



ELSEVIER

Journal of Chromatography A, 907 (2001) 201–209

JOURNAL OF
CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Influence of the carrier composition on thermal field-flow fractionation for the characterisation of sub-micron polystyrene latex particles

E.P.C. Mes¹, R. Tijssen, W.Th. Kok*

Polymer Analysis Group, Department of Chemical Engineering, University of Amsterdam, Nieuwe Achtergracht 166, 1018 WV Amsterdam, Netherlands

Received 19 July 2000; received in revised form 26 September 2000; accepted 3 October 2000

Abstract

A study on the influence of the carrier composition in a ThFFF system on the retention and thermal diffusion of sub-micron polystyrene latex particles has been carried out. Various factors that may influence retention were studied. These include: the type of electrolyte and surfactant, their respective concentrations, and the addition of an organic modifier. Particle retention is highly sensitive to small changes in the carrier composition. It is demonstrated that under the conditions applied, secondary effects, such as particle–wall and particle–particle interactions, are negligible. Addition of surfactants is required to minimise particle–wall interactions. Generally, retention increases at higher electrolyte concentration. Furthermore, the addition of acetonitrile (ACN) to an aqueous carrier leads also to an increased retention. The type of surfactant as well as its concentration is of influence on the retention time. The three surfactants that were studied, i.e., sodium dodecyl sulfate, Brij 35 and cetyltrimethylammonium bromide, showed significant differences in particle retention behaviour. The observed differences in retention in the carriers can be attributed to actual changes in thermal diffusion. D_T appears to be mainly determined by the interaction between the particle's surface and the carrier liquid, and is therefore highly sensitive to changes in the chemical composition of the particle surface and the carrier. Strong differences in size selectivity were found for different carrier compositions. This allows a relatively easy optimisation of the separation. On the other hand, it complicates the size and composition analysis of particles. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Thermal field-flow fractionation; Field-flow fractionation; Carrier composition; Surfactants; Latex particles

1. Introduction

Although originally conceived as a method to analyse polymers, field-flow fractionation (FFF) is

becoming increasingly popular to analyse particles. Where conventional polymer analysis methods such as size-exclusion chromatography fail, due to its open geometry FFF is perfectly able to separate particles in the range of a few nm up to 100 μm [1].

Up to now, most FFF applications for particle analysis involve flow field-flow fractionation (FlowFFF) [2,3] or sedimentation field-flow fractionation (SedFFF) [4,5]. For SedFFF, retention depends on the particle density and particle size

*Corresponding author. Tel.: +31-20-525-6539; fax: +31-20-525-5604.

E-mail address: wkok@its.chem.uva.nl (W.Th. Kok).

¹Current address: Dow Benelux N.V., Analytical Sciences Terneuzen, P.O. Box 48, 4530 AA Terneuzen, Netherlands.

whereas for FlowFFF retention depends only on particle size. A relatively new development is the use of thermal field-flow fractionation (ThFFF) for particle analysis [6]. Separation in ThFFF is governed by the ratio of the thermal diffusion and the normal molecular diffusion of the analytes (also known as the Soret coefficient). The normal diffusion coefficient D depends only on size and thermal diffusion coefficient D_T may depend on size but certainly on chemical composition. A straightforward correlation between D_T and chemical composition has been found for polymers [7,8] and composition-dependent retention has been shown for particles [9–11]. As a result, ThFFF can be used to separate macromolecules or particles according to size, but also to study their chemical composition.

A complication of FFF analysis of particles is the occurrence of secondary effects on retention and separation that are not accounted for in the standard FFF-retention model [12]. Steric exclusion effects, due to the finite size of particles, can be corrected for relatively easy. Particle–particle and particle–wall interactions, however, are more difficult to deal with [13].

A problem in ThFFF is that thermal diffusion is still an uncomprehended phenomenon [14]. Research on particle thermal diffusion is still in a very early stage and it is therefore difficult to predict the retention behaviour of a particle in a ThFFF system. From the few studies that have been performed on particles certain features are known. Especially in aqueous carriers, the presence of an electrolyte is necessary to achieve retention. It has been found that the retention of particles increases with electrolyte concentration [15,16]. Furthermore, thermal diffusion coefficients of particles tend to be much smaller than those of polymers [6]. Another marked difference is that the D_T value of particles often appears to be dependent on size whereas for polymers it is independent of size [17,18]. In all studies conducted on ThFFF of particles, D_T was found to be strongly dependent on both the particle and carrier composition. Especially the composition of the outer layer of the particle and the particle–carrier interface appears to determine the actual thermal diffusion [9]. Since surface effects dominate thermal diffusion, it has been suggested that D_T is related to the surface tension of the particles [11].

