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EFFECTS OF DETERGENT ON THE ELUTION PROFILES OF LATEX BEADS IN SEDIMENTATION FIELD-FLOW FRACTIONATION

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SUMMARY

An unexpected prolongation of retention was observed in an experiment on the retention behaviour of latex beads in constant field sedimentation field-flow fractionation (SFFF). The prolongation depended on the type and concentration of detergent used in the solvent stream, and also on the size of the solute particulates. These facts suggested that retention in SFFF strongly depends on the state of the interface between the column surface and the solvent stream. On application of Stern's electrical double-layer diffusion model to the state of the surface border of the column wall, the prolongation of retention behaviour was well explained. As a result, an additional modification to the SFFF retention equation can be made, and the empirical equation, $1/R = Cd_p^3 + Dd_p^6 + E$, is proposed, where R is the retention ratio, C , D and E are functional parameters determined by the type and concentration of detergents used in the solvent stream and d_p is the particle diameter.

INTRODUCTION

Field-flow fractionation (FFF) is a unique method for separation analysis which is capable of high-resolution separation of particulates and large macromolecules. Among the various FFF subtechniques, sedimentation field-flow fractionation (SFFF) is the most highly developed. Its usefulness for characterizing the size distributions of a wide range of particulates has been clearly established¹⁻⁹. The latest SFFF equipment permits the handling of particulates in the range of 0.005-100 μm^{10-16} .

In SFFF, sample particulates or macromolecules are introduced into a belt-shaped open channel which is suspended and rotated in a centrifuge, and are moved down the channel by the solvent stream. Particles that have an effective mass greater than that of the solvent streams are forced towards the outer wall and establish a specific layer thickness. This layer is of a different thickness for each distinct chemical or particulate species, and depends on the physical basis of the interaction between the force field and species and on the diffusion coefficient of each species.

The solvent stream which moves the solute particulates downstream flows along the channel axis according to a parabolic lamina flow. The flow velocity is greatest near the center of the channel. Thus the smaller particles which are carried along by the faster flowing regions will be eluted from the channel first, while the larger particulates forced into the relatively quiescent flow regions near the channel wall will be significantly retained. This is the basis for selective retention.

In an exceptional case, when the sample particles establish a layer on the wall and the effective radius of each particle is large enough to occupy a significant proportion of the channel thickness, the larger particles are eluted from the channel first. This is called steric SFFF, and particulates in the range of 1–100 μm are handled by steric SFFF^{3,15,16}.

The basic concept in SFFF is that retention of particulates is a direct function of particle mass¹. A discrepancy between the nominal size value and those derived from SFFF has been reported⁹. The manufacturer's values for the "0.220 and 0.312 μm " standards closely correspond with SFFF values, whereas the manufacturer's value for the "0.481 μm " sample is somewhat smaller than that found by a variety of SFFF measurements. In another paper², the electron microscopic value for a "0.44 μm " sample was reported to be smaller than that of SFFF value. Unexpected prolongation of the retention was also observed in our experiments on latex beads in SFFF. This behaviour was found to depend on the type and the concentration of detergent used in the solvent stream and on the mass of the solute particulates.

These discrepancies suggest that SFFF measurements may overestimate the size of the larger particulates. The retention behaviour of solute particles in SFFF would easily be affected by the state of the inner surface border of the channel wall. It is reasonable that detergents will affect the surface interaction and change the equilibration dynamics of solute particulates or the shape of the parabolic lamina flow. However, no parameter that could reflect the effects of detergent is included in the basic SFFF retention concept of Giddings *et al.*¹. To explain the unexpectedly large prolongation of retention caused by the detergent, we tried to apply Stern's diffusion model¹⁷ to the surface border of the accumulation wall to which the solute particles are forced by an external force-field.

In this paper we report the effects of detergents on retention behaviour of solute particles in SFFF.

THEORETICAL

The distribution of the solute layer across the channel in SFFF is expressed as

$$C = C_0 e^{-x/l} \quad (1)$$

where C is the solute concentration at a distance x from the wall, C_0 that at the wall and l is the mean thickness of the solute layer. The dimensionless mean layer thickness, λ , is

$$\lambda = l/W = D/UW \quad (2)$$

where W is the gap thickness of the channel, D the solute diffusion coefficient and U the mean lateral drift velocity. When $V_R/V_0 \gg 2$, the retention ratio, R , of the solute is given by

$$1/R = V_R/V_0 = W/6l = (\pi/36kT) (p_s - p_m)GWd_p^3 \quad (3)$$

where V_R is the retention volume of the eluted peak, V_0 the column void volume, p_s the density of the solute, p_m that of the solvent stream, G is the sedimentation force field, d_p the particle diameter, T the absolute temperature and k is Boltzmann's constant.

