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Flow field-flow fractionation of poly(ethylene oxide): effect of carrier ionic strength and composition[☆]

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Abstract

The effects of carrier ionic strength and electrolyte composition on the retention of poly(ethylene oxide) in aqueous flow field-flow fractionation have been investigated in this work. The study shows retention to be particularly sensitive to the presence of salts, as well as to the nature of the cation. Specifically, retention effects due to sample load are found to be very different in solutions containing potassium salts compared to those observed in solutions of the corresponding sodium salts. In a potassium-containing medium, the dependence of retention on sample mass is similar to that found previously for polyelectrolytes. This effect, which is particularly prominent for samples of low molecular mass, can be attributed to specific interactions between cation and polymer. Published by Elsevier Science B.V.

Keywords: Flow field-flow fractionation; Carrier ionic strength; Electrolyte composition; Poly(ethylene oxide)

1. Introduction

Poly(ethylene oxide) (PEO), also referred to as poly(ethylene glycol) (PEG), is a polymer widely studied for its unique properties and applications. Preparations with molecular masses ranging from 500 to a few thousand Da are reported in the literature to be of significant interest in biomedical applications due to their nonimmunogenicity and nontoxicity [1]. PEO is highly soluble in water but exhibits good solubility also in such organic solvents as acetonitrile, chloroform, toluene, methylene chloride, ethanol, and acetone. For this reason PEO is often thought of as an amphiphilic polymer. Interestingly, polymers with similar structure, such as poly(propylene oxide) and poly(methylene oxide) have very low solubilities in water at room temperature.

Studies of the behavior of PEO in aqueous solutions have shown the polymer to be highly hydrated, with an unusually large molecular volume as measured by size-exclusion chromatography (SEC). In fact, these volumes are significantly larger than those of other polymers with similar molecular mass [2,3].

[☆] This article is a report on the very last research carried out in the laboratory of Professor J. Calvin Giddings, who died in October 1996. M.-A.B. wishes to dedicate it to the person who showed her the harmony of nature through connections in science.

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A frequently used property of PEO in aqueous solution is its immiscibility with other polymers in the same solvent, which under certain circumstances leads to phase separation. This phenomenon has been widely exploited in biotechnology, where PEO is often used as a protein concentrating agent, and where protein separation in, e.g., PEO–Dextran aqueous two-phase systems has met with significant success. An altogether different application is its use to prevent the surface adsorption of protein by attaching PEO chains either to substrates in need of protection, or to the proteins themselves to prevent them from interacting with their natural macromolecular or particulate ligands, such as receptors or antibodies. Thus modified, the proteins can maintain their normal biological function, provided this function is not being hindered for steric reasons [4,5]. The modification does, however, significantly alter many properties. Thus, it has been observed that PEO–conjugated proteins show a reduced rate of clearance through the kidney, have altered electrophoretic mobilities due to charge shielding by the polymer layer, and show increased solubility in organic solvents, due to the polymer's solubility in such media.

An interesting observation is the reported capability of PEO to form complexes with metal salts [6], hence enabling their partition into various organic phases. This process is frequently referred to as ''phase transfer catalysis'' [7] since, during the partitioning, their counterions are carried along to maintain electroneutrality; poorly solvated in their new environment they become particularly reactive. The capability of ethers to form complexes with metal ions was first found for crown ethers, which are cyclic structures composed of 5–6 ethylene oxide units. The ion binding by crown ethers is cation selective, and depends on the size of the cavity for the center of the crown. A similar selectivity mechanism is reported for PEO polymers, and it has been related to the induction of a helical conformation with polymer cavities of well-defined sizes [8]. A steric mechanism has been invoked to explain the remarkable complexing power of PEO toward the potassium ion found in halogenated solvents as well as in water [9]. In addition, the molecular mass dependence of the catalytic power in phase transfer has been attributed to the possibility for longer flexible chain molecules to bind a greater number of ions [9,10]. Indeed, the degree of extraction of potassium per oxyethylene unit is found to be constant [9] in the 400–19 000 Da molecular mass range, suggesting that a constant number of repeat units per ion might be involved in the complexing process. Other authors prefer to interpret this evidence as being due to a more favorable type of coiling by PEO of higher molecular mass, but details regarding the differences in coil conformation are not given [10]. Flexible chain macromolecules do not have well defined dimensions; rather their size represents the average value of all possible conformations of equal free energy that the molecule may assume. The introduction of electric charges of the same sign into a polymer coil leads to an expansion of its dimensions due to charge repulsion. Therefore, the complexation of cations by the neutral PEO chain can be expected to expand its dimensions. Indeed, Bailey Jr. and Koleske [8] have reported that in forming complexes with certain metal ions, the PEO molecules achieve excluded volumes comparable to those otherwise seen for polyelectrolytes.

