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Effect of channel width on the retention of colloidal particles in polarization, steric, and focusing micro-thermal field-flow fractionation

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Abstract

The effect of the channel width on the performance of separation by micro-thermal field-flow fractionation (micro-TFFF) of the carboxylated polystyrene latex particles was studied by using the particles in diameter range from 100 nm to 3800 nm. It has been shown that the retention order follows the anticipated polarization, steric, and focusing mechanism in the corresponding size range and under the specific conditions, appropriate to each channel thickness. However, the attractive interactions of the particles with the accumulation wall can complicate the separation as has been proven by the experiments carried out by using the carrier liquids of different ionic strengths. Three channel thicknesses (0.025, 0.100, and 0.250 mm) were tested thus imposing the volumes of micro-channels of roughly 9, 37, and 92 μl. Such an experimental investigation has never been performed with respect to the applicability of the TFFF within an extended range of molar masses or particle sizes. The advantages and drawbacks of different channel widths are discussed with respect to the performance of separation of micro-TFFF but also by taking into account the practical requirements of the construction of the micro-TFFF channel. The principal finding is that very thin channel ($w = 0.025$ mm) substantially reduces the range of particle sizes or polymer molar masses that can effectively be separated due to the mixed separation mechanism, steric exclusion being effective from smaller particle size. The found dependence of the resolution on the imposed experimental conditions including the channel width has allowed the elucidation of some peculiar results published in the literature, which were contradictory with regard to the known theoretical and experimental findings.
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1. Introduction

The separation in field-flow fractionation (FFF) can be controlled by three basic mechanisms: *polarization*, *steric*, and *focusing*. The same holds true for a specific technique of micro-thermal field-flow fractionation (micro-TFFF) invented and proposed recently [1]. Some aspects of reducing the dimensions of the FFF channels were treated theoretically by Giddings [2] in 1993. The only experimental study aimed to increase the performance of the TFFF due to the reduced thickness of the channel was published in 1978 [3]. However, this pioneering work [3] concerned only the separation of the dissolved polymers up to the molar mass of 160 000 g/mol. A crucial role of the temperature drop ΔT in comparison with the channel thickness w and its impact on the performances of the TFFF was completely neglected [2,3] at that time. A detailed theoretical analysis of the advantages of miniaturization of TFFF with respect to the resolution and easier manipulation of the operational parameters accompanied by the appropriate experimental study was performed recently [1].

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Whenever the *polarization mechanism prevails*, the retained species form nearly exponential concentration profiles across the channel due to the thermophoretic flux between the hot and cold walls of the channel and the opposed diffusion flux generated by the formed concentration gradient. As long as the thermal diffusion coefficients are independent on the size of the retained species of identical chemical nature (displaying the thermophoretic mobility), larger species, having lower diffusion coefficients, are compressed closer to the accumulation wall (usually the cold wall) in a stream of lower longitudinal velocity of the carrier liquid flowing along the channel. The elution order is thus from the small to the large size retained species. Whenever the temperature drop, ΔT, applied across the channel is high enough and, consequently, the distances of the center of gravity of the established concentration profiles of the retained species from the accumulation wall are commensurable with the size of these species, the *steric exclusion mechanism dominates* the separation and the elution order is inverted. In order to exploit advantageously either polarization or steric exclusion mechanism for the separation, the experimental conditions must be chosen to avoid a simultaneous contribution of both mechanisms, otherwise low or zero resolution is obtained within the retention range around the inversion point. However, purely steric exclusion mechanism is operating only if the flow rate of the carrier liquid is not too high. If this is not the case, the separated species are exposed to *lift forces generating the focusing phenomenon*. This effect can also be exploited for the effective separations.

A supplementary complication of the above mentioned retention mechanisms can originate from the interactions of the separated species with the accumulation wall or from the mutual interactions between these species. Obviously, this effect can be more important at high ΔT because the retained species are more concentrated and compressed closer to the accumulation wall. Such interactions can be either attractive or repulsive and, consequently, they can lead to either increase or decrease of the retention in comparison with the retention in the case when only single of three basic mechanisms mentioned above comes into play.