To expand the applicability of ThFFF of particles and to exploit its main advantage over other FFF modes, namely the composition dependence, it is important to study the ThFFF particle retention mechanism and thermal diffusion. In the present study we focus on the influence of the carrier composition on the ThFFF retention and thermal diffusion of sub-micron polystyrene (PS) particles. The separation mechanism is investigated by varying factors in the carrier composition that may influence the retention. We studied the effect of surfactants and electrolytes on retention behaviour. The effect on the size selectivity and plate height was also examined. Furthermore, we studied the influence of adding acetonitrile (ACN) as an organic modifier to the aqueous carrier. Mole fractions up to 10% of ACN (approximately 25%, v/v) in water were used. The direct effect on the thermal diffusion coefficient of the PS latex particles was examined.

2. Theory

The standard FFF theory is based on the assumption that polymer molecules or particles act as non-interacting point masses. Under this condition, in a flat channel under laminar flow conditions in the presence of a force perpendicular to the flow, species are differentially migrating according to [19]:

$$\frac{t_R}{t_0} \approx \left[\frac{6D}{D_T \Delta T} \right]^{-1} \quad (1)$$

where t_R is the retention time, t_0 the void time, and ΔT the temperature difference between the channel walls. From the measured retention time, the thermal diffusion coefficient can be calculated when the diffusion coefficient is known.

Steric effects, however, can give rise to significant deviations from the standard FFF retention model. They are the result of the fact that particles with a finite size cannot approach the channel wall closer than the particle radius r . When steric effects are significant a corrected retention equation has to be used [20]:

$$\frac{t_R}{t_0} \approx \left(6 \left[\frac{D}{D_T \Delta T} + \frac{r}{w} \right] \right)^{-1} \quad (2)$$

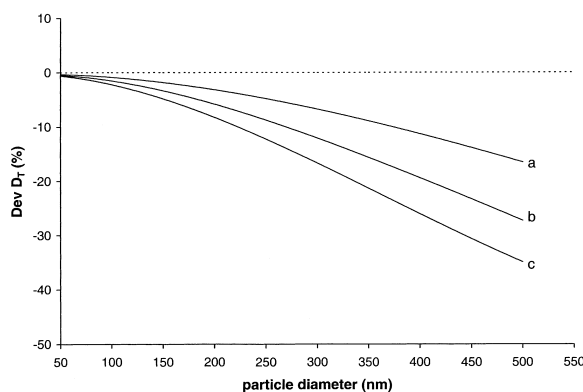


Fig. 1. Percent deviation between D_T of particles in water calculated with Eq. (1) and Eq. (2); (a) $\Delta T = 20$ K, (b) $\Delta T = 40$ K, (c) $\Delta T = 60$ K, $D_T = 4 \cdot 10^{-12}$ m²/s K, $T_c = 300$ K.

where w is the channel height. Neglecting the steric effects in particle analysis can lead to significant errors in the calculated D_T values. In Fig. 1, the deviations of the calculated D_T values from the true values, when steric effects are not accounted for, are shown as a function of the particle size. In our experiments, the corrections that had to be made amounted to up to 20%.

Both above-mentioned approximations (Eqs. (1) and (2)) are valid at sufficiently high retention times; $t_R/t_0 > 8$ (deviation from the exact FFF retention equation is less than 5%). At lower retention, the exact FFF retention equation, as given in Ref. [21], has to be used.

Details on the calculation of the thermal diffusion coefficient are given in the Experimental section.

For sub-micron particles other secondary effects may result from Van der Waals forces and electrostatic forces. Van der Waals forces and electrostatic forces are mainly manifest as particle–wall interactions. Hansen and Giddings showed that particle–particle interactions are usually negligible and they can be avoided relatively easily by using low sample concentrations [22].

