HIGH-SPEED MICRO-THERMAL FOCUSING FIELD-FLOW FRACTIONATION OF MICRON-SIZE PARTICLES

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Micron-size polystyrene-based latex particles were separated by using new micro-thermal field-flow fractionation (micro-TFFF). The order of retention from the largest to the smallest particles that appears at high field strength and high flow rate corresponds to the focusing mechanism which itself is a consequence of the lift forces acting on the particles. The mechanism of steric exclusion can only be effective at low flow rates of the carrier liquid. Whenever high-speed separation was performed, the focusing effect clearly dominated the FFF mechanism. This application of micro-TFFF in focusing mode to the separation of the particles is the first one published. As a result, micro-TFFF thus became a very universal technique for the separation of synthetic and natural macromolecules and of particles of various origin and size up to large (micron-size) diameter.

Keywords: Micro-thermal focusing field-flow fractionation; Separation of micron-size particles; Polymers; Macromolecules; Colloids; Polystyrene latex particles.

Micro-thermal field-flow fractionation (micro-TFFF) is a new technique proposed recently[1] and applied to separations of polymers and colloidal particles (see ref.[2] for review). The mechanism of separation in normal (polarization) micro-TFFF is based on the migration of the retained species due to temperature drop acting across the channel. Each retained species forms a quasi-steady state, nearly exponential concentration profile across the channel as a result of a balance between thermophoresis and the opposed diffusion flux. The carrier liquid flowing along the channel forms a nearly parabolic flow velocity profile across the channel. Larger species exhibiting lower diffusion coefficients are usually compressed closer to the accumulation wall in a zone of lower longitudinal velocity of the carrier liquid.

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As a result, the elution order is from the small- to the large-size species. If the field strength is high enough, the distance of the center of gravity of the concentration distribution of a retained species from the accumulation wall is commensurable with its size, the steric exclusion mechanism governs the separation, and the elution order is inverted. This mechanism was proposed by Giddings and Myers[3] and the method was called steric FFF. The other applications of steric FFF concerned exclusively sedimentation/steric FFF and flow/steric FFF (see references[4,5] for review).

Koch and Giddings[6] described a spectacular separation by sedimentation/steric FFF of a mixture of seven different polystyrene latex beads in the size range from 2 to 45 μm in the experiments performed at high field strengths and high flow rates in less than 4 min. As a matter of fact, under such experimental conditions, lift forces (observed experimentally earlier[7]) actively contribute to the separation, they play an active role of a focusing force, and the mechanism of separation is fully coherent with the model of focusing FFF originally proposed by Janča[8] and later by Giddings[9]. Ratanathanawongs and Giddings[10] described a conscious use of the focusing mechanism in flow FFF for high-speed separation of silica particles based on the action of the lift forces generating the focusing phenomena. Obviously, "steric" FFF represents rather an exceptional case of the separation mechanism because either various attractive forces between the retained species and the accumulation wall in a close proximity of the wall or the focusing lift force play a substantial role. In other words, whenever lift forces participate in FFF processes, the focusing mechanism operates and it is not the steric exclusion mechanism that accurately describes the retention in FFF. This preliminary communication demonstrates the first experimental results obtained by high-speed micro-thermal focusing FFF when separating large-size polymer latex particles.

THEORY

If the steric exclusion mechanism dominates the separation in FFF, the retention ratio is[11]:

$$R = 6\alpha(1 - \alpha) , \qquad (1)$$

where $\alpha = r/w$ is the ratio of the radius r of the separated species to the thickness w of the separation channel. For small α values, Eq. (*1*) reduces to a very simple relationship:

$$\lim_{\alpha \to 0} R = 6\gamma\alpha \,, \qquad (2)$$

where γ is a dimensionless factor accounting for some non-idealities, for example for the frictional drag, but it can also account for the intervention of the attractive or focusing lift forces. Correspondingly, γ can be higher or lower than unity. Equations (*1*) and (*2*) are rigorously valid only if the flow velocity profile formed inside the channel is parabolic. This is not the case of micro-TFFF because the viscosity varies with the temperature across the channel and the flow velocity profile is not parabolic. Nevertheless, the use of the approximate Eq. (*1*) is justified for a simple comparison of the theoretical dependence of the retention ratio *R* on the size *r* of the separated species with the experimental retention data and thus for a demonstration that the focusing mechanism was dominating in our experiments and that the mechanism of the purely steric exclusion is rather a very exceptional case.

On the other hand, the shape of the flow velocity profile which takes into account the variation of the viscosity with temperature can be calculated by using an approximate but simple third-degree polynomial relationship published by Belgaied *et al.*[12] based on previous proposal[13]:

$$\frac{v(x/w)}{\langle v(x/w)\rangle} = 6\left[(1+\kappa)(x/w) - (1+3\kappa)(x/w)^2 + 2\kappa(x/w)^3\right], \qquad (3)$$

where κ is a constant whose value is determined by the properties of the carrier liquid, the cold-wall temperature and the temperature drop ΔT, $v(x/w)$ is the velocity distribution across the channel, and $\langle v(x/w)\rangle$ is the average linear velocity of the carrier liquid inside the channel. A comparison of the isoviscous, parabolic flow velocity profile with a velocity profile calculated from Eq. (*3*) for model non-isothermal conditions is shown in Fig. 1. This figure will be used in the following discussion of our experimental findings to confirm rigorously that the steric exclusion mechanism can be effective only under very singular conditions and that it is the focusing mechanism that dominates the separations of large-size particles at high flow rates and high field strengths.