EXPERIMENTAL

The most important part of the SFFF equipment is the column assembly which is an instrumental limiting factor on the results. Our column assembly was designed to be mounted directly on a bottom-drive centrifuge and consists of two elements, a rotating column and a spinning sealing-joint. They are connected to each other by a newly designed universal joint fitted with magnetic couplings. The rotating column is made of stainless steel (SUS-304) and polyimide, and consists of two ring-shaped holders, a spacer and two disk-shaped mountings. The column was designed to enable the positioning of a surface strip on the accumulation wall of the ring-shaped outer holder^{18,19}. Its dimensions are 0.150 mm wide, 2.50 cm high and 62.8 cm long. The channel surface is highly polished and finished by lapping with 0.25- μm diamond powder (Minitoror, Tokyo, Japan). The sealing-point consists of a fixed and a spinning unit connected face to face and sealed with a replaceable sealing piece mounted between the faces²⁰⁻²².

The set-up is the same as used in our previous report²³. The following pieces of equipment were used, connected with 0.25-mm PTFE tubes: a solvent-delivery pump Model 6000A (Waters Associates, Milford, MA, U.S.A.); a proprietary four-way solvent-line exchanger; a sample injector Model 7125 (Rheodyne, CA, U.S.A.); the column assembly mentioned above and an UV detector Model 441 (Waters Associates). The centrifuge was a bottom-drive Type H-60S (Kokusan Co., Tokyo, Japan). A personal computer PC-9801 VM (NEC Co., Tokyo, Japan) and a electric power supply GP-035-15 (Takasago Co., Kawasaki, Japan) were used to monitor and control the spinning rate of the centrifuge.

The solvent stream was distilled water containing various concentrations of detergent and was prepared more than 24 h in advance of the experiment. The detergents used were Emulgen-PP150 (Kao Soap Co., Tokyo, Japan) and Aerosol-OT (Tokyo Chemical Industry, Tokyo, Japan). The sample solutes used were polystyrene latex beads (Dow Chemical, Midland, MI, U.S.A.), 0.232 and 0.312 μm in diameter, and poly(vinyltoluene) latex beads, 0.399 μm (Dow Chemical).

The spin rate of the centrifuge was kept constant at 1600 rpm using a personal computer both to monitor the rate and to control the electric power supply. The temperature of the spinning chamber was kept at 30°C and monitored. The sample size introduced was 2-4 μl of 0.1% of the latex beads. The relaxation time was 6 min. The flow-rate was 1.0 ml/min and the eluent was monitored at 254 nm.

