Characterisation of polymers and particles by asymmetrical flow field-flow fractionation

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CHAPTER 4

Assessment of the diffusional behaviour of polystyrene sulfonates in the dilute regime by hollow-fiber flow field-flow fractionation

ABSTRACT

Flow Field-Flow Fractionation (FFF) in a ceramic tubular membrane was applied to study the diffusional behaviour of sodium polystyrene sulfonate (NaPSS) as a function of salt and polyelectrolyte concentration in the dilute concentration regime. NaPSS standards were fractionated in an aqueous carrier liquid with salt concentrations of between 1 mmol l\(^{-1}\) and 100 mmol l\(^{-1}\) and injected amounts varying between 0.2 and 20 µg. A sharp increase of the apparent diffusion coefficients, due to peak overloading, occurs at a critical polyelectrolyte concentration at the accumulation wall. A model has been developed comparing repulsive electrostatic interactions, calculated from the Derjaguin-Landau-Verweij-Overbeek (DLVO) theory, to the thermal energy. It is shown that the observed critical concentration approximates the concentration of a closely packed lattice of hard spheres within an order of magnitude. The critical concentration appeared to be dependent on the salt concentration, but the influence of the molecular mass was less clear. Expressions for the migration velocity in overloaded channels have been derived and confirmed experimentally for the two lowest molecular masses. Deviations occur for high-molecular mass NaPSS, possibly due to mutual attraction. These experiments confirm the observations made by Small Angle Neutron Scattering and Dynamic Light Scattering that the ordering of polyelectrolytes occurs already in the dilute regime.
INTRODUCTION

A large variety of techniques has been utilized to measure static and dynamic properties of polyelectrolytes in solution, including osmotic pressure, viscosity, and birefringence measurements and light, x-ray and neutron scattering. As already predicted in 1976 by de Gennes et al. and confirmed experimentally with dynamic light scattering (DLS) measurements by Koene and Mandel, several concentration regimes for polyelectrolytes can be discerned. Complete phase diagrams for polyelectrolytes have been given by Kaji et al. and by Schiessel.

In the dilute regime, the focus of our interest, polyelectrolytes behave as flexible, charged chains with long-range Coulombic repulsion as the only source of mutual interaction. The Derjaguin-Landau-Verwey-Overbeek (DLVO) theory can be used to describe such systems. In this approach the polyions are modelled as hard, incompressible, charged spheres with a Coulombic interaction determined by the surface potential of the hypothetical spheres and the ionic strength of the surrounding solution.

Above a certain critical concentration within the dilute regime sudden changes of the static and dynamic properties of polyelectrolytes have been observed. A steep increase of the osmotic pressure and apparent diffusion coefficients are observed at concentrations well below the transition concentration between the dilute and semi-dilute regime. At higher polyion concentrations, in the semi-dilute regime, the apparent diffusion coefficients become independent of the molecular mass of the polyelectrolytes.

At polyelectrolyte concentrations higher than this critical limit in the dilute regime, a double exponential correlation function is measured with DLS. The correlation function is interpreted as consisting of a second diffusive or "slow" mode existing besides the normal "fast" diffusion. The increase of the fast diffusion coefficient is attributed to a coupled diffusion of the polyions and counterions in the solution. The slow mode correlation in DLS is interpreted as being caused by the presence of large domains or aggregates of polyionic chains. Small-angle X-ray and neutron scattering experiments (SAXS and SANS), conducted with a large variety of polyelectrolytes of synthetic as well as biological origin, also seem to indicate that ordered clusters of the polyelectrolyte ions are formed, in which the motion of the individual ions is coupled. The exact nature of these domains and the mechanism behind their formation is still not completely understood. Also, the transition of the ordinary mode (with a single exponential decay curve in DLS) to the extraordinary regime (with two modes of diffusion) and its dependency on, for example, the salt concentration of the solution and the molecular mass of the polyelectrolyte, is still a matter of dispute.