Generally, at low ionic strength (1–10 μM), a significant electrostatic repulsion between the particles and the wall is present [23]. At high ionic strength, however, the repulsive electrostatic force is strongly reduced and the opposing attractive Van der Waals forces prevail. In the extreme case, they may cause adsorption of the particles to the channel wall

[13,10]. In a solution of intermediate ionic strength (≈ 1 mM), the addition of a surfactant is usually sufficient to prevent these particle–wall interactions [23,24].

A source of error that can be of importance when mixtures of solvents are used as carrier is segregation of the mixture [25]. This effect can cause an extra field-dependent driving force, as the thermal diffusion is often dependent on the carrier composition. Generally, the segregation is negligible but it has been shown that occasionally this effect is large enough to give rise to non-ideal behaviour [26].

3. Experimental

3.1. Instrumentation

The ThFFF system was a laboratory-made apparatus. The channel dimensions were $460 \times 15 \times 0.125$ mm, giving a void volume of 0.6 ml. The channel flow was delivered by a Gynkotek (Model 300 C; Germering, Germany) high-performance liquid chromatography (HPLC) pump. A pulse damper and a flow restrictor were inserted directly behind the HPLC pump. Flow-rates from 0.25 to 0.45 ml/min were used. A six-port valve equipped with a 30- μ l loop was used for sample introduction. A 7- μ m in-line filter was inserted between the injection valve and the channel. The flow of the carrier was stopped for 6 min after sample injection to allow relaxation. The temperature drop was controlled by the FFF software that was obtained from FFFractionation (Salt Lake City, UT, USA). Temperature drops ranging from 15 K to 60 K were used. Cold-wall temperatures (T_c) ranged from 293 K up to 298 K depending on the temperature drop and the solvent composition. A UV detector (Model 757; Applied Biosystems, Ramsey, NJ, USA) operated at 254 nm was used for detection.

3.2. Chemicals and solutions

PS latex standards with diameters of 64, 107, 155, 202 and 356 nm were obtained from Polysciences (Eppelheim, Germany) and from Duke Scientific (Palo Alto, CA, USA). Original concentrations of the PS latex standards were in the order of 1–2.5 mg/

ml. All sample solutions were diluted prior to injection to a concentration of 1.25 $\mu\text{g/ml}$ and vortex-mixed thoroughly.

Sodium dodecyl sulfate (SDS) (Sigma–Aldrich, Steinheim, Germany), polyoxyethylene laurylether (Brij 35) (Acros, Geel, Belgium), and *N*-cetyl-*N,N,N*-trimethylammoniumbromide (CTAB) (Merck) were used as anionic, non-ionic and cationic surfactants, respectively. For the experiments involving mixed carriers, tetrabutylammoniumperchlorate (TBAP) (Acros) was used as an electrolyte, SDS was used as a surfactant, and HPLC-grade ACN (Rathburn, Walkerburn, UK) was used as an organic modifier.

3.3. Calculation of thermal diffusion coefficients

D has been calculated from the particle radius by using the Stokes–Einstein equation:

$$D = \frac{kT}{6\pi\eta r} \quad (3)$$

where k is Boltzmann's constant, T the absolute temperature, and η the carrier viscosity.

Data on the viscosity of liquids and its dependency on the temperature have been taken from the literature [27]. To calculate viscosities for the water–ACN mixtures, the method of Lobe has been used [28,29]. For the temperature, the estimated value in the centre of gravity of the sample zone was used. A previously developed algorithm [30] has been used to account for the temperature dependency of the viscosity and the thermal conductivity of the carrier for the calculation of D and D_T . Furthermore, corrections were performed for the steric particle–wall effects. The values of the calculated viscosities of the mixtures and their respective derivatives at room temperature are given in Table 1.

Table 1
Viscosities of water–ACN mixtures and the temperature dependency at 298 K

Mol% ACN	$10^3 \eta$ (Pa s)	$10^5 d\eta/dT$ (Pa s/K)
0	0.894	–1.98
5	0.998	–2.29
10	0.981	–2.29

4. Results and discussion

4.1. Secondary effects

In order to ascribe changes in retention time under different experimental conditions to actual changes in thermal diffusion, secondary effects such as particle–particle interactions, particle–wall interactions (with the exception of steric effects), and, for mixed carriers, segregation of the mixture have to be ruled out.