Field-flow fractionation (FFF) is by now a well-established technique in the arsenal of separation methods. In a great number of applications, this family of analytical techniques has demonstrated its ability to yield accurate measurements of the hydrodynamic dimensions of macromolecules and colloids in a broad range of particle sizes [11,12]. The FFF separation takes place in a single phase, and results from the differential partitioning of solute into fluid laminae moving at different velocities inside a thin separation channel. Because of the channel's parallel plate configuration the distribution of fluid velocities is parabolic, and partitioning is accomplished through the external application of a force field in the direction perpendicular to the eluent flow. Thus, a differential interaction with the field translates into a differential migration of solute through the channel. The weak forces required for separation in these systems, the slight shear forces to which a sample is subjected during its passage through the channel, and the limited opportunities for surface adsorption, contribute to make the FFF techniques especially suitable for polymer separation and characterization.

In the particular FFF subtechnique used for the present study, namely flow FFF, a flow of liquid is established in the direction perpendicular to that of the eluent. This flow transports all solutes, regardless of size and composition, with the same velocity in the direction of the semi-permeable accumulation wall in the channel. For each component this accumulation is offset by a component-specific diffusive flux away from the wall, so that at equilibrium one obtains a size dependent partitioning of sample into the eluent flow profile. Due to the universal nature of this separation mechanism, flow FFF can be considered as the most versatile of all the FFF techniques.

Standard FFF theory, developed for point masses at infinite dilution, rules out a priori any effect on retention due to the amount of injected sample. However, experimental evidence, acquired since the first FFF investigations, has shown a variety of effects that may be related to the amount of sample injected per analysis. Such non-ideal behavior has been reported for colloidal [13] as well as polymeric [14–24] samples. Specifically, polymer retention was found to be affected by sample size in thermal FFF [15,16,24] as well as in conventional flow [17–20] and hollow fiber flow FFF [21–23]. The influence of injection size on retention in conventional flow FFF has been recorded both for organic and aqueous carriers. From this work it appears that neutral polymers analyzed in organic solvents always show increased retention with increased sample amount, regardless of the type of FFF used for the analysis. The magnitude of the perturbation does however appear to be a strong function of the nature of the polymer–solvent system, in particular of the molecular mass of the polymer. Indeed, both in flow FFF [17] and in thermal FFF [15,16,24] the mass effects on retention are more pronounced the higher the molecular mass of the sample.

In the FFF analysis, a sample is injected into a thin channel, typically with rectangular cross section. Immediately upon injection it becomes subjected to the influence of the externally applied field and proceeds to concentrate at one of the channel walls. Equilibration under the field is best performed in the absence of longitudinal, or eluent flow. This stop-flow procedure [25], however, induces a considerable local increase in sample concentration compared to that of the injected sample, c_{inj}. Indeed, it has been shown [17] that the wall concentration approximately equals c_{inj}/λ, where λ represents the dimensionless thickness of the equilibrated sample zone (see Eqs. (3) and (4) below). Since typical λ-values are in the range of 0.01–0.1, the relaxation procedure may give rise to local sample concentrations that are increased more than 10-fold.

Polymeric samples are particularly prone to intermolecular entanglement at elevated concentrations, and for most polymer–solvent systems one can identify a critical concentration above which the solution can no longer be treated as a collection of individual macromolecules. In this concentration regime, generally referred to as ‘‘semi-dilute’’, the polymer rather behaves as a gel-like collection of clusters of varying size. The threshold concentration for transition into the semi-dilute regime depends on the degree of polymerization, i.e., on molecular mass, and on the square of the characteristic parameter d/l representing the ratio of thickness to length for the polymer chain [26–28]. Thus, zone formation in FFF may generate sample concentrations at the accumulation wall exceeding by many times the critical level for polymers of higher molecular mass [17].

The effects of sample size in aqueous flow FFF are rather different from those seen in non-aqueous solvents. As concerns the carrier, the often high solvating power of water can be significantly modulated by the addition of simple electrolytes. The water-soluble polymeric samples, in turn, vary greatly in their physicochemical properties, from polyelectrolytes to amphiphilic and uncharged polymers that may or may not undergo hydrolytic degradation. In aqueous systems, polymer–solvent interactions may hence vary greatly in both type and strength. Early studies of polymer analysis by aqueous FFF indicated that increased sample loads as a rule were associated with reduced retention, any time the sample was moderately or highly charged. This was true regardless of the FFF technique used, and regardless of whether spherical particles [13] or flexible chain polyelectrolytes [18–23] were under investigation. Variations in ionic strength were shown to affect the retention to degrees that depended on sample composition, conformation, and charge density. These experimental observations were attributed to the volume exclusion effect, i.e., to the exclusion of some part of the sample molecules from the volume immediately adjacent to the accumulation wall. By this mechanism, component particles are forced away from the wall and into regions where the fluid velocity is higher. This, in turn, increases the average migration velocity of the zone. The effect of sample size on retention in aqueous FFF is thus related to the effective molecular volume, which increases with the charge of the eluting particle or molecule, i.e., with interparticle repulsion and the double layer thickness. For flexible chain macromolecules, coil expansion due to intraparticle repulsion may dramatically contribute to an increase in molecular volume.