RESULTS AND DISCUSSION

Effects of detergents on retention

Fig. 1 shows fractograms of latex beads in constant field SFFF. The concen-

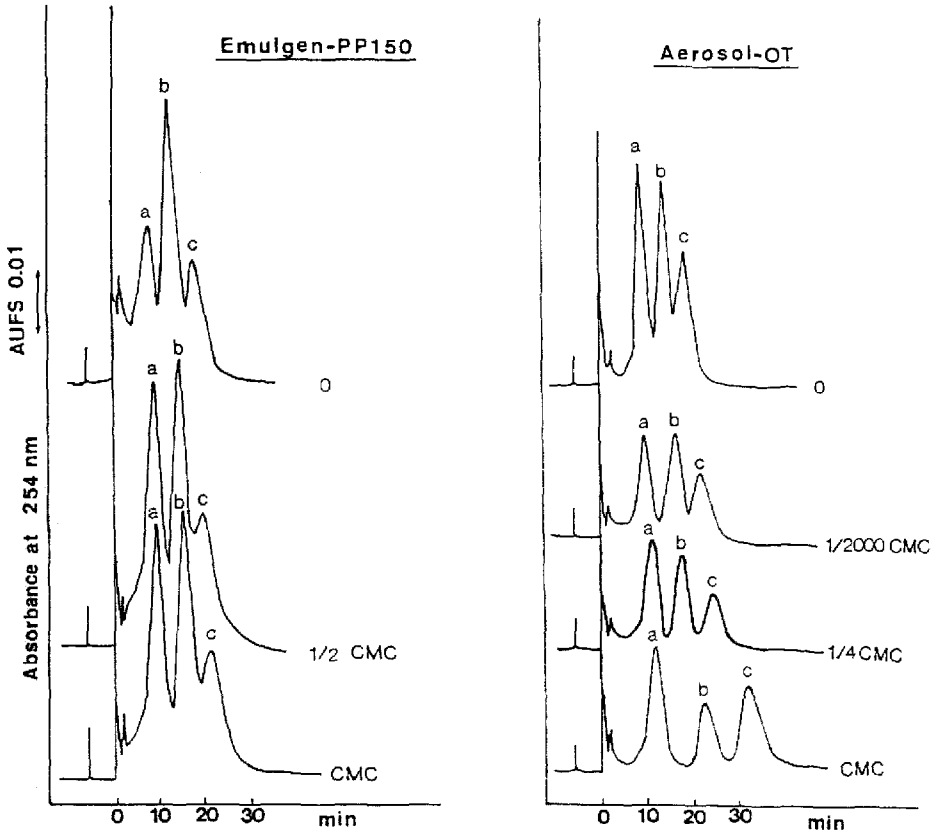


Fig. 1. Fractograms of latex beads on a stainless-steel column. Channel: stainless-steel surface (SUS-304); gap = 150 μm , width = 25 mm; length = 628 mm; radius = 100 mm. Eluting conditions: relaxation time = 6 min; flow-rate = 1.0 ml/min; spin rate = 1600 rpm. Eluents: Emulgen-PP150 and Aerosol-OT. Solutes: a = polystyrene latex, 0.232 μm ; b = poly(vinyltoluene), 0.399 μm ; c = polystyrene latex, 0.312 μm .

tration of detergent in the solvent stream was varied, other eluting conditions being constant, *i.e.*, the solvent stream flow-rate was 1.0 ml/min, the relaxation time was 6 min and the spin rate was 1600 rpm. The left-hand part of Fig. 1 shows that various concentrations of Emulgen-PP150 have little effect on the retention behaviour of latex beads, whereas the right-hand part shows that Aerosol-OT induces a fairly large prolongation of the retention depending on the concentration. These findings suggest that latex beads were variously retained depending on the type of detergent and the concentration. A useful check on the effect of detergent on retention can be made by means of a plot of $1/R$ versus the detergent concentration.

In Fig. 2 plots of $1/R$ versus the critical micelle concentration (CMC) of detergents are shown. The nominal diameter of poly(vinyltoluene) beads is 0.399 μm . As they are less dense than polystyrene latex beads, for the purposes of this discussion the diameter has been corrected to 0.281 μm , the same as for polystyrene latex beads. It is found that $1/R$ increases little with increasing concentration Emulgen-PP150.

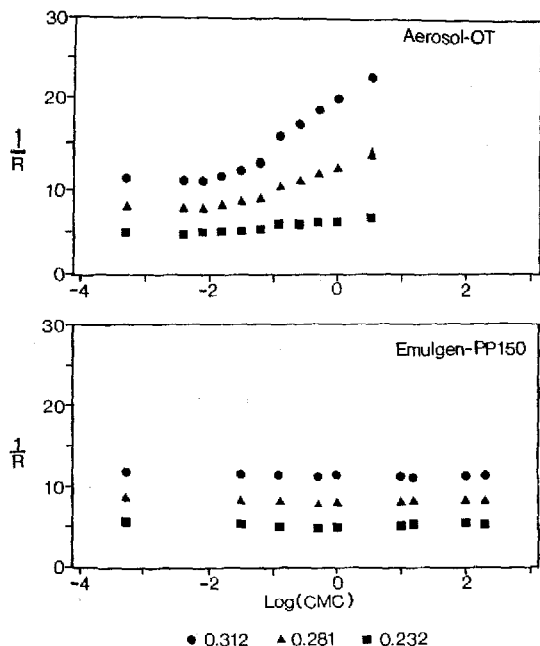


Fig. 2. Plots of $1/R$ versus detergent concentration in the mobile phase obtained from the fractogram under the same conditions as in Fig. 1. The nominal diameter of the poly(vinyltoluene) beads, $0.399 \mu\text{m}$, was corrected to that of polystyrene latex beads, $0.281 \mu\text{m}$.

whereas with Aerosol-OT, $1/R$ values increases markedly at concentrations greater than $\text{CMC}/64$ ($10^{-5} M$). This shows clearly that the retention of latex beads in SFFF depends on the concentration of Aerosol-OT in the solvent stream.

Fig. 3 shows plots of $1/R$ versus particle volume, d_p^3 , for the respective concentrations of each detergent. According to eqn. 3, such plots should be linear with a slope determined by the particle mass, d_p^3 . The points for Emulgen-PP150 do fall on a straight line. However, the slopes of the empirical lines for Aerosol-OT are inconsistent with the theory. The increase of retention caused by Aerosol-OT depends not only on the detergent concentration but also on the particle mass.

