value of 0.49 for \( b \)) an apparent polydispersity of \( 1.003 \pm 0.003 \) (95% CI, 8 df) was calculated. This result shows that instrumental band broadening in the present HF5 system is negligible.
Figure 2. Fractionation of ferritin in ACES buffer. The numbers with the peaks denote the aggregation number. $F_{in} = 1.5 \text{ ml min}^{-1}$, $F_{cr} = 0.38 \text{ ml min}^{-1}$, void time = 0.1 min. Detection: UV at 280 nm.

Figure 3. Reciprocal of the plate number for ferritin as a function of $1/(u_c^2 \tau)$. 
Performance in organic solvents

The resistance of the membrane to several organic solvents was tested by trial flow FFF runs of a 400 kDa PS standard. The membrane was indeed resistant to dichloromethane (DCM), methyl ethylketone (MEK), ethyl acetate and tetrahydrofuran (THF). Notably, a fiber could be used for at least five days in any of these solvents, without loss of performance. In contrast, the membrane appeared not to be resistant to toluene and cyclohexane: with these solvents the standard was not recovered from the fiber.

The suitability of HF5 for quantitative analysis in organic solvents was investigated by measuring the effect of the injected mass on the peak area and peak height of a high-molar mass (2MDa) PS standard in ethyl acetate. The injected mass was varied between 0.5 and 5 µg and the inlet flow and cross-flow were set at 0.4 and 0.07 ml min\(^{-1}\), respectively, which resulted in a retention time of 10 minutes. It was observed that the dependence of the peak area on the injected mass was linear over the complete range, with a value of 0.992 for \(r^2\). In contrast, the effect on the peak height was close to linear with injected masses of up to 2 µg, while the points above 2 µg deviated from the straight line. This curvature is caused by peak overloading, which was observed as a distortion of the peak shape at these mass loads. The recovery of the standard, calculated from the extinction coefficient, peak area and outlet flow rate, was determined to be 96 ± 5% (95% CI, 11 df). The complete recovery and linearity of the peak area show that the method can be used for quantitative analysis of high-molar mass compounds.

From the height of the peak at the onset of overloading, with 2 µg injected, \(c^*\) was calculated to be 2.5 g l\(^{-1}\), taking into account a dilution factor \((c_{\text{det}}/c_0)\) of 1450 (Equation 6). From the same fractograms a concentration detection limit \(c_{\text{LOD}}\) \((S/N = 3)\) of 0.29 mg l\(^{-1}\) was estimated. It can be calculated with Equation 7 that under optimal conditions, i.e., at a minimum sample load and maximum cross flow, 360 plates can be obtained in 10 minutes for this polymer. With the flow rates as applied in the current set of experiments, 60 plates can be obtained in 10 min for a monodisperse standard (Equation 5). In our experiments only 40 plates were generated. This corresponds to a polydispersity of the standard of 1.1 (Equation 8), which is much smaller than the value of 1.3 supplied by the manufacturer. It has been suggested previously that the polydispersities reported by manufacturers may be conservative estimations.\(^{12}\)