In the present work we wish to examine the flow FFF retention of the neutral PEO polymer in aqueous electrolytes of different ionic composition. Specifically, we wish to test the technique's ability to detect such conformational shifts as might originate from a complexation by the polymer of certain cations, specifically the potassium ion.

2. Retention theory

Flow FFF is the most versatile of the FFF techniques, and one of the two mainly applied to the separation of soluble polymers. As in the case of other separation techniques one can define a dimensionless retention ratio R as the ratio between the average velocities of the sample zone and the carrier fluid, respectively. This is a universal parameter that measures the sample's response to the applied field. Due to the inverse relationship between time and velocity, the retention ratio may be simply expressed as the ratio between two times, namely that needed for a non-retained component to elute from the channel, or void time t^0, and the average residence time, or retention time, t_r:

$$R = \frac{t^0}{t_r} \tag{1}$$

The retardation of sample molecules in FFF arises from their confinement to an exponentially distributed concentration plug, whose mean thickness is determined on the one hand by the interaction of the sample particles with the applied field, and on the other by the dispersing flux due to diffusion. The mean thickness is described as:

$$\ell = \frac{D}{U} \tag{2}$$

where U is the average field-induced velocity and D is the diffusivity, i.e., the transport parameter characterizing the magnitude of the diffusive flux. The measured retention ratio R reflects the relationship between this layer thickness and the thickness of the flow channel, w. For the sake of convenience a reduced layer thickness λ can be defined as:

$$\lambda = \frac{\ell}{w} \tag{3}$$

Cast in terms of this parameter, R takes the following form [17]:

$$R = \frac{t^0}{t_r} = 6\lambda \cdot \left[\coth\left(\frac{1}{2\lambda}\right) - 2\lambda \right] \tag{4}$$

It is evident from Eqs. (2), (3) and (4) that an observed retention, through parameter λ, is an explicit function of the sample's diffusion coefficient D and drift velocity U:

$$\lambda = \frac{D}{Uw} \tag{5}$$

In the case of flow FFF, the drift velocity U, i.e., the linear cross-flow velocity, is one and the same for all components in the channel, and D is therefore the only sample specific quantity in Eq. (5). For convenience, this expression can be rewritten in terms of readily measurable experimental quantities. This is done by first recognizing that the channel thickness is scaled to the two other dimensions, i.e., the breadth, b, and length, L, through the void volume $V^0 (w = V^0/bL)$. The second step involves the realization that U is simply the volumetric cross-flow $\dot{V}_c$ per unit area of the accumulation wall $(U = \dot{V}_c/bL)$. Thus:

$$\lambda = \frac{DV^0}{\dot{V}_c w^2} \tag{6}$$

The Stokes–Einstein equation expresses the relationship between the diffusivity D of a spherical particle and its hydrodynamic radius R_h in a medium with viscosity η:

$$D = \frac{kT}{6\pi\eta R_{\mathrm{h}}} \tag{7}$$

Although the above equation describes the behavior of single particles at infinite dilution, it is a good model for the transport coefficient even in moderately concentrated suspensions, provided the viscosity remains constant and the suspension/solution remains ideal. By combining Eqs. (6) and (7) and rearranging terms one arrives at an explicit relationship between the (measurable) parameter λ and the characteristic dimension of the analyte:

$$\lambda = \frac{kT}{6\pi\eta R_{\mathrm{h}}} \cdot \frac{V^0}{\dot{V}_{\mathrm{c}} w^2} \tag{8}$$

If molecular mass, rather than size, is the desired outcome of a polymer analysis by flow FFF the diffusion coefficient in Eq. (6) can be expressed in terms of molecular mass M through use of Eq. (9):

$$D = AM^{-b} \tag{9}$$

where A and b are parameters characteristic of a given solvent–solute pair. In this manner, one can formulate the following relationship between retention parameter λ and sample molecular mass:

$$\lambda = AM^{-b} \cdot \left(\frac{V^0}{\dot{V}_{\mathrm{c}} w^2} \right) \tag{10}$$