EXPERIMENTAL

The apparatus for micro-TFFF consisted of an intelligent pump model PU-980 (Jasco, Japan), an injection valve model 7410 (Rheodyne, U.S.A.) with a 1-μl loop, a UV-VIS variable wave-

length spectrophotometric detector model UV-975 (Jasco, Japan) equipped with a 1-μl cell, and an integrator Model HP 3395 (Hewlett–Packard, U.S.A.). The versatile micro-TFFF channel was designed in our laboratory and fabricated by Lascialfari, SARL (La Rochelle, France). The dimensions of the microchannel used in this work were 0.1 × 4 × 96 mm. The cold-wall temperature was controlled and kept constant by using a compact, low-temperature thermostat Model RML 6 B (Lauda, Germany). The electric power for heating cartridge was regulated with an electronic device designed and built up in our laboratory. The temperatures of the cold and hot walls were measured with a digital thermometer (Hanna Instruments, Portugal) equipped with two thermocouples. An aqueous solution of 0.1% of detergent Brij 78 (Fluka, Germany) and of 0.02% of NaCl was used as the carrier liquid.

Spherical carboxylated polystyrene latex particles (PS) were used in this study[14]. All latex samples were prepared by polymerization of styrene using 4,4′-azobis(4-cyanopentanoic) acid as initiator (0.2 wt.%) at the temperature 353 ± 1 K. In order to prepare PS particles of diameters over 1 μm, dispersion polymerization of styrene was carried out in the presence of poly(vinylpyrrolidone) as a polymer stabilizer in an ethanol/water mixture (93 vol.%) or in 100% ethanol[15]. After 4–7 h, a conversion of about 99% was reached. The latexes were washed by successive centrifugation and redispersion in water (at least three times). Finally,

TABLE I
Particle sizes of polystyrene latexes

Polystyrene latex	Average diameter by TEM, nm	Average diameter by QELS, nm
PS1	1000	1045
PS2	2300	2300
PS3	3800	3769

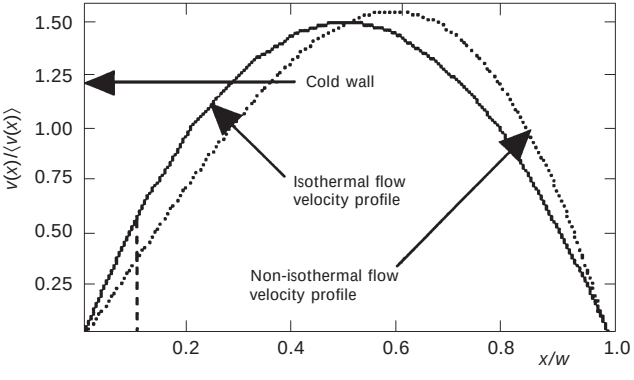

FIG. 1
Flow velocity profiles formed under isoviscous (isothermal) and non-isoviscous (non-isothermal) conditions calculated from Eq. (*3*): non-isoviscous (·····) and isoviscous (——) flow velocity profiles

the samples were treated by ultrasound and kept in 10% water suspensions. The average particle sizes measured by quasi-elastic light scattering (QELS) and transmission electron microscopy (TEM) are given in Table I.

The average particle diameters of all studied particles were measured by QELS using a Zetamaster (Malvern Instruments Ltd., Malvern, Worcestershire, U.K.) apparatus, and by TEM using a JEM 100 S microscope (Jeol, Japan). The analysis of micrographs of more than 100 particles of each sample gave their mean (by weight) diameter. The polydispersity index of all latex samples was lower than 1.02, indicating highly uniform particles.

RESULTS AND DISCUSSION

The results of separation of a mixture of three different-size polystyrene latex particles, namely 1000, 2300, and 3800 nm, obtained by micro-TFFF at three different flow rates, 0.1, 0.5, and 1.0 ml/min, and at the temperature drop ΔT = 50 K are shown in Fig. 2. In all three cases of different flow rates, an optimized injection-stop-flow procedure was applied. It consisted of the injection of a sample at a very low flow rate (0.01 ml/min) during 1.5 min

