

Effects of ionic strength of eluent on size analysis of sub-micrometre particles by sedimentation field-flow fractionation

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ABSTRACT

Sedimentation field-flow fractionation (SdFFF) has a high resolution over a wide range of particle size compared with other methods of sub-micrometre particle size determinations, and has the great advantage that the fractional collection is sorted by the particle mass. However, the retention behaviour in SdFFF depends strongly on the experimental parameters, especially the ionic strength of the eluent. The sizes calculated from the experimental results of SdFFF are underestimated if an eluent with low ionic strength is used, compared with those obtained by quasi-elastic light scattering spectroscopy, owing to the interparticle repulsion. There is a maximum value of the ionic strength of the eluent for particle size analysis, because rapid flocculation of particles occurs at high electrolyte concentrations. Further, hardly any difference in the retention times was found in SdFFF using different anionic surfactant solutions as the eluent.

INTRODUCTION

Field-flow fractionation (FFF) is a single-phase chromatography-related technique in which an externally applied field is allowed to interact with suspended particles under motion in a channel. Sedimentation FFF (SdFFF) is an FFF technique that uses centrifugal force for the external field, and is well suited to the characterization and fractionation of particulate materials and soluble samples in the colloid size range.

The classical theory of FFF, developed mainly by Giddings and co-workers, was presented as a method for conversion from retention time to particle size^{1–3}. Another contribution to SdFFF technology was made by Yau and Kirkland^{4,5} of DuPont through the development of a commercial instrument. They also implemented a special programming technique which reduces the analysis time, and introduced

a conversion equation to obtain the size by integration of the classical theory. However, the applicability of the classical theory is limited, because the interactions among particles and the wall of the channel due to local particle concentration, surface charge of the particles and the ionic strength of the eluent are not included in this theory.

In studies of the effects of the eluent, Hoshino *et al.*⁶ reported the unexpected prolongation of the retention at high concentrations of Aerosol OT (AOT), which was explained by the difference in the state of the interface between the channel wall and the solvent stream depending on the concentration and the kinds of the surfactants. Hansen and Giddings⁴ included the particle-wall interaction in their calculation, and found that the retention time depends on the electrostatic properties of the particles and of the channel wall, by comparison of the calculation with the experimental results. We evaluated the DuPont SdFFF instrument⁷, and found small effects of the surfactants in the eluent on size measurements by SdFFF, quasi-elastic light scattering (QELS) spectroscopy and transmission electron microscopy. We concluded that these small effects might be explained by the influence of the surface charge of the particles and the pH or ionic strength of the eluent.

This paper describes the importance of the ionic strength of the eluent, based on a comparison of size measurements by SdFFF and QELS spectroscopy.

THEORETICAL

In the classical theory, the retention factor R of a particle of diameter D_p is defined as the ratio of the elution time for an unretained peak, t_0 , to that for particles, t_R , as usual in chromatography. The following relationship between R and λ was obtained by Hovingh *et al.*⁸:

$$R = t_0/t_R = 6 \lambda \{ \coth[1/(2 \lambda)] - 2 \lambda \} \quad (1)$$

λ is expressed by the Stokes-Einstein relationship and the force generated in a centrifuge in the case of SdFFF:

$$\lambda = 6kT/(D_p^3 \pi \Delta \rho G w) \quad (2)$$

where k is the Boltzmann constant, T is the absolute temperature, w is the channel thickness, G is the field strength and $\Delta \rho$ is the density difference between the particle and the eluent.

For highly retained sample components, simplifying approximations employed in obtaining eqn. 1 are valid, as shown by Giddings⁹. Caldwell² recommended the following second-order approximation:

$$R = 6 \lambda (1 - 2 \lambda) \quad \text{for } R < 0.5 \quad (3)$$

In field-programmed SdFFF, the retention factor R becomes a function of time, depending on the field strength at that time:

$$t_0 = \int_0^{t_R} R(t) dt \quad (4)$$

The time-dependent retention $R(t)$ is still expressed by eqn. 1 if the rate of change of the field is slow so that each particle can move to the equilibrium position which is determined by the force of the field and the Brownian motion of the particle. The DuPont SdFFF instrument uses the time-delayed exponential decay force-field programming mentioned by Yau and Kirkland⁴. That form of the centrifugal force field is