3. Experimental

3.1. Apparatus

The flow FFF system from FFFractionation (Salt Lake City, UT, USA) employed for the PEO separations contained a channel with a length, L of 29.75 cm, a breadth, b of 2.0 cm, and a thickness, w, of 0.0254 cm. This channel was cut out from a sheet of mylar, which served as a spacer, and was clamped between two hollow plexiglass blocks, each accommodating a cross-flow reservoir behind a ceramic frit. The sample components are impeded from escaping through the accumulation wall by the presence of an ultrafiltration membrane, placed flat on the corresponding frit. In the present study a PLGC-regenerated cellulose membrane from Millipore (Bedford, MA, USA) was used. The HPLC pumps used to deliver cross-flow and longitudinal flow were respectively an Eldex Metering CC-100-S-PK pump (Eldex Labs., Napa, CA, USA) and a Beckman Model 110B pump (Beckman Instruments, Berkeley, CA, USA). The liquid carrier enters and exits the channel from two separate ports, at flow-rates that are selected based on a number of parameters such as the type of sample, the desired retention level, and the expected resolution. The exact match between incoming and exiting flow-rates in both the transport and the field line is accomplished through the use of back-pressure regulators (Alltech Associates, Deerfield, IL, USA) placed at both outlets. An on-line tee union with a septum is used as the injector port. This type of injector has the advantage of allowing virtually any injection volume. A zero dead volume filter from Upchurch (Oak Harbor, WA, USA) was placed between the injector and the channel inlet. The elution curves were registered by an Optilab Interferometric detector from Wyatt Technology (Santa Barbara, CA, USA). Data storage and analysis were done by computer, using in-house software. The stop-flow procedure was applied to all runs and the stop-flow time was taken as the time required to sweep out one void volume across the membrane wall.

3.2. Reagents and samples

The liquid carriers used in this study were doubly distilled deionized water and aqueous solutions of sodium sulfate (0.025 M) and potassium sulfate (0.025 M), respectively. The samples were PEO of the following molecular masses: 4240, 12 000, 41 500, 250 000, 590 000, and 990 000; they were all purchased from Polymer Standard Service (Silverspring, MD, USA). The samples were in all cases dissolved in the same liquid as that serving as carrier. Typical sample concentrations ranged from 0.1 to 1.0 g/l. For the extrapolation of sample characteristic parameters from the experimental observations, the measured temperature of the experiment was always considered.

4. Results and discussion

Although the present study focuses on low-molecular-mass PEO because of the wide biomedical and biotechnical application of these particular polymers, a mixture of four high-molecular-mass PEO samples was also analyzed to examine the performance of the flow FFF system. The baseline separation of four fractions of the polymer with molecular masses of 12 000, 250 000, 590 000, and 990 000 Da, shown in Fig. 1, was carried out in an aqueous solution of Na_2SO_4 (0.025 *M*). Fig. 2, in turn, illustrates typical elution curves for three lower-molecular-mass PEO samples. Here, the elution was performed in deionized (DI) water under a longitudinal flow of 0.28 ml/min and a cross-flow of 1.71 ml/min.

In principle, the study of any retention-derived parameter may give information on the effect of sample size in FFF. Variations in retention ratio *R*, or the corresponding elution time/volume, with sample load are often reported in the literature [17–21,24] but other related parameters such as the reduced layer thickness λ, or the sample property responsive to the field, reveal load effects as well [14,22,30]. The polymer characteristic which determines the level of retention in flow FFF is the translational diffusion coefficient, and this parameter is chosen here to monitor the influence of load on the effective hydrodynamic size of the polymer. In the case of polymeric samples, shifts in retention may be interpreted as shifts in effective molecular mass, according to Eq. (10).

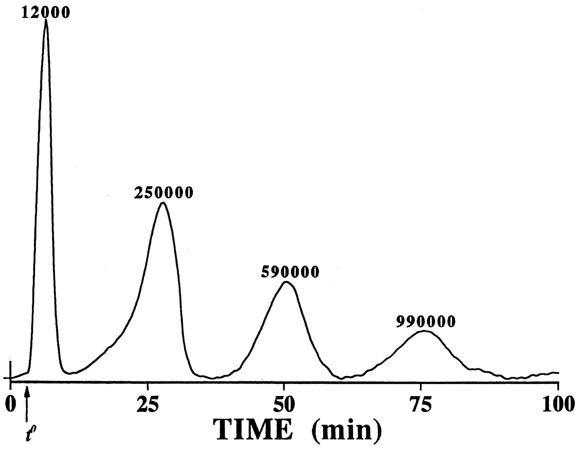

Fig. 1. Flow FFF separation of a mixture of four poly(ethylene oxide) polymers of the nominal molecular masses shown in the figure. The aqueous 0.025 *M* Na_2SO_4 solution was supplied at a channel flow-rate of 0.28 and crossflow-rate of 0.57 ml/min. Sample load was about 2 μg for each polymer.