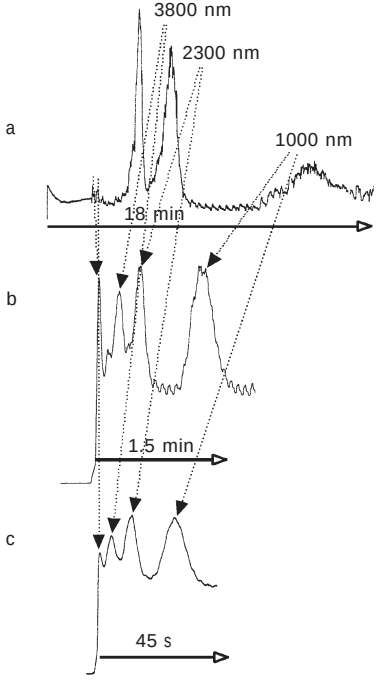

FIG. 2

Fractograms of a mixture of three PS latex samples obtained under various experimental conditions: flow rate 0.01 ml/min during the 1.5-min injection period, stop-flow time 1 min, ΔT = 50 K, T_c = 299–305 K; flow rate (ml/min): 0.1 (a), 0.5 (b), 1.0 (c)

followed by a 1-min stop-flow period for relaxation. A low flow rate applied during the injection allows to minimize the band broadening at the beginning of separation and the stop-flow time allows to establish a quasi-steady-state concentration distribution of the retained species across the channel and thus to minimize the band broadening caused by relaxation processes. It can clearly be seen in Fig. 2 that a good separation of three polystyrene latex particles was obtained at each flow rate. A significant increase in flow rate (by a factor of 10) does not result in a substantial decrease in resolution. This can be explained by the fact that the focused zones are very narrow in agreement with the theory of focusing phenomenon[16] and thus the band broadening due to the mass transfer in the direction across the channel between the streamlines of different longitudinal velocities is low. Consequently, high-resolution micro-thermal focusing FFF can be achieved even at very high flow rates. The time of the micro-thermal focusing FFF run was thus as short as 3 min including the time of the injection-stop-flow procedure while the separation alone took only 45 s. Nevertheless, our investigation continues in this direction with the goal not only to optimize the operational parameters but to justify such an optimization theoretically.

Figure 3 shows the plot of the experimental retention ratios *versus* particle diameter obtained from the fractograms shown in Fig. 2 and the same dependence calculated theoretically from Eq. (*1*). It is obvious that in all three cases of different flow rates the experimental data deviate from the

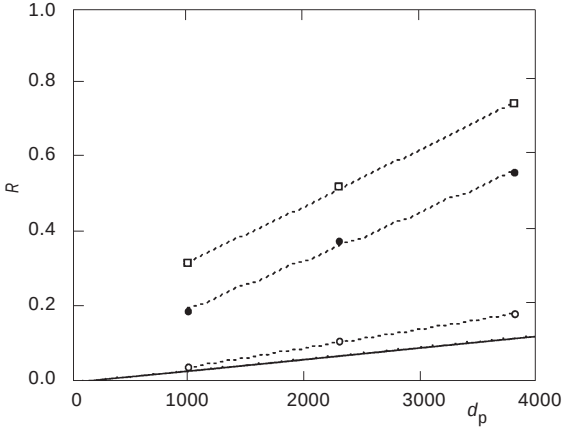

FIG. 3

Theoretical and experimental dependence of the retention ratio R on particle diameter d_p. Flow rate (ml/min): 0.1 (○). 0.5 (●), 1.0 (□). Theoretical curve (——) was calculated from Eq. (*1*) corresponding to the pure steric exclusion mechanism

theoretical curve thus indicating that the focusing mechanism dominates the retention. It has to be stressed that, with regard to the difference between isoviscous and non-isoviscous flow velocity profiles shown in Fig. 1, the theoretical retention ratios calculated by taking into account the non-isoviscous flow velocity profile should be lower (see, for example, lower streamline velocity at $x/w = 0.1$ for non-isoviscous profile in comparison with the isoviscous one in Fig. 1). The corresponding theoretical R (retention ratio) *versus* d_p (particle diameter) curve should thus be below that shown in Fig. 3, which means that the differences between experimental data and the theory that considers steric exclusion model as appropriate to describe the retention in the relevant cases are, in fact, more important in comparison with those shown in Fig. 3.

An obvious question arises, whether the steric exclusion can accurately correspond to the separation mechanism if the flow rate should be still lower than that applied in the experiment the result of which is shown in Figs 2 and 3. We have carried out such experiments but when decreasing the flow rate, an incomplete recovery of the injected amount of samples was observed up to complete and irreversible retention. This means that the attractive interactions (adsorption) between the retained particles and the accumulation wall became effective and in such a case it is hard to imagine that the purely steric exclusion mechanism can be considered as accurate to describe the retention.

CONCLUSION

High-speed, high-resolution micro-thermal focusing FFF was performed for the first time to separate micron-size polystyrene-based latex particles. It has been demonstrated by comparison of experimental results with the theory of steric FFF that such a theoretical model is inappropriate for most of the separations under the relevant experimental conditions. The focusing mechanism dominates the retention of the species that undergo a strong interaction with the applied field (temperature drop in our case) and the steric exclusion mechanism can be effective in very limited cases and still only under the condition that the other attractive interactions do not enter into play.

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