Experimental retention equation

It is clear that, depending on the concentration or the type of detergent in an aqueous solvent stream, fairly large discrepancies are found between the experimental retention values and those predicted. Aerosol-OT, an anionic surfactant, prolongs the retention of latex beads, more noticeably for larger beads and at higher detergent concentrations. This means that the condition of the channel surface, the shape of the parabolic lamina flow and the equilibration dynamics of solute beads will be dependent on the concentration and type of detergent.

To account for these experimental observations, regression analysis was performed on the data points shown in Fig. 2 using an empirical equation, $1/R = Ad_p^3 + B$, where A is the coefficient of first term in eqn. 3 and B is a constant that expresses the experimental error. Fig. 4 shows plots of the coefficient A and constant

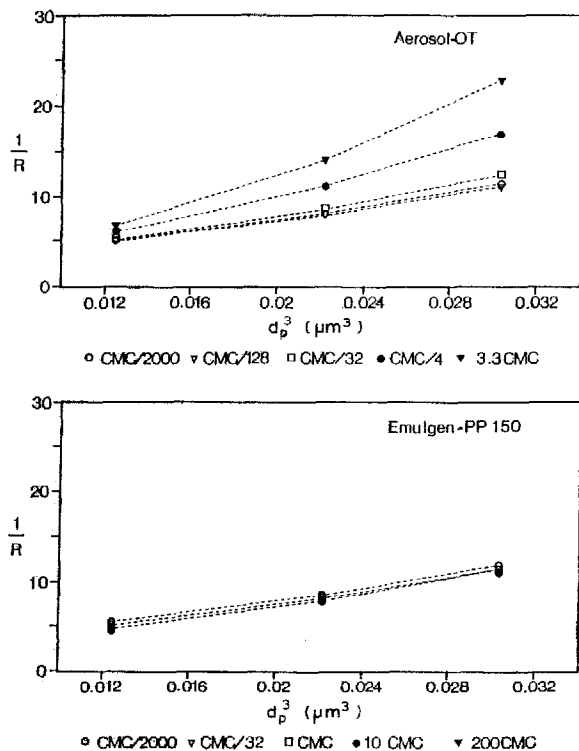


Fig. 3. Plots of $1/R$ versus mass (d_p^3) of latex beads. Conditions as in Fig. 2.

B versus the CMC concentration for Emulgen-PP150 and Aerosol-OT, respectively. The data for Emulgen-PP150 show little dependence on the concentration, and are roughly in accord with the theory. However, parameters A and B for Aerosol-OT showed larger deviations depending on the concentration, and the magnitude of the deviations was quite inconsistent with those calculated according to Giddings. Eqn. 3 cannot explain the prolongation of retention which is caused by the difference in the type of detergent, the strength of the detergent and the size of the solute particle.

It may well be that the different findings for Emulgen-PP150 and Aerosol-OT correspond to the variety of states at the accumulation surface caused by the detergents. Two possible explanations for these different findings. One is the presence of friction between the channel surface and the solvent stream which causes parabolic lamina flow between the two parallel plates of the channel. As is well known, a detergent changes the surface tension of a solvent, in other words, it changes the energy level of the solvent surface. It can be readily assumed that as the "wetting" phenomenon increases, the profile of parabolic lamina flow becomes sharp with a constant flow-rate. The second possibility is adsorption of solute particles on the Stern layer formed on the channel surface which depends on the type and concentration of surfactant in the solvent stream. The force field that induces the adsorption of solute particles will affect the final formation of solute layer together with the external force field caused by the centrifugal force.

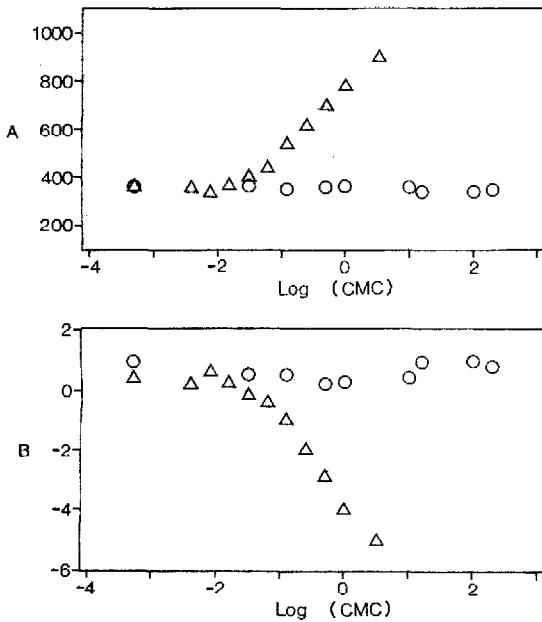


Fig. 4. Plots the parameters *A* and *B* versus detergent concentration. The parameters were calculated from the plots in Fig. 2 by regression analysis. Δ = Aerosol-OT; \circ = Emulgen-PP150.