Flow field-flow fractionation has been developed as an analytical separation technique for the fractionation and characterisation of polymers in solution. In flow FFF a separation channel with a semi-permeable wall is used. Through the channel a carrier liquid is pumped in the axial (length) direction, while at the same time a so-called cross-flow is maintained through the semi-permeable wall. Macromolecular compounds that are injected into the channel as analytes cannot pass through the semi-permeable wall and are concentrated into a thin layer of solution close to this
wall. The thickness of this layer depends on the velocity of the cross-flow and on molecular diffusion of the analyte compounds. Since the axial flow is laminar, this thickness determines the (average) velocity of the analyte in the axial direction. Thus in flow FFF macromolecular compounds are separated on the basis of differences in molecular diffusion rates. The separation mechanism of flow FFF is such that it provides a direct method to determine the molecular diffusion as originally defined by Fick in 1855.\textsuperscript{23} Ideally, the molecular diffusion coefficient data that are obtained by flow FFF can be translated into molecular size data through the Stokes-Einstein theory.

Hollow-fiber flow FFF was experimentally developed by Jönsson and Carlshaf in 1989.\textsuperscript{24} We have used a hollow-fiber flow FFF set-up, newly developed in our laboratory, with a tubular ceramic ultrafiltration membrane as the separation channel. While previously employed polymeric fibers may be subject to swelling in the solvent,\textsuperscript{25} the rigid structure of the ceramic support ensures stable channel dimensions.

Generally, low analyte concentrations are used in flow FFF, so that for neutral polymers apparent diffusion coefficients are obtained that approach the values at infinite dilution.\textsuperscript{26} However, several studies have shown that flow FFF applied to polyelectrolytes is very susceptible to overloading.\textsuperscript{27-29} When flow FFF is used to obtain data on the molecular size distribution of a polyelectrolyte sample, care should be taken that the amount of analyte injected in the channel stays below a certain limit. An increase in concentration will lead to electrostatic repulsion of the analyte from the solution layer close to the accumulation wall.

The overloading phenomena that are observed in flow FFF of polyelectrolytes are obviously connected to the steep increase of apparent diffusion coefficients above a certain critical concentration, as has also been observed with other techniques. Therefore, flow FFF appears to be a very suitable technique to study such diffusional behaviour. We have used flow FFF to estimate the critical concentration of poly(styrenesulfonate) standards with different molecular masses in solutions with a wide range of ionic strengths. The experimental results obtained have been compared to theoretical predictions from the DLVO theory for charged hard spheres. The model developed for this purpose elaborates on previous applications of DLVO theory to flow and sedimentation FFF for particles.\textsuperscript{30-32}
EXPERIMENTAL

Instrumental

Tubular ceramic ultrafiltration membranes were purchased as a Keraset-400 module (Rhône Poulenc, Lyon, France) composed of 19 parallel channels with a length of 40 cm and an inner diameter of 2.5 mm. A molecular weight cut-off of 15 kDa is given by the manufacturer. A single channel with a length of 11 cm was cut out of the module and polished to a cylindrical shape with an outer diameter of ca. 4.2 mm.

Connections from standard 1/16" PEEK tubing to the channel were made via two 1/16" stainless steel zero-dead-volume unions (Swagelok, Solon, OH, USA). In order to fit them to the ceramic tube, they were cut in half and a small cylinder was soldered to the end. The connectors were glued to the ends of the channel with Araldite 2014 epoxy resin (Ciba-Geigy, Basel, Switzerland).

A scheme of the instrumental set-up with an enlarged picture of the separation channel is shown in Figure 1. The channel was mounted in a stainless-steel tube that was subsequently filled with the carrier liquid. A Spectroflow pump (Applied Biosystems, Ramsey, NJ, USA) delivered the flow in the axial direction ($F_{in}$). A P-6000 syringe pump controlled by a P-500-plus controller (Pharmacia, Uppsala, Sweden) was connected to the stainless steel encasing so that it could control the cross-flow ($F_{cr}$).