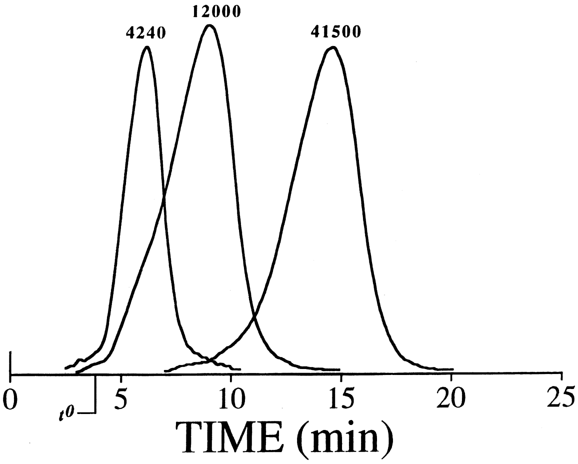

Fig. 2. Superimposition of the elution curves of three PEO polymers of nominal molecular masses 4240, 12 000 and 41 500. The carrier was doubly-distilled deionized water. Flow-rates were $\dot{V}=0.28$ ml/min and $\dot{V}_c=1.71$ ml/min.

As stated in Eq. (7), the hydrodynamic size of a molecule or particle is the only species specific parameter with influence on the translational diffusivity in an infinitely dilute solution. It must therefore be argued that any load dependent variations in FFF retention must be due to non-ideal behavior of the solute. This non-ideality could be due to concentration effects on the local viscosity; it could also derive from attractive or repulsive forces between solute molecules that become amplified at the high degrees of crowding present in a compressed and highly retained zone. The retention-derived diffusivity should, therefore, be treated as an apparent diffusion coefficient, D_{app}.

In polymer theory, two different types of diffusion coefficients are generally recognized, namely the tracer (or self) and the cooperative (or mutual) diffusion coefficient, labeled D_t and D_{coop}, respectively. They are related to different diffusive mechanisms. While the former refers to the motion of a single labeled particle winding through a uniform medium of indistinguishable particles, the latter describes the net transport that results from random thermal motion in a concentration gradient. For polymers the two coefficients may be very different, although they are expected to coincide for infinitely dilute solutions. It is clear that different techniques for measuring diffusion coefficients may yield very different values for the same system if they respond to and detect different diffusive effects [31]. The cooperative diffusion coefficient is recognized as having the same structure as the Stokes–Einstein coefficient, and it is expected to increase with the polymer volume fraction [32]. Since the FFF mechanism involves a diffusive flux in a concentration gradient, the retention-derived diffusion should be of the cooperative type and should be expected to increase with concentration.

Plots illustrating the effect of sample load on the retention-derived parameter D_{app} in different carrier solutions are shown in Figs. 3, 4 and 5. The samples in these illustrations are PEO fractions of three different molecular masses (4240, 12 000, and 41 500, respectively), analyzed in carriers of three different compositions. Each data point is the average of 3–7 measurements obtained under the same experimental conditions. Data reduction for each set of points was obtained as the least-squares best fit. From these figures it appears that sample load has little or no effect on retention of the two higher-molecular-mass fractions, regardless of carrier composition. By contrast, the sample of the lowest molecular mass behaves differently in all three carriers. In DI water and in 0.025 M K_2SO_4 the change in D_{app} with sample size follows a pattern similar to that reported previously for polyelectrolytes at low ionic strength [19,20,29], i.e., a steadily increasing apparent diffusion coefficient with increased sample load. The same relationship between D and concentration, as that reported in the Figs. 3 and 4 for the 4240 sample, is found by quasi-elastic light scattering for polystyrene sulfonate

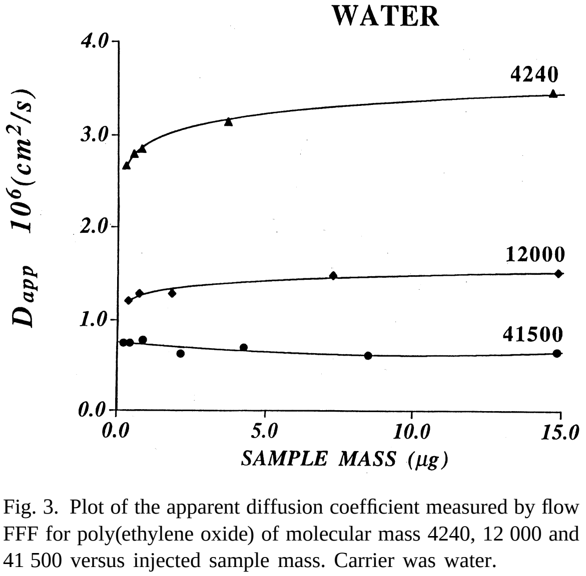

Fig. 3. Plot of the apparent diffusion coefficient measured by flow FFF for poly(ethylene oxide) of molecular mass 4240, 12 000 and 41 500 versus injected sample mass. Carrier was water.