The distance between the accumulation and depletion walls of the separation channel or, in other words, the width, w, of the channel proper, plays an active role as concerns the appearance of the steric exclusion mechanism. Obviously, when decreasing w, the effect of steric exclusion starts (the inversion point is shifted) at smaller size of the retained species. We have studied recently the effect of the channel width on the retention in micro-TFFF with regard to minimum width of the micro-TFFF channel that should be respected in order to avoid a deterioration of the separation of macromolecules or particles within as large molar mass or particle size ranges as reasonable but the experimental investigation was carried out with a channel of only one given width w [4]. In this paper, a detailed experimental investigation of the influence of the channel width and of the flow rate on the retention is described. Thus, the domains of the action of each of three above mentioned basic separation mechanisms as well as of supplementary interactions are elucidated.

2. Theory

The retention ratio R describing the simultaneous action of the polarization and steric exclusion mechanisms is [5]:

$$R = 6(\alpha - \alpha^2) + 6\lambda(1 - 2\alpha)\left[\coth\left(\frac{1 - 2\alpha}{2\lambda}\right) - \frac{2\lambda}{1 - 2\alpha}\right] \quad (1)$$

where $\alpha = r/w$ is the ratio of the radius r of the separated species to the width w of the separation channel and λ is a dimensionless retention parameter defined by:

$$\lambda = \frac{k_B T}{6\pi\eta r D_T \Delta T} \quad (2)$$

where k_B is Boltzmann constant, T the temperature, η the viscosity of the carrier liquid, D_T the coefficient of thermal diffusion, and ΔT is temperature drop across the micro-TFFF channel. Eqs. (1) and (2) are rigorously valid only if the flow velocity profile formed inside the channel is parabolic. This is not the case in micro-TFFF because the viscosity varies with the temperature across the channel and thus the flow velocity profile is not strictly parabolic and also the coefficient D_T can be temperature dependent. However, we can neglect this effect in our following considerations.

The result of model calculation, performed with the use of Eqs. (1) and (2), and by imposing the virtual operational parameters corresponding to the real conditions applied in the present experiments and the thermal diffusion coefficient determined previously [4], is shown in Fig. 1. It represents the dependence of the inverse value of the retention ratio $1/R$ on the radius r of the separated species—the colloidal polystyrene (PS) based particles in our present case. The curves calculated for three different values of $w = 0.023$, 0.100, and 0.250 mm and shown in Fig. 1 will serve to
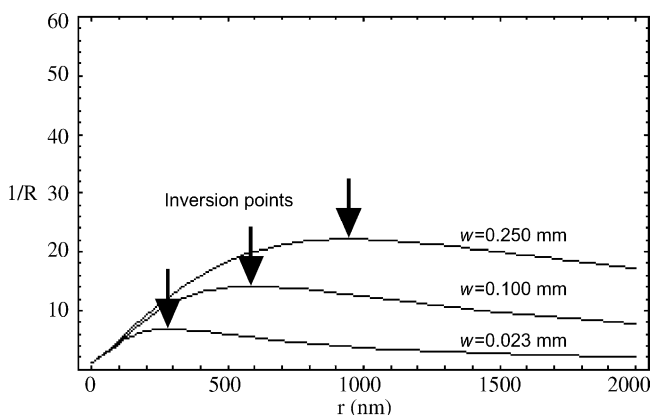

Fig. 1. Theoretical dependence of the inverse retention ratio $1/R$ on the particle radius r calculated from Eqs. (1) and (2) by using $w = 0.023$, 0.100, and 0.250 mm, $T_C = 303$ K, $\Delta T = 40$ K, viscosity of water, $\eta = 0.008$ P (g cm^{-1} s^{-1}) at 303 K, Boltzmann constant k_B, and $D_T = 3.85 \times 10^{-8}$ K^{-1} cm^2 s^{-1} (empirical value measured previously [4]).

compare the experimental data with the theoretical prediction and thus to estimate the casual contribution of the lift forces and/or of other particle–particle or particle–wall interactions mentioned in Section 1.