Figure 1. Scheme of the instrumental set-up of the flow FFF instrument. The enlargement shows a photograph of the channel with the tubing connected to it. Arrows indicate the directions of the flows during fractionation.
Both the cross-flow and flow through the detector were recycled to the solvent reservoir with a volume of 1 l. Samples were injected via a 5 µl injection loop on a 7010 injection valve (Rheodyne, Berkeley, CA, USA). UV detection at 226 nm was performed using a Spectroflow 757 UV detector (Applied Biosystems, Ramsey, NJ, USA). Detector signals were amplified by a model 113 pre-amplifier (Princeton Applied Research, Princeton, NJ, USA) and transferred by a home-built A/D converter to a personal computer.

**Chemicals and solutions**

NaPSS standards (Polymer Standard Service, Mainz, Germany) were reported by the manufacturer to have a polydispersity smaller than 1.1. Demineralised water and p.a. sodium chloride were used to prepare the carrier liquid. Samples were prepared by dissolving the polymer in the carrier liquid and stored at 4°C.

**Procedure**

All experiments were carried out at room temperature. After injection, the analyte was flushed with 75 µl of carrier liquid towards the channel (corresponding to the calculated dead volume in front of it) to decrease the time required for relaxation. Then, during a relaxation period of 8 minutes, \( F_m \) and \( F_c \), were set to 0.02 and 0.2 ml min\(^{-1} \), respectively. The fractionation was started by increasing the axial and cross-flow to the desired values. Depending on the ionic strength and molecular mass, the inlet flow rate was adjusted so that at the highest injected mass the fractionated peak was still separated from the void peak. \( F_c \) was kept at 0.15 ml min\(^{-1} \).

**THEORY**

**DLVO model**

In the DLVO model, a polyelectrolyte is considered as a hard, incompressible, charged sphere with a hydrodynamic radius \( (n_t) \). When introduced in a flow FFF channel, two forces act on the particle: Hydrodynamic forces, induced by the cross-flow, driving the particles towards the accumulation wall and repulsive electrostatic forces (particle-particle interactions). At low concentrations, the average distance between the particles is large and the cross-flow can concentrate the sample in a solution layer close to the wall with an average thickness determined by the thermal energy of the particles. As the concentration is increased, the particles move closer to each other and repulsive interactions increase. When repulsive forces exceed the thermal energy, the sample can no longer be contained in the thin solution layer. *Ergo*, peak overloading will occur. In that case, the sample is repelled to average faster velocity zones and a change in retention time and peak shape occurs. The point where the repulsive energy is equal to the thermal energy is, according to the definition by de Gennes *et al.*, the transition point into an ordered or "lattice" state.
Hence, in flow FFF the point where overloading starts to take effect is directly related to the transition into an ordered state.

In a solution, charged particles carry a surface potential \( \psi_0 \) that is screened by oppositely charged counterions in the solution over the Debye screening length. The expression for the electrostatic repulsive energy \( (E_r) \) between two particles at a distance of \( 2x \) in a 1:1 electrolyte has been derived by Honig and Mul on the basis of DLVO theory.\(^{33}\) Substituting \( r_n \) for the particle radius gives for polyelectrolytes:

\[
E_r = 2 \pi r_H \varepsilon \varepsilon_0 \gamma e^{-2\kappa(x-r_n)}
\]

where \( \varepsilon \) the relative permittivity of the solvent and \( \varepsilon_0 \) the vacuum permittivity. \( \kappa \) is the inverse of the Debye screening length:

\[
\kappa = \left( \frac{2 - \frac{Iq^2}{ee_0 kT}}{2 - \frac{Iq^2}{ee_0 kT}} \right)^{1/2}
\]

where \( q \) is the elementary charge, \( I \) the ionic strength of the solvent, \( k \) the Boltzmann constant and \( T \) the temperature. \( \gamma \) is a potential function derived from the surface potential of the polyelectrolyte:

\[
\gamma = \frac{4kT}{q} \tanh \left( \frac{q}{4kT \Psi_0} \right)
\]

From the electrophoretic mobility of NaPSS ions, 41.2 \( 10^{-9} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1} \), as given by Cottet and Gariel,\(^{34}\) it follows that \( \psi_0 \) is in the order of 52 mV. A value of 48 mV is calculated for \( \gamma \). The surface potential is independent of the ionic strength of the solution and the molecular mass.