To check for particle–particle interactions, samples were injected at different concentrations (see Fig. 2). The results show that overloading effects, and therefore particle–particle interactions, can be neglected at the injection concentration used in this study (1.25 $\mu\text{g/ml}$).

Particle–wall interactions (repulsion or adsorption) will have a strong influence on peak widths. To study possible particle–wall interactions, the plate heights experimentally found were compared with the theoretically expected plate heights. Under ideal circumstances, the plate height is determined by the non-equilibrium (mass-transfer) contribution and is given by:

$$H = \frac{\chi w^2 \langle v \rangle}{D} \quad (4)$$

where $\langle v \rangle$ is the mean linear flow velocity and χ a function of the so-called dimensionless retention

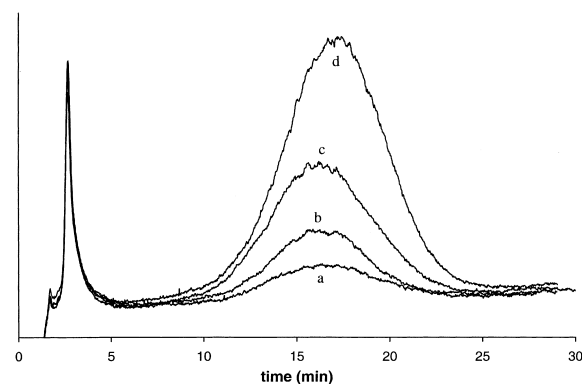


Fig. 2. Elution profiles at different sample loads of PS latex 202 nm at $\Delta T = 60$ K; V_{inj} : 30 μl , C_{inj} : (a) 0.3 $\mu\text{g/ml}$, (b) 0.6 $\mu\text{g/ml}$, (c) 1.3 $\mu\text{g/ml}$, and (d) 2.6 $\mu\text{g/ml}$. Carrier: phosphate buffer (2.5 mM) + 1 mM SDS.

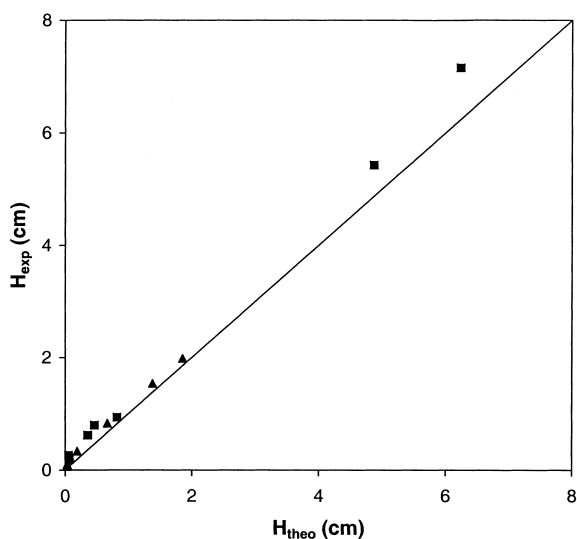


Fig. 3. Experimentally found plate heights (H_{exp}) versus theoretical plate heights (H_{theo}) of (■) PS latex 202 nm and (▲) PS latex 356 nm in several carrier compositions.

parameter λ , which is defined as $D/(D_T \Delta T)$. To a good approximation χ can be calculated as $24\lambda^3(1 - 10\lambda + 28\lambda^2)/(1 - 2\lambda)$ [31]. In Fig. 3, the experimental plate height values of PS latex 202 nm and PS latex 356 nm in several carrier compositions are plotted against the theoretical values. Overall, it was found that the experimental values were only slightly higher than the ideal theoretical values. Taking into account the precision of the measurements, we can safely assume that secondary effects can be neglected [32].

When mixed solvents are used as carrier, solvent segregation may give rise to an (apparent) field-dependent D_T value. Such effects can easily be detected by performing the analysis at several temperature gradients. The D_T values obtained should be independent of the strength of the used field; any deviations indicate non-ideal behaviour. Calculation of the thermal diffusion coefficients at different temperature drops (15, 20 and 30 K) showed that, in accordance with theory, D_T was independent of ΔT . Therefore, it may be concluded that possible segregation of the carrier can be neglected.