The adsorption of the solute on the Stern layer should depend on the ratio of the solute concentration at the Stern layer to the volume of the solute cloud. The effective retention volume in SFFF, V_e , might be expressed as

$$V_e = \varphi_0 V_R [1 + \varphi_1 (C_0 e^{-a/l}) / (\int_0^w C_0 e^{-x/l} dx)] \tag{4}$$

where a is the distance of the Stern layer from the accumulation wall, and φ_n is a parameter expressed as a function of the physicochemical properties of the surfactant. When $l/W \ll 1$, $a/l \ll 1$, eqn. 4 becomes:

$$V_e = \varphi_0 V_R (1 + \varphi_1/l) \tag{5}$$

Considering eqn. 3, the effective retention ratio, $R' = V_0/V_e$, can be simply expressed as

$$1/R' = Cd_p^3 + Dd_p^6 \tag{6}$$

where C and D are functions of the physicochemical properties of the detergent and of the size of the solute particles. This equation is different from eqn. 3. Thus regression analysis was performed on the data points shown in Fig. 2 by use of the new empirical equation derived from eqn. 6, $1/R = Cd_p^3 + Dd_p^6 + E$, where E is a constant representing the experimental error.

Fig. 5 shows plots of C , D and E for Emulgen-PP150 and Aerosol-OT. Parameter D for Aerosol-OT showed a strong dependence on the detergent concentration,

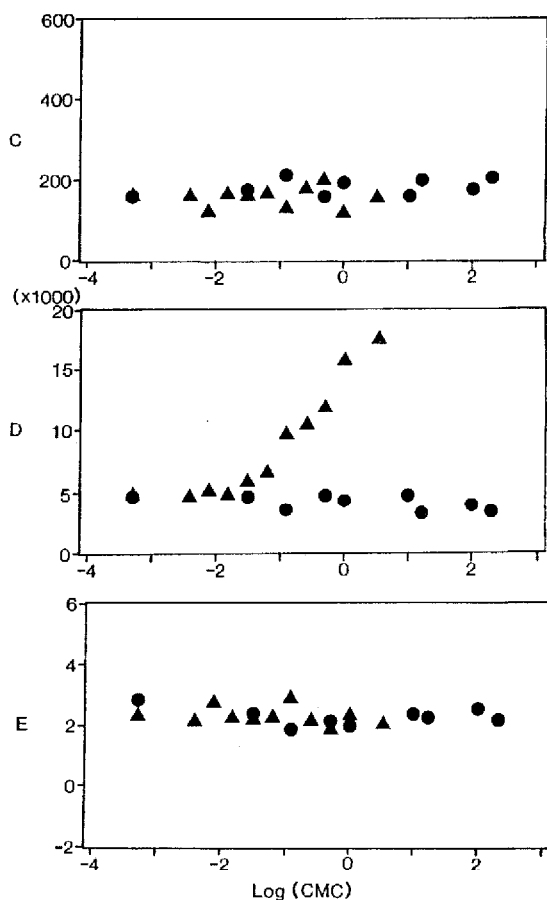


Fig. 5. Plots of the parameters C , D and E versus detergent concentration. The parameters were calculated from the plots in Fig. 2 by regression analysis. \blacktriangle = Aerosol-OT; \bullet = Emulgen-PP150.

i.e., the ability of Aerosol-OT to retain latex beads is strongly dependent on its concentration. As for parameter C , a similar dependence on the concentration was shown in Aerosol-OT and Emulgen-PP150. Most probably, C is related to the surface tension between the channel surfaces and the solvent stream, and D to the interaction between the surfaces of the channel and the type of solute.

Using the two parameters based on the shape of the parabolic flow and the Stern layer model, it is possible to explain that the particles were retained more strongly with increasing detergent concentration, and that larger particles tend to be retained more strongly in SFFF at higher concentrations of ionic detergents. Several papers^{2,9} here reported a discrepancy in particle size between the nominal size and the SFFF value as obtained 0.1% anionic detergent in the solvent stream. Considering our results, it is possible that the SFFF values are overestimates.

A modification of Giddings' theory is proposed. In our preliminary experiment on columns with several kinds of channel surface, a concentration dependences of retention was observed. Further study is required to confirm this, but it is worthwhile being aware of these effects of detergents on retention in all of other FFF methods.

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