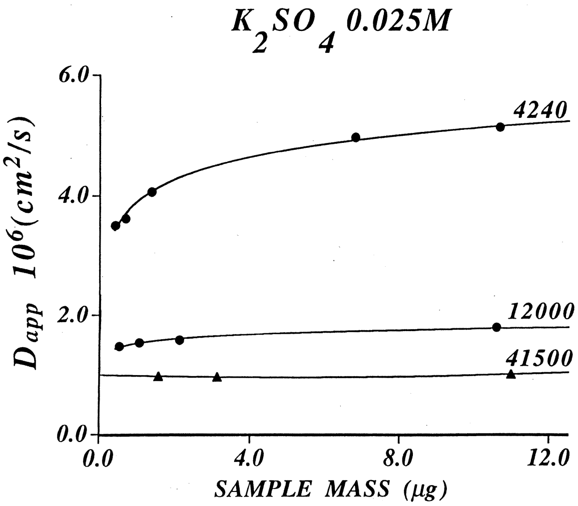

Fig. 4. Plot of the apparent diffusion coefficient for the same PEO sample as Fig. 3 versus injected sample mass. A 0.025 M aqueous solution of potassium sulfate was used.

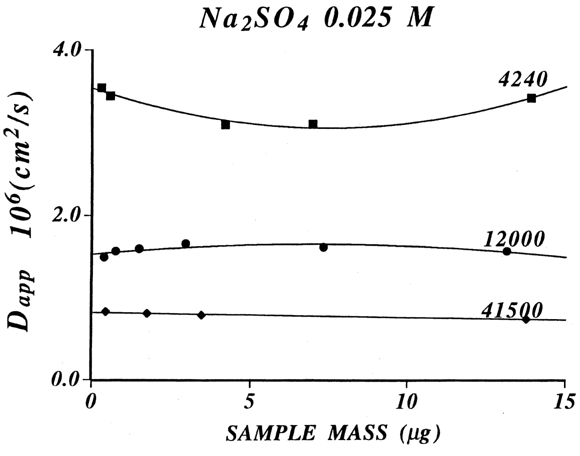

Fig. 5. Same type of plot as in Fig. 3 but using a 0.025 M aqueous solution of sodium sulfate.

[33,34] at almost the same ionic strength as the one used here [33]. The actual value of this coefficient for a given polymer concentration is, however, considerably lower in pure water than it is in the K_2SO_4 environment. In 0.025 *M* Na_2SO_4, on the other hand, the value for D_{app} begins at the smallest loads by being comparable to that seen in the potassium-containing environment. However, rather than increasing with increasing loads, it decreases over a wide mass range and appears to pass through a shallow minimum around 7 μg of injected mass. Using a load of 5 μg as a basis for comparison, one finds the values for D_{app} to be 3.3, 4.8, and $3.1 \cdot 10^{-6}$ cm^2 s^{-1} in DI water, 0.025 *M* K_2SO_4, and 0.025 *M* Na_2SO_4, respectively.

In DI water, the uncharged PEO molecule can be expected to behave as a random coil whose diffusivity would increase with increasing concentration due to excluded volume effects, as postulated by de Gennes [32]. For the sample of lowest molecular mass the apparent diffusivities in Fig. 3 are entirely in accord with this model. The addition of salt to the carrier could be expected to reduce its calibre as a solvent for the polyether. Such a reduction would lead to a contraction of the polymer coil and an increased diffusivity compared to the condition in DI water. This is indeed observed for the lowest loads of the 4240 Da PEO in Figs. 3–5, where in the limit of zero load the value for D_{app} is extrapolated to $2.5 \cdot 10^{-6}$ cm^2 s^{-1} for DI water compared to $3.5 \cdot 10^{-6}$ cm^2 s^{-1} for either of the salt solutions. For the samples of intermediate and higher molecular mass the effect is similar, although less pronounced.

Although the values of the diffusion coefficient measured at the lowest concentration in the two salt solutions, are very close, the 4240 Da PEO responds very differently to increased polymer concentration in the two media. In the carrier containing 0.025 *M* Na_2SO_4 a gradual increase in load results in a lowering of D_{app}, as seen in Fig. 5. Such an effect could be explained by a tendency to aggregate that is due to the reduced solvent power. It is not present for either of the higher-molecular-mass samples.

By contrast, the behavior in 0.025 *M* K_2SO_4 shows the opposite trend of an increasing D_{app} at increased sample loads. Clearly, the polymer responds very differently to the potassium ion than it does to the sodium ion. As discussed in the introduction, the PEO is known to form helical coordination complexes around certain cations [6,8], notably the potassium ion [9]. In so doing, it would become converted into a pseudo-polyelectrolyte.