For comparison, in Figure 2 the repulsive force between two 17 kDa NaPSS molecules as a function of the intermolecular distance is shown together with the thermal energy. A salt concentration of 10 mmol l\(^{-1} \) and a hydrodynamic radius of 4.1 nm (calculated from its diffusion coefficient) were used in the calculations. Since the repulsive energy increases rapidly with decreasing distance, there is a distinct minimum of approach \( (x_{\text{min}}) \) between adjacent polyelectrolytes where the two energy curves cross in Figure 2. The minimum distance of approach can be found as the distance where the repulsive energy equals \( kT \):

\[
x_{\text{min}} = 2r_H - \frac{1}{\kappa} \ln \left( \frac{kT}{2\pi r_H \varepsilon \varepsilon_0 \gamma} \right)
\]

From the closest packing of spherical particles the critical concentration \( c^* \) (in g l\(^{-1} \)) can be obtained as:

\[
c^* = 0.74 \frac{3M}{1000 \pi \frac{3}{4} x_{\text{min}}}
\]

where \( M \) is the molecular mass and \( N_A \) Avogadro's number.
Figure 2. Energy of repulsion between two 17 kDa NaPSS molecules as predicted by the DLVO theory compared to the thermal energy, kT; \( c_s = 10 \text{ mmol l}^{-1} \).

In order to calculate \( c^* \) at a given ionic strength, the value of the hydrodynamic radius of the polymer is required. \( r_H \) can be determined from the diffusion coefficient obtained by flow FFF, given that the system is not overloaded (see Equation 10 below). The Stokes-Einstein equation relates the hydrodynamic radius of the macromolecule to its diffusion coefficient under unperturbed conditions \( (D_0) \):

\[
r_H = \frac{kT}{6\pi \eta D_0}
\]

where \( \eta \) is the dynamic viscosity of the solvent.

**Elution in flow FFF**

In flow FFF without overloading effects, a macromolecular compound is concentrated in a thin layer of solution close to the fiber wall. The concentration of the compound follows an exponential profile as a function of the distance \( z \) to the accumulation wall:

\[
c(z) = c_0 \cdot \exp \left( -\frac{z}{\ell_0} \right)
\]

where \( c_0 \) is the concentration at the accumulation wall. \( \ell_0 \) is the characteristic layer thickness which is determined by the diffusion coefficient of the compound and the velocity of the cross-flow:

\[
\ell_0 = \frac{D_0}{u_{cr}}
\]
Here, $u_0$ is the linear velocity of the cross-flow through the wall. At sufficiently high retention, under the influence of the parabolic axial flow in a hollow fiber the axial velocity $v_0$ of the compound becomes:

$$v_0 = \frac{4\ell_0}{R} \bar{v}$$

(9)

where $R$ is the radius of the fiber and $\bar{v}$ the average axial flow velocity. According to the standard theory on flow FFF the retention time $t_r$ of the compound can be approximated by the equation:\(^{24}\)

$$t_r = \frac{R^2}{8 D_0} \ln\left( \frac{F_{\text{in}} - \zeta F_{\text{cr}}}{F_{\text{in}} - F_{\text{cr}}} \right)$$

(10)

Here, $\zeta$ denotes the distance of the relaxation point from the inlet of the fiber relative to the length of the fiber; $\zeta$ is equal to the ratio of $F_{\text{in}}$ and $F_{\text{cr}}$ during relaxation (0.1 in our case). Equation 10 allows the diffusion coefficient at infinite dilution to be calculated from the retention time under unperturbed conditions.