3. Experimental

The apparatus for micro-TFFF consisted of an intelligent pump model PU-980 (Jasco, Japan), an injection valve model 7410 (Rheodyne, USA) with a 1 μl or 5 μl loop, a UV–vis variable wavelength detector model UV-975 (Jasco, Japan) equipped with the 1 μl cell, and an integrator Model HP 3395 (Hewlett-Packard, USA). The versatile micro-TFFF channel was designed in our laboratory and fabricated by Lascialfari, SARL (La Rochelle, France). The dimensions of the micro-channel used in this work were w mm × 4 mm × 96 mm. The main channel walls were made of multi-layer composites with either Cr or Ti upper layer forming the inner surfaces of the channel. Three widths w = 0.023, 0.100, and 0.250 mm of the channel were determined by the thickness of the applied Mylar foil. 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 by an electronic device designed and built up in our laboratory. The temperatures of the cold and hot walls were measured by Digital thermometer (Hanna Instruments, Portugal) equipped with two thermocouples. An aqueous solution of 0.1% detergent Brij 78 (Fluka, Germany) and 3 mM NaCl was used as the principal carrier liquid. In some cases only a 0.1% detergent Brij 78 dissolved in deionized water was used without NaCl or the carrier liquid contained an increased concentration of NaCl, so that its composition was 0.1% of the detergent Brij 78 and 50 mM NaCl in deionized water. The temperature drop across the channel was always $\Delta T = 40$ K in order to keep the retention ratio R unchanged in channels of different widths. The temperature of the cold wall was always T_C = 301 K. The flow rates (in ml/min) were chosen in such a way to have the series of identical average linear velocities (in cm/s) in all three channels with w = 0.023, 0.100, and 0.250 mm.

Table 1
Size characteristics of the studied carboxylated polystyrene particles

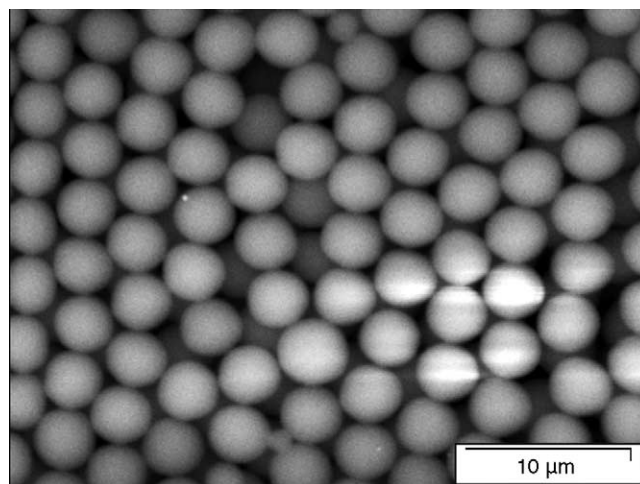| Sample number	Particle diameter (μm)
PS 100	0.100
PS 250	0.250
PS 360	0.360
PS 530	0.530
PS 720	0.720
PS 1000	1.000
PS 1360	1.360
PS 1900	1.900
PS 2300	2.300
PS 2500	2.500
PS 3200	3.200
PS 3800	3.800

Fig. 2. Scanning electron microscopy picture of the particles of PS 3800 sample.

Spherical carboxylated polystyrene latex particles (PS) of very narrow particle size distribution (PSD) were used in this study. Their synthesis and characterization was described previously [6–10]. The average particle sizes measured by quasi-elastic light scattering (QELS) and transmission electron microscopy (TEM) are given in Table 1. In order to demonstrate the narrow PSD and a spherical form of the particles, a picture of the largest size PS sample (particle diameter 3.8 μm) obtained by scanning electron microscopy (SEM) is shown in Fig. 2. The other samples exhibit similar narrow PSDs.

4. Results and discussion

4.1. Effect of channel width on the retention

Our previous experiments [11] were devoted to the measurement of the dependence of the retention ratio on the particle radius within a wide range of particle sizes from 100 nm to 3800 nm at different temperature drops ΔT = 5, 10, 15, 20 and 40 K and at the flow rates varying from 0.01 ml/min to 0.5 ml/min. Later [12], the range of the flow rates was extended up to 1.0 ml/min. In all previous experiments [11,12], an optimized injection-stop-flow procedure was applied. The injection at low flow-rate was carried out during some period of time followed by stop-flow time and then by the elution at a chosen flow rate. We have adopted this optimized injection-stop-flow procedure in the present work with some variations taking into account three different widths of the channel. The exact injection-stop-flow procedure is notified at each particular experiment.