In the flow FFF analysis of polyelectrolytes referred to above [19,20,29] the effect of sample load on retention was found to be negligible at high salt concentration but becoming increasingly pronounced the lower the ionic strength. This phenomenon could be explained in part by the fact that excluded volume effects, caused by both intra- and inter-molecular charge repulsion at low ionic strength, become progressively smaller as the concentration of the added electrolyte increases. Similar concentration effects on polyelectrolyte diffusion have been observed by quasi-elastic light scattering for highly charged polystyrene sulfonates in solutions of low and medium ionic strength [33,34]. It could be argued that the $PEO \cdot nK^+$ polycation might behave in a similar way, which would explain the results of the lower molecular mass sample displayed in Fig. 4. This effect does not seem to be pronounced in the case of the higher-molecular-mass samples, which behave very similarly in the sodium and potassium containing solutions.

The presence of strong interactions between the polymer and the potassium ion seems to be substantiated by a separate analysis of the collected retention data. Specifically, the exponential relationship between a macromolecule's diffusion coefficient and molecular mass expressed in Eq. (9) allows a linear correlation to be made between the corresponding logarithms with a slope equal to $-b$. Clearly, the same power dependence should also characterize the relationship between hydrodynamic size and molecular mass. The log–log plots constructed from data obtained in the three different carrier liquids are shown in Figs. 6–8. It is worth mentioning that an excellent linear correlation ($r^2 =$ 0.998–0.996) is found for the flow FFF measurements of the higher loads shown in the Figs. 6 and 7, despite the fact that the model for the size-molecular mass relationship is not expected to hold in this low molecular mass range. A similar correlation has been found by others for PEG with molecular masses as low as 1500 [35].

It is known from the physical chemistry of flexible chain macromolecules [32] that molecular conforma-

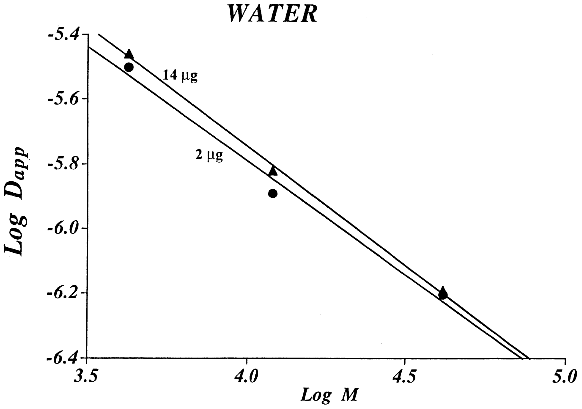

Fig. 6. Log–log correlation between the measured diffusion coefficient and molecular mass for PEO shown for different sample sizes in water. r^2 for the lower and higher injected mass was, respectively, 0.987 and 0.998.

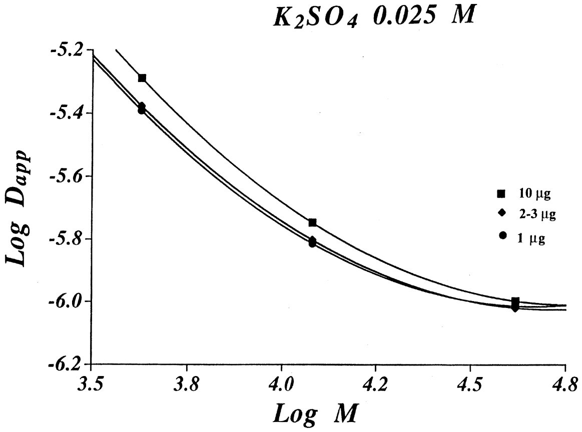

Fig. 8. The log D_{app} versus log *M* for the poly(ethylene oxide) in 0.025 *M* aqueous potassium sulfate is shown with the least-square best fit.

tion and hydration determine the value of parameter *b* in Eq. (9), a value expected to increase with enhanced solvation and molecular asymmetry. The values of 0.70 and 0.73 for this parameter, determined for the three PEO samples analyzed in water (Fig. 6), indicate an open, possibly asymmetric, configuration. The presence of Na_2SO_4 lowers the *b*-value to the level expected for a statistical coil in a good solvent and without strong shape asymmetry ($b = 0.61$–0.66). By contrast, no linear correlation was seen in the log–log plots relating size to molar mass for PEO in K_2SO_4 (see Fig. 8). However a two-point estimation for the lower molecular masses in Fig. 8 indicates *b* for short PEO chains to have a large, negative value (around -0.9), in turn suggesting an expanded or rigid structure in the presence of potassium ions. Although this *b*-value is highly speculative, one must conclude from the curvature of the full plots that conformational differences exist between polymer chains of different lengths. The coordination of the polymer chain around a potassium ion would impose order, and would by definition reduce the entropy of the system. Although this loss would be compensated for by the binding energy between ion and chain, it may be that such compensation only can occur for short chains while the loss of configurational entropy for longer chains might be great enough to make their structuring prohibitive.