$$G(t) = G_0 \quad \text{for } t \leq \chi \quad (5)$$

$$G(t) = G_0 \exp[-(t - \chi)/\tau] \quad \text{for } t > \chi \quad (6)$$

where G_0 is the initial sedimentation force field, τ is the exponential decay time constant and χ is an arbitrary delay time before decreasing the centrifugal field. In order to carry out the integration of eqn. 4, eqn. 3 can be used as the retention equation. The relationships between the retention time and the particle mass are as follows:

$$\text{for } t \leq \chi: \\ m = 3\phi t_R/t_0 \{1 + \sqrt{1 - [4t_0/(3t_R)]}\} \quad (7)$$

$$\text{for } t > \chi: \\ m = 3\phi\tau/t_0 \left\{ (\chi - \tau)/\tau + \right. \\ \left. + \exp[(t_R - \chi)/\tau] \left\{ 1 + \sqrt{1 - \frac{2t_0/3\{2\chi - \tau + \tau \exp[2(t_R - \chi)/\tau]\}}{\{(\chi - \tau) + \tau \exp[(t_R - \chi)/\tau]\}^2}} \right\} \right\} \quad (8)$$

If $\chi = \tau$, as in the DuPont SdFFF instrument, eqn. 8 is simplified⁴:

$$m = \frac{3\phi\tau}{t_0} \cdot \exp\left(\frac{t_R - \tau}{\tau}\right) \left(1 + \sqrt{1 - \frac{2t_0}{3\tau} \left\{ 1 + \exp\left[\frac{-2(t_R - \tau)}{\tau}\right]\right\}} \right) \quad (9)$$

where

$$\phi = kT/[G_0 w(\Delta\rho/\rho_s)] \quad (10)$$

$$m = \pi \rho_s D_p^3/6 \quad (11)$$

and ρ_s is the particle density. Eqns. 7 and 9 were used to calculate the equations for conversion from retention time to particle diameter in this paper.

EXPERIMENTAL

The DuPont SdFFF instrument was used, and the particle size was obtained by the modified calculation software based on the above theoretical consideration, which was reported previously⁷. The flow-rate during the analysis period was 2 ml/min. The

eluent was monitored with a normal UV detector at 254 nm. The delay/decay time constant, τ , was chosen to be 10 min when the instrument worked in the mode of the time-decayed exponentially decayed programming technique (TDE mode or TDE-SdFFF)^{4,5}. The non-equilibrium effects, which arise from slow mass transport between flow lines of different velocity, can be neglected if the delay/decay time constant is more than 10 min⁷.

In order to measure the particle diameter by QELS spectroscopy (Model N4MD instrument; Coulter Electronics), the liquids eluted from SdFFF were each fractionated into 4-ml volumes when the detector showed signals for particles. The mean particle size and the standard deviation for the fractionated samples were obtained by the unimodal calculation procedure with this spectroscopic method. The accuracy of this measurement should be very good because of the narrow size distribution of the particles in the sample.

AOT solution (10%) (Fisher Scientific, Fair Lawn, NJ, U.S.A.) was used to prepare the eluent. The water for dilution was prepared by distillation and passage through a Milli-Q system (Millipore).

RESULTS AND DISCUSSION

Polystyrene latex

The retention times of polystyrene latex beads (G0301; Japan Synthetic Rubber, Tokyo, Japan) were measured in the TDE mode with an initial centrifugal speed of 3000 rpm using 0.1% and 0.001% AOT solution as the eluent. The mean size was calculated as 269 nm from the retention time (28.3 min) at the peak absorbance of the fractogram of the result with 0.1% ($2.2 \cdot 10^{-5}$ mol/l) AOT solution. This value is in good agreement with the mean size of the fractionated sample, 268 nm, in QELS spectroscopic analysis, which was collected from the outlet of the SdFFF instrument during the retention time period between 27.5 and 29.5 min. This is in agreement with several literature reports¹⁰⁻¹³ that the results of size analysis by SdFFF with 0.1% AOT solution agreed well with those obtained by QELS spectroscopy or transmission electron microscopy.