The first series of experiments was carried out in a channel with w = 0.250 mm at three different flow rates and the retentions were compared with the data obtained previously [11] by using the channel whose w = 0.100 mm. In order to allow a direct comparison of the experimental data obtained with the use of the channels of different widths w, the flow
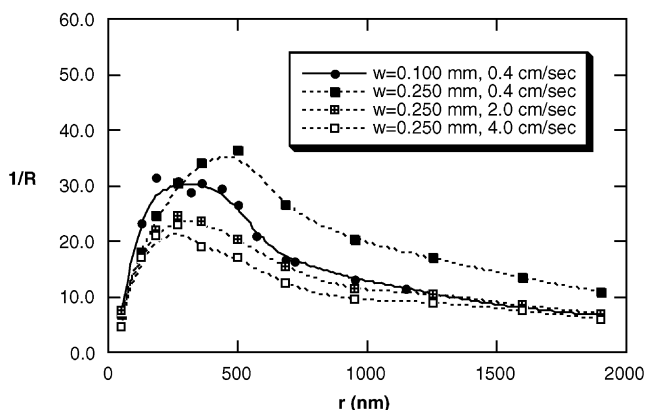
Fig. 3. Comparison of the experimental retention data showing $1/R$ vs. r dependences measured in micro-TFFF channels of $w = 0.100$ and 0.250 mm at $\Delta T = 40$ K and at different average linear velocities of the carrier liquid. The injected volumes were 1 μl at 0.010 ml/min during 1 min in $w = 0.100$ mm channel and 5 μl at 0.025 ml/min during 40 s in $w = 0.250$ mm channel. Stop-flow times were 1 min in $w = 0.100$ mm channel and 6 min in $w = 0.250$ mm channel.

Fig. 4. Effect of ionic strength on $1/R$ vs. r dependence measured in micro-TFFF channel of $w = 0.250$ mm at $\Delta T = 40$ K and at the average linear velocity of the carrier liquid 0.4 cm/s. The carrier liquid contained different concentrations of NaCl. The injected volume was 5 μl at 0.025 ml/min during 40 s and stop-flow time was 6 min.

rates applied in the experiments were adjusted in such a way that the established average linear velocities (in cm/s) were identical for all channels of different w values. The results are shown in Fig. 3. The experimental points and the corresponding best-fit curves of the $1/R$ versus r dependence shown in Fig. 3, obtained at the lowest average linear velocity 0.4 cm/s of the carrier liquid, agree quite well with the corresponding theoretical curves in Fig. 1 for both channels of $w = 0.100$ and 0.250 mm in the part corresponding to the dominating steric exclusion mechanism. On the other hand, in the range where only the polarization mechanism should be operating, the experimental data deviate from the theoretical curves in an important manner. The particles are retained more than predicted by the theory. It might be caused by the attractive interactions with the accumulation wall (adsorption). It seems that a simple frictional drag of the particles in contact with the accumulation wall is not at the origin of higher retentions because larger particles retained by steric exclusion mechanism do not exhibit such an increase of the retention. The other deviation from the theoretical curves is in the position of the maximum of the $1/R$ versus r dependence, or in other words, the experimental inversion point is shifted in comparison with the theoretical prediction. The experiments described in the following paragraph are aimed to clarify the causes of these deviations. General decrease of the retention (decrease of the $1/R$ value shown in Fig. 3) in the experiments carried out at higher average linear velocities of the carrier liquid indicates the appearance of lift forces. This effect increases with the increasing flow rate within the whole range of the particle sizes.

Some experiments were performed with other compositions of the carrier liquid, at the lowest average linear velocity in the channel with $w = 0.250$ mm. The results are shown in Fig. 4. An increase of the NaCl concentration to 50 mM results in a substantial increase in the retention around the
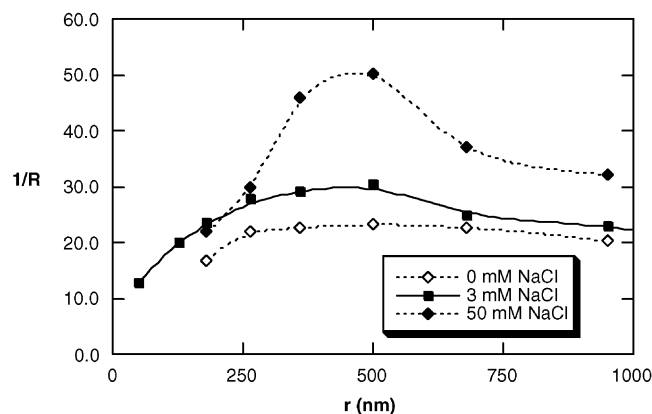
inversion point and within the range of steric exclusion mechanism. The particles are no more protected by the electrostatic double-layer so that they can approach closer to the accumulation wall and closer to each other. As a result, the particles are adsorbed on the accumulation wall. The adsorption was also confirmed independently by a noticeable decrease in sample recovery (decrease in peak area at the same injected amount of the sample in comparison with the experiments performed in a carrier liquid containing only 3 mM NaCl). On the other hand, when the deionized water containing only the detergent Brij 78 was used as carrier liquid, the experimental retentions of the PS samples are very close to the theoretical $1/R$ versus r dependence within the whole investigated size range (compare Fig. 4 and Fig. 1). It seems that under these conditions, the retention is governed by an appropriate mechanism, anticipated by the theory for each corresponding size range. Obviously, a difference between the real particle size and apparent particle size due to the electrostatic double-layer is negligible with regard to relatively large average sizes of the investigated particles.