As was shown in the previous paragraph, the DLVO theory predicts a steep increase of the mutual repulsion of polyelectrolyte molecules when the critical concentration $c^*$ is exceeded. For hollow-fiber flow FFF this implies that the exponential concentration profile close to the wall will be disturbed when too much material is introduced in the channel. The starting point of overloading in hollow-fiber FFF corresponds to the point where the concentration of the macromolecular compound on the inner surface of the fiber reaches $c^*$. This concentration may be obtained from the peak concentration in the detector at the onset of overloading. From the flux equations that can be set up for the system the relation between the detector concentration $c_{\text{det}}$ and the wall concentration at the end of the channel $c_0$ can be calculated:\(^{27}\)

$$c_{\text{det}} = \frac{8 \ell_0^2}{R^2} c_0$$

(11)

The value of $\ell_0$ or $D_0$ required to estimate $c^*$ can be acquired from the retention time in the absence of overloading (Equation 10).

When more material is introduced in the channel the concentration profile may take approximately a form as is shown in Figure 3. Close to the wall a layer with a thickness of $\ell^*$ is formed containing the maximum concentration $c^*$ of the compound, and on top of that the normal exponential layer with thickness $\ell_0$. The thickness of the layer $\ell^*$ will depend on the amount of the compound introduced into the fiber.

The average axial velocity of the compound will be increased compared to the situation without overloading effects. An overloaded peak can be seen as a collection of zones, each moving with a specific velocity $v^*$. $v^*$ is dependent on the concentration profile in the zone, which is reflected in $c_{\text{det}}$. 
Figure 3. Concentration profile as a function of the distance $z$ from the accumulation wall under unperturbed (a) and overloaded conditions (b).

It can be shown that the velocity of such a zone with a concentration profile as depicted in Figure 3 (curve b) is:

$$v^* = \left( \frac{\ell_0^2 + \ell^* \ell_0 + 1/2 \ell^*^2}{\ell_0^2 + \ell^* \ell_0} \right) v_0$$

(12)

where $v_0$ is the axial velocity of the compound under unperturbed conditions. The detector concentration and the maximum wall concentration and layer thickness are related by:

$$c_{\text{det}} = 8 \frac{\ell_0^2 + \ell^* \ell_0 + 1/2 \ell^*^2}{R^2} c^*$$

(13)

Equations 12 and 13 can be used to analyze a series of data on peak retention times and detector concentrations obtained under overloaded conditions.

RESULTS AND DISCUSSION

In order to validate the system four standards were fractionated with different combinations of $F_{in}$ and $F_{cr}$. The salt concentration ($c_s$) of the carrier liquid was 10 mmol l$^{-1}$ and the injected mass 1 $\mu$g. $F_{in}$ was varied between 0.5 and 2 ml min$^{-1}$, $F_{cr}$ between 0.05 and 0.3 ml min$^{-1}$. In Table 1, the resulting mean diffusion coefficients, calculated with the use of Equation 10 and the relative standard deviations (RSD) of
the diffusion coefficient of the four standards are presented. Also, the average recoveries are given, i.e., the ratio of the measured peak area and the expected value based on the injected amount. It can be seen in Table 1 that the recovery for all standards is complete within the expected experimental error (e.g., injection volume or outlet flow rate), indicating that there is no significant leakage in the system. Notably, the relative standard deviation of the mean D₀ is smaller than 5% for all standards.

Generally, diffusion coefficients of polymers follow the well-known relation:

\[ D_0 = A \cdot M^{-b} \]  

where A and b are empirical constants. The constant b is a measure of the solvent quality and state of the polymer. It can vary between 0.5 for a bad solvent and 0.6 for a good solvent. Linear regression of the results in Table 1 yields \( \log A = -7.8 \pm 0.11 \) and \( b = 0.58 \pm 0.02 \). This is in good agreement with constants obtained by analysing data from literature: \( \log A = -7.8 \pm 0.18 \), \( b = 0.56 \pm 0.02 \) and \( \log A = -7.6 \pm 0.2 \), \( b = 0.61 \pm 0.04 \) as measured with flow FFF and DLS, respectively. Our system is obviously well suited to determine diffusion coefficients of NaPSS.