The repeatability of the system was evaluated by repetitive fractionations of a standard mixture containing 1 µg of PS 100 and 400 kDa in ethyl acetate. Six consecutive injections were performed on six different fibers. In Table 2, the average recovery, calculated diffusion coefficient and number of plates obtained with each fiber are presented. While on average the 400 kDa standard is completely recovered, only about 57% of the 100 kDa standard is eluted. Apparently, part of the latter standard is lost through the pores of the membrane. It is conceivable that the molecular weight cut-off, which is given as 30 kDa for a charged solute in water, is larger for an uncharged polymer, and swelling of the pores of the membrane in organic solvent cannot be ruled out.
Table 2. Repeatability of calculated diffusion coefficient, recovery and number of plates for 100 kDa and 400 kDa PS standards. 
$F_w = 0.6 \text{ ml min}^{-1}$ and $F_{cr} = 0.26 \text{ ml min}^{-1}$. Values are given as the mean ± standard deviation (n=6).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Recovery (%)</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
<th>VI</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>PS 100 kDa</td>
<td></td>
<td>50 ± 3</td>
<td>55 ± 2</td>
<td>61 ± 3</td>
<td>64 ± 3</td>
<td>56 ± 2</td>
<td>59 ± 2</td>
<td>57 ± 2</td>
</tr>
<tr>
<td>D ($10^{11} \text{ m}^2 \text{s}^{-1}$)</td>
<td>6.38 ± 0.05</td>
<td>6.06 ± 0.02</td>
<td>6.29 ± 0.03</td>
<td>6.01 ± 0.08</td>
<td>6.80 ± 0.06</td>
<td>6.52 ± 0.02</td>
<td>6.34 ± 0.12</td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>52 ± 2</td>
<td>48 ± 2</td>
<td>42 ± 2</td>
<td>39 ± 1</td>
<td>34 ± 1</td>
<td>58 ± 1</td>
<td>45 ± 4</td>
<td></td>
</tr>
<tr>
<td>PS 400 kDa</td>
<td></td>
<td>108 ± 3</td>
<td>96 ± 2</td>
<td>101 ± 3</td>
<td>106 ± 2</td>
<td>99 ± 3</td>
<td>111 ± 2</td>
<td>104 ± 3</td>
</tr>
<tr>
<td>D ($10^{11} \text{ m}^2 \text{s}^{-1}$)</td>
<td>3.06 ± 0.03</td>
<td>2.91 ± 0.01</td>
<td>3.02 ± 0.01</td>
<td>2.91 ± 0.02</td>
<td>3.26 ± 0.01</td>
<td>3.16 ± 0.01</td>
<td>3.05 ± 0.06</td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>107 ± 4</td>
<td>178 ± 3</td>
<td>107 ± 4</td>
<td>81 ± 3</td>
<td>115 ± 5</td>
<td>108 ± 3</td>
<td>116 ± 13</td>
<td></td>
</tr>
</tbody>
</table>
Extrapolation of literature data obtained by dynamic light scattering experiments gives respective diffusion coefficients of 7.1 and $3.4 \times 10^{-11}$ m$^2$ s$^{-1}$. The average diffusion coefficients calculated for the 100 and 400 kDa standards were 6.34 and $3.05 \times 10^{-11}$ m$^2$ s$^{-1}$, respectively. The values from our experiments are approximately 10% lower than these literature values. A realistic explanation of this discrepancy may be that the actual radius of the fiber, when operated in an organic solvent, differs from the nominal value used for the calculation of $D$ (Equation 2). Apparently, calibration of the HF5 system is still required. This is supported by the observation that when the 100 kDa standard is used as an internal standard, for the 400 kDa standard a diffusion coefficient of $(3.42 \pm 0.02) \times 10^{-11}$ m$^2$ s$^{-1}$ (95% CI, 5 df) was found, which is equal to the literature value. The repeatability of the separation efficiency, expressed as the number of plates, was satisfactory. For the 400 kDa standard, with most of the fibers around 110 plates are obtained.