Last series of experiments was carried out in a channel whose $w = 0.023$ mm. The results are shown in Fig. 5. The $1/R$ versus r dependence of the same series of PS particles obtained in the channel with $w = 0.100$ mm is given again in Fig. 5 for comparison. The $1/R$ versus r dependence obtained in thinnest channel ($w = 0.023$ mm) exhibits higher than theoretically expected retentions within the whole range of the particle sizes at the lowest average linear velocity (0.4 cm/s) of the carrier liquid (compare the experimental data in Fig. 5 with the corresponding theoretical curve in Fig. 1). This is again probably due to the adsorption of the particles on the accumulation wall because they are concentrated closer to the wall at the same temperature drop $\Delta T = 40$ K in comparison with the above described experiments in channels of larger widths. Some chaotic recovery of the injected samples, which was observed by using this channel width can serve as a proof of partial adsorp-
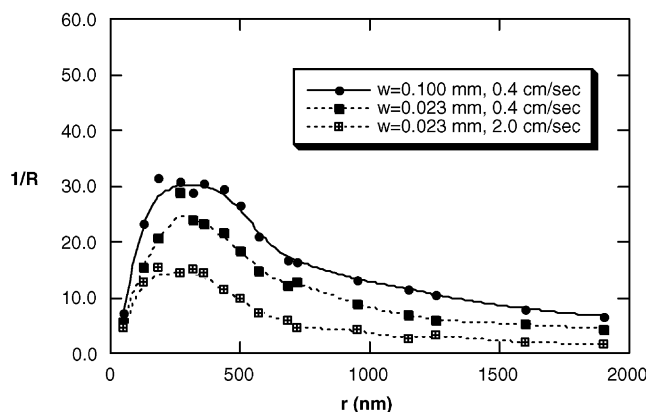
Fig. 5. Comparison of the experimental retention data showing $1/R$ vs. r dependences measured in micro-TFFF channels of $w = 0.023$ and $0.100\,\text{mm}$ at $\Delta T = 40\,\text{K}$ and at different average linear velocities of the carrier liquid. The injected volume was $1\,\mu\text{l}$ at $0.010\,\text{ml/min}$ during $1\,\text{min}$ followed by stop-flow time of $1\,\text{min}$ in both channels.

tion of the retained particles on the accumulation wall. The $1/R$ versus r dependence obtained at higher average linear velocity ($2.0\,\text{cm/s}$) of the carrier liquid is very close to the theoretical curve shown in Fig. 1 within the whole range of the particle sizes. Nevertheless, it must be a pure coincidence of some kind of "equilibrium" between the attractive forces (adsorption) and lift forces. As the adsorption cannot be fully controlled, it is preferable to carry out the separations in micro-TFFF under more convenient conditions (lower linear velocities and thicker channels) allowing to avoid (or at least to minimize) the uncontrolled processes and mechanisms, which might be irreproducible thus providing the results of the fractionation uncertain. Very detailed study of the effect of the experimental conditions, such as the ionic strength, published by Shiundu et al. [13] indicates and confirms that the choice of the convenient operational conditions is very important for a successful strategy of separation by TFFF.