However, the retention time for 0.001% ($2.2 \cdot 10^{-7}$ mol/l) AOT solution was 22.2 min. The dependence of the retention time behaviour on the AOT concentration in the eluent is the same as that reported by Hoshino *et al.*⁶. They explained this phenomenon by a change in the profile of the parabolic laminar flow in the channel and/or a change in the interaction between particles and the wall of the channel, and they suggested that the size obtained from FFF methods could be overestimated at high AOT concentrations in the eluent. However, our results with SdFFF and QELS spectroscopy indicated that particles in 0.001% AOT solution are eluted earlier than the time predicted by the classical theory.

The difference in AOT concentration causes not only a difference in the absorbed states of AOT on the particle surfaces and on the channel wall, but also differences in the ionic strength, because AOT is an anionic surfactant. Fractograms of latex (G0301) are shown in Fig. 1. The concentration of AOT in the eluent was 0.001% and sodium chloride was added in order to investigate the effect of the ionic strength of the eluent. When the sodium chloride concentration was 0.1 mol/l, the detector did not record a particle signal, which suggested particle agglomeration in the channel. Fig. 1a

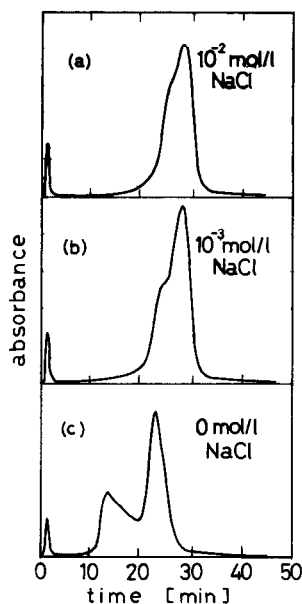


Fig. 1. Effect of the concentration of electrolyte added to 0.001% AOT solution on fractograms of polystyrene latex. TDE mode; initial centrifuge speed = 3000 rpm; delay/decay time constant = 10 min; sample concentration = 0.1 wt.-%; flow-rate = 2 ml/min.

is for 0.001% AOT and 0.01 mol/l sodium chloride. The fractogram was same as that when 0.1% AOT solution was used as the eluent without any sodium chloride added. When the concentration of sodium chloride was decreased further, the retention time of the peak absorbance became smaller, indicating a smaller particle size. The shoulder on the left-hand side of the fractogram was more pronounced at 10^{-3} than at 10^{-2} mol/l sodium chloride, and finally a double peak signal of the fractogram was obtained, as shown in Fig. 1c, when 0.001% AOT solution without sodium chloride was used as the eluent. The retention times of the peak absorbance in Fig. 1a, b and c correspond to particle sizes of 265, 262 and 220 nm, respectively. However, fractions collected at the times represented by the above peaks corresponded to particle sizes of 266, 267 and 275 nm, respectively, in QELS spectroscopic analysis.

We conclude that the difference between the sizes of the particles in SdFFF analysis shown in Fig. 1a–c is caused by the effect of the ionic strength of the eluent. With the eluent containing 0.1 mol/l sodium chloride, rapid flocculation among the particles might have occurred owing to the high concentration of electrolyte. On the other hand, the results which gave a smaller size in SdFFF analysis at low electrolyte concentration are probably due to interparticle electrostatic repulsion as a result of the long-distance effect of the electric double layer of the particles, together with the high local solid concentration. This, in turn, moves the particles further from the wall and thus results in a decrease in the retention time in SdFFF. However, interparticle forces are not experienced in QELS spectroscopic analysis, as the solid concentration is much lower.

The reason is not clear why the double peaks on the fractogram appear when

TABLE I

RETENTION TIMES OF COLLOIDAL SILICA IN VARIOUS AEROSOL OT ELUENTS

Experimental conditions: TDE mode; initial centrifuge speed = 10 000 rpm; delay/decay time constant = 10 min; sample concentration = 25% (w/w); flow-rate = 2 ml/min.