**Applications in organic solvents**

In Figure 4 fractograms are presented of different polymer standards in a variety of organic solvents. Detection was performed by ELSD. The injected masses varied between 0.2 and 1 μg. $F_i$ (0.5 to 0.75 ml min$^{-1}$) and $F_m$ (in the order of 0.2 ml min$^{-1}$) were set as a compromise between resolution and signal-to-noise ratio. Void times were in the order of 0.3 min. Figure 4A and B show the fractionation of polybutadienes and polyisoprenes with DCM as the solvent. Excellent separations of these standards were obtained. These results show that non-cured rubber-polymers can be separated with flow FFF. HF5 may prove to be valuable for the determination of microgels (large cross-linked structures) in rubbers, which is generally troublesome with Size Exclusion Chromatography. Work on this subject is currently being carried out in our laboratory. Figure 4C shows a fractionation of two poly (tert-vinylbutyl) ketones in MEK. The peak of the 380 kDa standard is relatively broad compared to the 80 kDa standard, as a result of the difference in polydispersity (1.15 vs. 1.04, reported by the manufacturer). A good separation of four poly(methylmethacrylate) standards over a molar mass range of between 80 kDa and 1.4 MDA was obtained in THF (Figure 4D). Due to the relatively large polydispersities of the 333 and 610 kDa standards, these were not completely resolved. Also, a separation of four PS standards in THF was performed. A plot of log $D$ vs. log $M$ from this result displayed a strong curvature, while a linear relationship is expected. This pointed towards adsorption of the solutes to the membrane wall. A linear relationship between log $D$ and log $M$ was obtained after addition of 1 mmol l$^{-1}$ ammonium acetate to the mobile phase, a well-known remedy against adsorption. A fractogram of four PS standards in ethyl acetate is presented in Figure 4E. All standards, covering a mass range of over one order of magnitude, are nearly base-line separated. The results in Figure 4 underline the enhanced versatility that is achieved with the PAN membrane. In particular THF and DCM are commonly applied in polymer analysis because they are potent solvents for a wide range of polymers.
Figure 4. Fractionation of synthetic polymers in various organic solvents with ELSD detection.

A: Polybutadienes in dichloromethane. $M_p = 120$ kDa (1) and 330 kDa (2).
B: Polyisoprenes in dichloromethane. $M_p = 60$ kDa (1), 295 kDa (2) and 590 kDa (3).
C: Poly (tert-vinyl butylketones) in MEK. $M_p = 80$ kDa (1) and 380 kDa (2).
D: PMMA’s in THF. $M_p = 88$ kDa (1), 333 kDa (2), 610 kDa (3) and 1400 kDa (4).
E: Polystyrenes in ethyl acetate. $M_p = 100$ kDa (1), 400 kDa (2), 900 kDa (3) and 2 MDa (4).
A broad PS SRM 706 reference standard, dissolved in THF, was fractionated and detected with on-line UV and MALS detection. The MALS detector measures light scattering at multiple angles during short time intervals. In combination with a concentration detector, molar masses can directly be obtained for each time interval.26,33-35. In Figure 5 the UV trace and 90° signal of the MALS detector are shown. From the extinction coefficient and the refractive-index increment (dn/dc) of PS in THF (0.194 ml g⁻¹) the molar mass was calculated. The molar mass trace displays a separation of over an order of magnitude. A molar mass value at the concentration maximum (Mₚ) of 280 kDa was measured, which is in good accordance with the value provided by the manufacturer (271 kDa). A plot of the root-mean-square radius vs. time (not shown here) displayed a slight curvature. An artefact in the UV signal after ca. 30 minutes disturbed the signal from the high-mass end of the distribution and thereby prevented an accurate determination of the polydispersity. Nonetheless, Figure 5 shows that MALS detection can in principle be coupled to HF5. This combination will be very useful because MALS offers an on-line and direct way to measure molar mass distributions without the need for suitable standards.

**Figure 5.** Fractionation of a broad SRM 706 PS standard in THF with MALS and UV detection. The 254 nm UV signal (1), 90° scattering signal (2) and molar mass (3) are shown. Fᵢₚ = 0.4 ml min⁻¹, Fᵢᵣ = 0.22 ml min⁻¹, void time = 0.6 min.
CONCLUSIONS

In this work, the consistently successful application of organic solvents in flow FFF is presented for the first time. The PAN hollow-fiber membrane is stable in a wide variety of solvents. The fact that solvents such as THF, dichloro methane and MEK can be used implies a vast broadening of the application range of flow FFF. Obviously, the PAN membrane can be a useful tool in other (analytical) applications that require ultrafiltration in hydrophobic organic solvents. HF5 may prove to be particularly valuable for the analysis of large polymeric structures such as microgels that are difficult to handle with packed columns. There is still room for improvement of the system. A reduction of the inner diameter of the fiber (possibly combined with an increased fiber length to suppress instrumental band broadening) can lead to a significant enhancement of the performance. This should result in HF5 becoming a full and established member of the FFF family.
REFERENCES