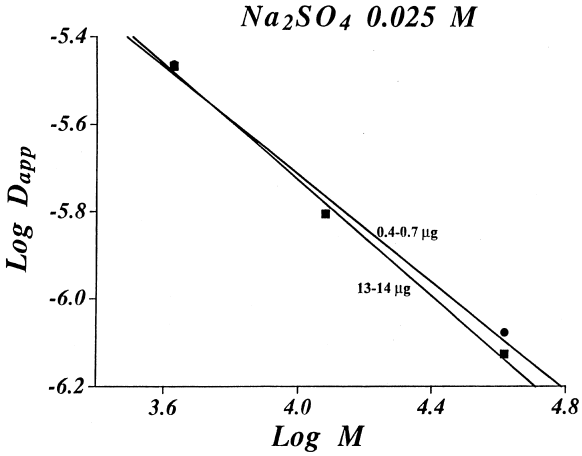

Fig. 7. Same plot as in Fig. 6 obtained in 0.025 *M* aqueous sodium sulfate. r^2 for the lower and higher injected mass was, respectively, 0.987 and 0.996.

5. Conclusions

The flow FFF technique is capable of generating highly reproducible, size selective fractionation patterns for polymeric samples. Since the level of retention is an explicit function of the effective diffusivity of the polymer, the technique can be used to perform comparative studies of the hydrodynamic sizes of polymers under different solvent conditions. Specifically, such studies can shed light on the goodness of a particular solvent and its influence on the hydrodynamic behavior of polymers of different molecular mass under different levels of crowding.

For the case of PEO chains of different molecular masses it has been possible to systematically collect retention data in pure water, as well as in two solutions of low and identical ionic strength, whose ionic components are Na_2SO_4 and K_2SO_4, respectively. From the retention-derived apparent diffusion coefficients, D_{app} of the lowest injected load, one can conclude that the hydrodynamic size of each examined polymer sample is larger in pure water (D_{app} smaller) than in either of the salt solutions. Not unexpectedly, the water is, therefore, found to be the better solvent for this non-ionic polymer. The relative goodness of these solvents with respect to PEO was also probed in an entirely different way, still making use of D_{app}-values from flow FFF. In this approach D_{app} was plotted as a function of molecular mass, M, in log–log plots whose slopes, $-b$, were taken as measures of the polymer–solvent interaction. Given that a b-value of 0.5 describes the ideal random coil present in a Θ-solvent, numerical values larger than 0.5 are indicative of more expanded coil-structures, i.e., better solvents. The values of 0.73 in DI water and 0.66 in 0.025 M Na_2SO_4 for comparable PEO concentrations determined here, again support the notion of pure water as the better solvent.

The lowest molecular mass fraction of PEO (4240) was found to behave very differently in the two dilute salt solutions, depending on whether the cation was sodium or potassium. While the influence of polymer concentration on D_{app} in the sodium containing medium was slight, and fully in accord with what could be expected for a modest solvent, the behavior in the potassium solution was similar to that seen previously for polyelectrolytes. Notably, the value for D_{app} increased significantly with an increase in PEO concentration, a behavior reminiscent of the charge repulsion seen when concentrating highly charged polystyrene sulfonate. In addition, the log–log plot of D_{app} versus M was no longer linear, as had been the case for the sodium-containing medium, but was strongly curved with a high slope value (departure from a random coil configuration) at the low M in the presence of potassium ions. Together, these observations support the notion of the potassium ions forming coordination complexes with the ether oxygens in this flexible polymer, thereby generating somewhat more rigid helical structures. The effect is only seen in the lower molecular mass range, suggesting the entropic penalty for such structuring to be too large for PEO chains with molecular masses in excess of around 10 000 Da.

6. Nomenclature

A	Constant relating D to M^{-b}
b	Exponent in diffusion coefficient expression
b	FFF channel breadth
D	Diffusion coefficient
D_{app}	Apparent diffusion coefficient
D^0_{app}	Apparent diffusion coefficient at zero concentration
D_{coop}	Cooperative diffusion coefficient
D_t	Tracer diffusion coefficient
k	Boltzmann constant
ℓ	Characteristic thickness of solute zone
l	Length of a polymer chain
L	Channel length
M	Polymer molecular mass
R	Retention ratio in Eq. (1)
R_h	Particle hydrodynamic radius
t^0	Void time
t_r	Retention time
T	Absolute temperature
U	Field-induced velocity
$\dot{V}_c$	Volumetric cross flow-rate
V^0	Void volume
w	Channel thickness

Greek characters

η	Fluid viscosity
λ	Retention parameter

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