Parameter	Run No.										
	SYTON-14	SYTON-33	SYTON-10	SYTON-11	SYTON-13	SYTON-25	SYTON-27	SYTON-26			
AOT (mol/l)	$2.2 \cdot 10^{-5}$	$2.2 \cdot 10^{-5}$	$2.2 \cdot 10^{-5}$	$2.2 \cdot 10^{-5}$	$2.2 \cdot 10^{-5}$	$2.2 \cdot 10^{-7}$	$2.2 \cdot 10^{-7}$	$2.2 \cdot 10^{-7}$			$2.2 \cdot 10^{-7}$
HCl (mol/l)	$9.9 \cdot 10^{-3}$	0	0	0	0	$1.0 \cdot 10^{-2}$	0	0			0
NaOH (mol/l)	0	0	0	$2.6 \cdot 10^{-4}$	$6.0 \cdot 10^{-4}$	0	0	0			0
NaCl (mol/l)	0	$1.0 \cdot 10^{-2}$	0	0	0	0	0	$1.0 \cdot 10^{-2}$			0
pH	2.0	4.0	4.0	6.0	9.7	2.0	5.5	5.7			
<i>I</i> (mol/l) ^a	$9.9 \cdot 10^{-3}$	$1.0 \cdot 10^{-2}$	$1.0 \cdot 10^{-4}$	$2.6 \cdot 10^{-4}$	$6.0 \cdot 10^{-4}$	$1.0 \cdot 10^{-2}$	$1.0 \cdot 10^{-2}$	$1.0 \cdot 10^{-2}$			$2.0 \cdot 10^{-6}$
1/ <i>k</i> (nm) ^b	3.1	3.1	31	19	12	3.1	3.1	215			
Retention time (min):											
1st peak	49	48	46	45	46	48	48	27			
2nd peak	55	54	52	52	52	54	54	33			
3rd peak	60	59	57	57	57	59	59	38			
Particle size (nm):											
1st peak	83.4	80.6	75.4	73.0	75.4	80.6	80.6	40.0			
2nd peak	102	92.2	92.2	92.2	92.2	98.5	98.5	48.9			
3rd peak	120	116	109	109	109	116	116	57.8			

^a *I* = Ionic strength of the eluent.

^b 1/*k* = Thickness of electric double layer of particle.

0.001% AOT solution without sodium chloride was used as the eluent, as shown in Fig. 1c. The particle size corresponding to the first peak in SdFFF analysis is 160 nm. In contrast, a mean size of 290 nm was obtained by QELS spectroscopy using the fractionated sample from SdFFF at the retention time of the first peak absorbance. The size indicated by QELS spectroscopy, 290 nm, is larger than the values obtained above. The large size for the first peak in QELS spectroscopy together with its smaller SdFFF retention time may be due to the lower density of this material and/or stronger repulsion forces. We require further information on the behaviour of the particle and the channel wall in different eluents if this phenomenon is to be clarified.

The relationship between the retention time of the latexes and the ionic strength of the eluent is shown in Fig. 2. These experiments were carried out with a five-component mixture of standard latex for the eluents consisting of 0.1% or 0.001% AOT solution with or without 0.01 mol/l sodium chloride. The sizes calculated from the retention times at 0.01 mol/l ionic strength, which means that the eluent with 0.01 mol/l sodium chloride added was used, were 74, 110, 180, 300 and 550 nm. The results obtained by QELS spectroscopy indicated 75, 111, 170, 296 and 598 nm, respectively. It is concluded that the size calculated from the retention time in SdFFF analysis may underestimate the true value owing to electrostatic repulsion among the particles if the ionic strength is not adjusted.

Colloidal silica

For SdFFF analysis of inorganic materials such as silica with a smaller size and a broad distribution, it is important to establish the effect of surface charge of the particles, and also the effect of the eluent composition. Both effects will appear strongly because a higher sample concentration is needed owing to the low sensitivity of the detector for smaller particles. Generally, the surface charge of inorganic particles is described in terms of zeta potential or sol stability, which are known to be strongly dependent on pH. The isoelectric point (zero point of charge) of colloidal silica is at *ca.* pH 2. At higher pH the surface charge becomes increasingly negative, until at *ca.* pH 10 dissolution occurs. SdFFF results used colloidal silica (Syton W50; Monsanto, London, U.K.) appear to be little influenced by the pH of the eluent consisting of 0.1% AOT, as indicated in Table I. The ionic strength in the eluent was calculated from the concentrations of AOT, hydrochloric acid, sodium hydroxide and