4.2. Effect of channel width on the resolution

The theory predicts [1] that the resolution can be more effectively manipulated by the variation of ΔT in comparison with the variation of w. This follows from the relationship:

$$R_s = \left(\frac{1}{8w}\right)\sqrt{\frac{L D_T^3 \Delta T^3}{6\langle v \rangle}}\left|\frac{1}{D_1} - \frac{1}{D_2}\right| \qquad (3)$$

Eq. (3), derived theoretically [1], shows that the shortening of the channel length L accompanied by the appropriate decrease of the average linear velocity $\langle v \rangle$ of the carrier liquid keeps the resolution unchanged. The $D_{1,2}$ are diffusion coefficients of two resolved species. It is obvious that the increase of ΔT has a more important impact on the resolution than a proportional decrease of w, both changes at the same necessary increase of the heat flux across the channel. The validity of Eq. (3) was verified by the experiments [14]. As
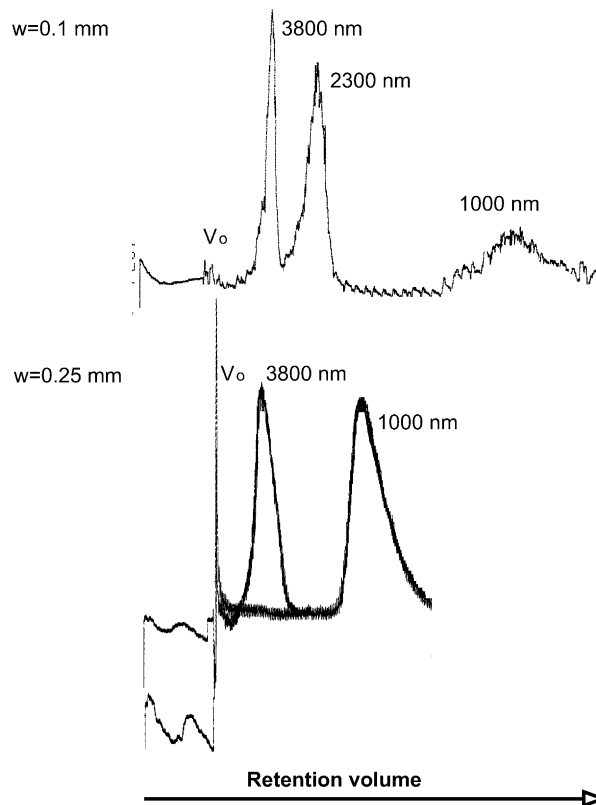

Fig. 6. Fractogram of the mixed sample of PS 3800, PS 2300, and PS 1000 obtained at $\Delta T = 40\,\text{K}$ and at the average linear velocity of the carrier liquid $0.4\,\text{cm/s}$ in micro-TFFF channel with $w = 0.100\,\text{mm}$ and the superposition of the fractograms of simple PS 3800 and PS 1000 samples obtained under the identical experimental conditions but in micro-TFFF channel with $w = 0.250\,\text{mm}$. The injected volumes were $1\,\mu\text{l}$ at $0.010\,\text{ml/min}$ during $1\,\text{min}$ in $w = 0.100\,\text{mm}$ channel and $5\,\mu\text{l}$ at $0.025\,\text{ml/min}$ during $40\,\text{s}$ in $w = 0.250\,\text{mm}$ channel. Stop-flow times were $1\,\text{min}$ in $w = 0.100\,\text{mm}$ channel and $6\,\text{min}$ in $w = 0.250\,\text{mm}$ channel.

a result, a decrease of the channel width does not represent an advantageous solution but it can even complicate the separation of larger size species due to a possible simultaneous intervention of polarization and steric exclusion mechanisms.

On the other hand, an increase of the channel width should not a priori lead to an advantageous extension of the range of particle sizes that could effectively be separated in steric mode. Such an increase should be accompanied by an increase in channel breath in order to keep the aspect ratio width/breath constant. If this condition is not respected, the resolution can decrease as can be seen in Fig. 6 showing a decrease of the resolution of some PS particles separated in steric mode micro-TFFF with increasing channel width and by keeping ΔT and the average linear velocity identical in both cases.