For the diffusional study, four standards were fractionated in a carrier liquid with six different salt concentrations of between 1 and 100 mmol l⁻¹. At each salt concentration five or six fractionations of the respective standards were carried out; the injected masses ranged between 0.2 and 20 μg.

Typical examples of the results that were obtained are depicted in Figure 4. Figure 4A shows a superposition of five fractograms of the 17 kDa standard with different injected amounts at a salt concentration of 30 mmol l⁻¹. The trend shown in Figure 4A is illustrative of the diffusional behaviour. First, with sample loads below 2.5 μg, the peaks remain symmetrical and the retention time remains approximately constant. Upon increasing the sample load, the peak shape changes to triangular, and the peak top shifts towards shorter retention times. In accordance with the presented model, there is a threshold concentration, reached with an injected mass of between 1 and 2.5 μg, above which overloading starts to occur.

Figure 4B shows fractograms at a lower salt concentration of 2 mmol l⁻¹. Evidently, due to insufficient screening of the repulsive forces, the cross-flow is unable to concentrate the polyelectrolytes into a narrow solution zone. While, for example, an injected mass of 1 μg in Figure 4A generates a nearly symmetrical peak, the peak shape in Figure 4B is strongly asymmetrical.

For each fractogram apparent values of the diffusion coefficient (Dₑₚₚ) and the concentration c₀ were calculated from the retention time and detector signal at the peak maximum, using Equations 10 and 11, respectively. In Figure 5 the results for the four standards at salt concentrations of 100 and 2 mmol l⁻¹ are shown. In this type of plot the effect of the concentration of the polyelectrolyte at the accumulation wall becomes very pronounced.
Table 1. Average diffusion coefficients and recoveries of four polystyrene sulfonates and the relative standard deviations of the mean $D_0$. Values for the 17 and 46 kDa standards are based on six measurements, for the 123 and 356 kDa standards on five measurements. Conditions: see text.

<table>
<thead>
<tr>
<th>M (kDa)</th>
<th>$D_0$ ($10^{-11}$ m² s⁻¹)</th>
<th>RSD (%)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>17</td>
<td>6.0</td>
<td>2.7</td>
<td>102</td>
</tr>
<tr>
<td>46</td>
<td>3.6</td>
<td>3.3</td>
<td>108</td>
</tr>
<tr>
<td>123</td>
<td>1.72</td>
<td>1.9</td>
<td>101</td>
</tr>
<tr>
<td>356</td>
<td>1.03</td>
<td>4.0</td>
<td>85</td>
</tr>
</tbody>
</table>

Figure 4. Superposition of five fractograms of the 17 kDa NaPSS sample. Injected amounts are 0.2, 1, 2.5, 5, 10 and 20 μg, respectively. $c_s = 30$ mmol l⁻¹ (A) and $c_s = 2$ mmol l⁻¹ (B). For other conditions: see text.

Figure 5. Apparent diffusion coefficient versus polymer concentration at the concentration wall at the peak maximum. Legend: (♦) 17 kDa; (X) 46 kDa; (●) 123 kDa; (■) 356 kDa. $c_s = 10$ mmol l⁻¹ (A) and 2 mmol l⁻¹ (B). Dashed lines indicate the diffusion coefficients from Table 1; drawn lines are added to guide the eye. For other conditions: see text.
The threshold value c* was extracted from the double log-plots, such as Figure 5A and 5B, as the point where D_{app} starts to increase. For the lower salt concentrations it is problematic to determine the critical point, since c* is not reached with the smallest amount injected (see, e.g., Figure 5B). The injected mass could not be lowered further, because this made it difficult to determine the exact retention time and peak height. In these cases, the diffusion coefficient under non-overloading conditions was estimated by the value of D_0 at a salt concentration of 10 mmol l^{-1} (Table 1). Although this is not entirely correct (due to chain expansion or coil to rod transition, the diffusion coefficient can change significantly) the values are assumed to be fair estimates. Also, the r_H values substituted into the DLVO model were calculated from the values of D_0 in Table 1.