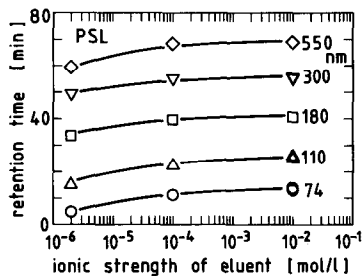


Fig. 2. Relationships between retention time and ionic strength of eluent, I , for a five-component mixture of standard latexes in TDE-SdFFF analysis. Initial centrifuge speed = 10 000 rpm; time delay/decay constant = 10 min; flow-rate = 2 ml/min. $I = 10^{-6}$ mol/l, 0.001% AOT solution; $I = 10^{-4}$ mol/l, 0.1% AOT solution; $I = 10^{-2}$ mol/l, 0.01 mol/l NaCl added to 0.1% or 0.001% AOT solution.

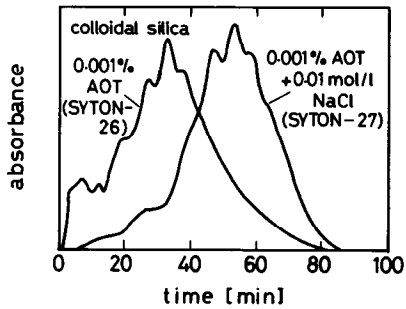


Fig. 3. Fractograms of colloidal silica in TDE-SdFFF analysis. Experimental conditions as in Table I.

sodium chloride and from the measured pH value, and then the thickness of the electric double layer of the particle, *i.e.*, the reciprocal of the Debye-Hückel parameter, was calculated (Table I). The retention time could be correlated with the ionic strength and the thickness of the electric double layer.

Run number SYTON-26, which was performed at low AOT concentration, shows a strong decrease in the retention time. When sodium chloride was added to this eluent (SYTON-27), the SdFFF retention was brought back to normal, as shown in Fig. 3 and Table I. Also, the SdFFF retention is normal at low pH near the isoelectric point (SYTON-25). Moreover, the fractions collected at the first and the third peak absorbances in these three runs show about the same mean sizes in QELS

TABLE II

RETENTION TIMES OF COLLOIDAL SILICA IN VARIOUS SURFACTANT ELUENTS

Experimental conditions as in Table I.

Parameter	Run No.					
	SYTON-23	SYTON-20	SYTON-22	SYTON-21	SYTON-32	SYTON-30
HMP ^a (mol/l)	$3.3 \cdot 10^{-3}$	$3.3 \cdot 10^{-3}$	0	0	0	0
SPP ^a (mol/l)	0	0	$2.2 \cdot 10^{-3}$	$2.2 \cdot 10^{-3}$	$2.2 \cdot 10^{-5}$	$2.2 \cdot 10^{-5}$
HCl (mol/l)	$1.0 \cdot 10^{-2}$	0	$1.0 \cdot 10^{-2}$	0	0	0
NaCl (mol/l)	0	0	0	0	$1.0 \cdot 10^{-2}$	0
pH	2.0	6.0	2.0	9.0	7.0	7.0
Retention time (min):						
1st peak	50	49	49	48	47	34
2nd peak	56	55	55	54	53	41
3rd peak	61	60	60	59	59	46
Particle size (nm):						
1st peak	86.2	83.4	83.4	80.6	78.0	50.6
2nd peak	105	102	102	98.5	95.3	63.9
3rd peak	124	120	120	116	116	75.4

^a HMP = Sodium hexametaphosphate; SPP = sodium pyrophosphate.

spectroscopic analysis, *i.e.*, 90 and 120 nm, respectively. These results indicate that the retention time depends on the ionic strength of the eluent. On the other hand, the surface charge effects of particles, that is, the effect of the pH of the eluent, may not be so significant, as shown by the same ionic strength data in Table I.

Table II shows the effect of the anionic surfactant in the eluent. Although the ionic strength could not be calculated exactly because of the lack of dissociation constants of the two surfactants, the ionic strengths in the experiments listed in Table II except run SYTON-30 are $\geq 10^{-2}$ mol/l. For these eluents hardly any difference in retention time was found. The ionic strength in run SYTON-30 is calculated to be of the order of 10^{-5} mol/l. The retention time in this instance is much shorter than in other instances. This fact may also indicate the importance of the ionic strength of the eluent.

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