We have already clearly demonstrated [14] how the resolution can conveniently be tuned by the variation of the operational parameters and how a high-resolution of the separation of colloidal particles on our micro-TFFF channel [14] can be achieved. Fig. 7 represents an additional explicit demonstration of what is (and what is not) possible when separating a
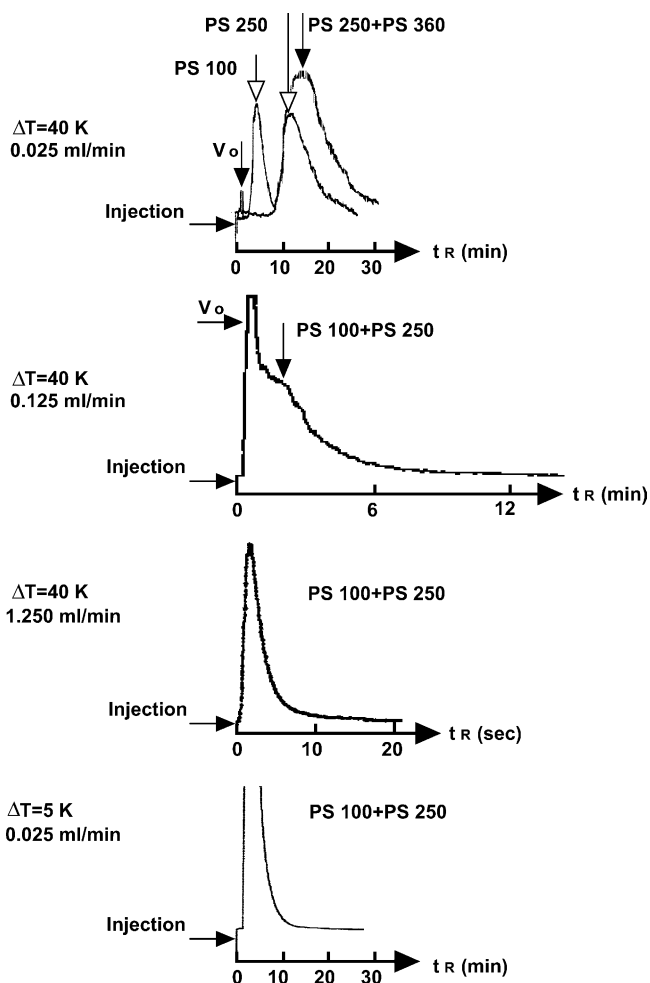
Fig. 7. Fractograms of the mixed samples of PS 100 with PS 250, and PS 250 with PS 360, obtained at different ΔT values and different average linear velocities of the carrier liquid in micro-TFFF channel with $w = 0.023$. The injected volume was 1 µl at 0.010 ml/min during 1 min followed by stop-flow time of 1 min.

mixture of the particles of different diameters by using micro-TFFF channel with $w = 0.023$ mm. The results demonstrated in Fig. 7 are not surprising but interesting with a special regard to a paper [15]. Fig. 7 thus shows that the mixture of PS 100 and PS 250 samples can be well separated at $\Delta T = 40$ K and at very low flow rate 0.025 ml/min. Contrary to this good separation, a superposed fractogram of the mixture of PS 250 and PS 360 samples shows that no separation under identical conditions was achieved. If the flow rate was increased only five times to 0.125 ml/min by keeping the same $\Delta T = 40$ K, the separation of the PS 100 and PS 250 mixture was highly deteriorated. Further increase of the flow rate to 1.250 ml/min at $\Delta T = 40$ K resulted in no separation of this mixture. Not surprisingly, a decrease of the temperature drop to $\Delta T = 5$ K did not result in a separation even at the lowest applied flow rate 0.025 ml/min. Our correctly performed and described experiments did not confirm at all very peculiar observation reported in [15]. The detailed comments of the problematic results reported in paper [15] were published

recently [16]. As a result, with respect to the theoretical and experimental results reported in this paper, it seems that a tendency [16,17] to propose the construction of the micro-TFFF channels with the reduced thickness of the order of 0.025 mm does not represent an advantage but rather the potential complications with regard to a possible interference of the mixed polarization and steric exclusion mechanisms leading to a deterioration of the separation. The channels of intermediate thicknesses, lying between 0.100 and 0.250 mm, are the most universal for the applications within a wide range of polymer molar masses and particle sizes and much more versatile as to the easy manipulation within an extended range of the operational variables.

5. Conclusion

The study of the effect of channel width on the retention and resolution in micro-TFFF of the colloidal and large size particles demonstrated the rational limits of the variation of this dimension of separation channel. The experimental conditions were chosen to cover the whole range of three basic separation mechanisms—polarization, steric, and focusing. The intervention of each one was clearly determined. Moreover, the potential intervention of secondary mechanisms resulting from particle–particle or particle–wall interactions was also elucidated. Some experiments were carried our under the conditions identical or close to those described recently in the literature but the peculiar published results were not confirmed.

Acknowledgements

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