In Figures 6A and 6B, the experimentally determined c* values for the 17 and 123 kDa samples are presented with the c* values according to the DLVO model, obtained as outlined previously. For comparison, the transition of the dilute to semi-dilute regime calculated on basis of scaling relations is shown. It should be noted, however, that due to the large number of scaling lengths, the applicability of scaling theory to flexible polyelectrolytes is disputable. On the other hand, it is clear that the concentrations in our experiments fall well into the dilute regime and therefore the increase in D_{app} is not due to a transition into the semi-dilute regime.

The DLVO model predicts the critical concentration within an order of magnitude, and the measurements follow the trend predicted by the model. These results give evidence for the occurrence of ordering in polyelectrolyte solutions, since the model directly relates the critical concentration to a crystal lattice at the point where the repulsive energy equals the thermal energy. Without the applied cross flow, it is not possible for the molecules to freely diffuse in the solution, since the intermolecular distance cannot be smaller than x_{min}. This may give rise to the formation of clusters of polyelectrolytes diffusing at a distance of around x_{min}.

![Figure 6](image_url). Critical concentrations versus salt concentration for NaPSS standards with molecular masses of 17 kDa (A) and 123 kDa (B). Drawn line: c* according to the DLVO model. Dashed line: Transition from the dilute to semi-dilute regime according to scaling theory. ●: Experimentally determined c*.
Interestingly, in flow FFF a strong influence of the salt concentration on the critical concentration was found, as was predicted on basis of the DLVO theory. From SANS and SAXS experiments on DNA fragments no consensus has been reached about the effect of the salt concentration on the average spacing between polyelectrolytes.\textsuperscript{19-21} Flow FFF experiments on similar samples may be helpful in assessing more precisely these phenomena. The results in Figure 6 oppose the findings of Kaji \textit{et al.}\textsuperscript{5}, who derived on the basis of scaling theory a critical concentration of 3.34·N\textsuperscript{-2}, where N is the degree of polymerisation. In this equation, the ionic strength is not accounted for.

In Figure 7 the effect of the molecular mass on \(c^*\) at three different salt concentrations is presented. Here, the values calculated by the model are compared with the measured \(c^*\) values. With the exception of \(c_s = 10\) mmol l\textsuperscript{-1}, the model is not quantitative in predicting the effect of \(M\) on \(c^*\) and the expected trends are not displayed in the measurements. There is a strong indication that the high-molecular mass standards deviate from the behaviour of hard incompressible spheres. At elevated salt concentrations, the measured \(c^*\) exceeds the value calculated from the DLVO model for the two highest molar masses.

Similar conclusions can be drawn from applying the DLVO model to data from literature. Sedláček has measured the ionic strength dependence of the splitting of diffusion coefficients into a fast and slow mode in DLS.\textsuperscript{12} Using a 5 g l\textsuperscript{-1} NaPSS solution, it was found that above a critical salt concentration the slow mode disappeared. When applying the DLVO model to his data, we find for the 5 and 47 kDa standard that his experimental conditions (salt and polyelectrolyte concentrations) where the splitting occurs are close to the conditions where ordering in the solution starts to occur. For the 1200 kDa sample however, the DLVO model predicts an onset of ordering at a much lower concentration of the polyelectrolyte than the concentration at which the splitting of diffusion coefficients was observed. As seen in our experiments, the model tends to underestimate the \(c^*\) value of higher molecular masses. It is conceivable that the chains of the flexible NaPSS become entangled as the molecular mass increases. It has been observed from static light scattering and SANS experiments that the spacing between adjacent 780 kDa NaPSS molecules scales differently from the spacing between 18 kDa NaPSS, proteoglycan and t-RNA molecules, that scale as hard spheres.\textsuperscript{19} Attraction between similarly charged polyelectrolytes, which is under discussion in recent literature, cannot be ruled out.\textsuperscript{39-42} Mutual attraction between polyelectrolytes is not included in the model used in this work.
Figure 7. Effect of molecular mass on $c^*$ at three different salt concentrations. Drawn lines show the predictions by the DLVO model and markers show the measured values of $c^*$. $c_s = 1 \text{ mmol l}^{-1}$ (a and □); $c_s = 10 \text{ mmol l}^{-1}$ (b and ×); $c_s = 100 \text{ mmol l}^{-1}$ (c and ○).

In the previous section, the dependencies of the velocity $v^*$ and $c_{\text{det}}$ on the layer thicknesses $\ell_0$ and $\ell^*$ with injected masses above the critical concentration have been derived. Using the values of $\ell_0$ and $c^*$ determined in the previous experiments, the relation between $v^*/v_0$ and $c_{\text{det}}$ can be calculated from Equation 12 and 13 by varying $\ell^*$. In Figure 8, the theoretical curves of $\log (v^*/v_0)$ versus $\log (c_{\text{det}})$ for the 17, 46 and 356 kDa standards are combined with the experimentally determined velocities and concentrations in the detector. For the latter, we used the concentrations and retention times at the peak maximum, as these values can be determined most accurately. The salt concentration was 10 mmol l$^{-1}$.

Clearly, there is an excellent agreement between the measured velocities and the theoretical expectations for the 17 and 46 kDa standards. From this Figure and the agreement between the DLVO model and experimental data (Figure 5) it can be concluded that the diffusional behaviour of the low-molecular mass standards is comparable to that of hard incompressible spherical particles. In contrast, for the 356 kDa standard the experimental velocities are lower than expected on basis of $c_{\text{det}}$. A possible explanation for the deviating behaviour of this high-molecular mass polyelectrolyte may be that chain entanglement or attraction (also discussed within context of Figure 7) results in a layer $\ell^*$ which is smaller than expected on theoretical grounds. As a consequence, the velocity increase is less than expected when $c_0$ exceeds $c^*$.
Figure 8. Double logarithmic plot of relative velocity versus concentration in the detector for NaPSS standards of 17 kDa (●), 46 kDa (×) and 356 kDa (■); cᵣ = 10 mmol l⁻¹. Injected masses: 0.2, 1, 2.5, 5, 10 and 20 µg (for M = 17 and 46 kDa) and 2.5, 5, 10 and 20 µg (M = 356 kDa), respectively.

CONCLUSIONS

This work shows that flow FFF is an excellent tool to study the diffusion of macromolecular compounds. The critical concentration at which overloading of poly(styrenesulfonates) occurs agrees within an order of magnitude to the concentration of a closely packed lattice. While the behaviour of low-molar mass NaPSS can adequately be described by hard-sphere repulsion, deviations of high-molar mass compounds may possibly be explained by attractive forces. Qualitatively, these results agree with experiments conducted with DLS and SAXS. It would be interesting to apply our method to polyelectrolytes other than NaPSS.

Unfortunately, a direct comparison between flow FFF data and DLS or SAXS is as yet not possible. Most of the applications of the latter techniques are carried out in salt-free solutions or at polyelectrolyte concentrations in the order of 1 to 50 g l⁻¹, while the values in our experiments are of at least an order of magnitude lower. It is a general advantage of flow FFF that only a low amount of analyte is required and no extensive sample preparation is necessary, because flow FFF is relatively insensitive to contaminants. Moreover, since the retention mechanism of flow FFF is directly based on the displacement of macromolecules due to their diffusion in a solution, it provides a direct way to study diffusion as originally defined by Fick, without accompanying artefacts.
REFERENCES