4.2. Influence of the carrier composition

Preliminary experiments with PS latex particles

showed that the presence of an electrolyte in the carrier is required to achieve reasonable retention. For all carrier compositions used it was found that retention increases with electrolyte concentration. Unfortunately, the use of higher electrolyte concentrations also occasionally resulted in irreproducible retention times and decreasing peak areas, indicating particle adsorption on the channel wall. The addition of a surfactant could prevent these interactions.

The surfactant commonly used in FFF studies on solid particles is FL-70 (Fisher Scientific), which is a mixture of anionic and non-ionic components. The composition of FL-70 is, however, not accurately defined and this surfactant is, therefore, not very well suited in studies of the retention mechanism. In our study we used three different surfactants, i.e., SDS (anionic surfactant), Brij 35 (non-ionic surfactant), and CTAB (cationic surfactant). It was found that the retention behaviour of PS latex particles was highly dependent on the type of surfactant used. With CTAB as the surfactant, broad and irreproducible peaks eluted, which could be explained as caused by adsorption of the particles to the wall.

Much better results were obtained with SDS. The addition of SDS prevented adsorption to the wall but also caused a decrease of retention (see Fig. 4A). It is highly unlikely that electrostatic repulsion is the cause of the decrease in retention. The average particle-wall distance ($\approx 5\text{--}20 \mu\text{m}$) is much larger than the electric double layer thickness around the particle, which is in the order of 1–10 nm. Therefore, an actual change in the thermal diffusion is most likely the cause of the observed effect. The decrease in thermal diffusion appears to be directly related to the surfactant concentration at the particle surface.

The retention behaviour of latex particles as function of the non-ionic Brij 35 surfactant concentration is shown in Fig. 4B. It can be seen that the retention is almost independent of the Brij concentration. This different behaviour compared to SDS, however, can also support the assumption that D_T is related to the surfactant concentration on the particle's surface. The critical micelle concentration (CMC) of Brij 35 is much lower than that of SDS, viz. about 0.1 mM for Brij 35 and about 8 mM for SDS. The particle's surface is, therefore, already

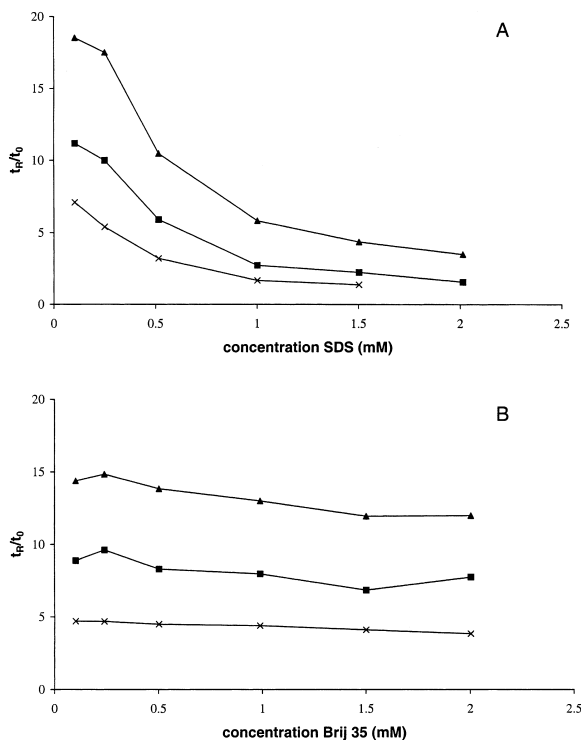


Fig. 4. (A) Influence of SDS concentration on particle retention time; (x) PS latex 107 nm, (■) PS latex 202 nm, (▲) PS latex 356 nm; flow-rate=0.45 ml/min; carrier: Tris buffer (1 mM, pH 8); $\Delta T=45$ K and $T_c=295$ K. (B) Influence of Brij 35 concentration on particle retention time; (x) PS latex 107 nm, (■) PS latex 202 nm, (▲) PS latex 356 nm; flow-rate=0.45 ml/min; carrier: Tris buffer (1 mM, pH 8); $\Delta T=30$ K and $T_c=294$.

saturated with Brij 35 molecules in the concentration range studied [33], and any further increase in the bulk surfactant concentration only increases the number of micelles and will not affect the surfactant concentration on the particle's surface [34,35].

In Fig. 5 the dependency of retention of PS latex 202 nm on the electrolyte concentration is depicted. In the presence of SDS the retention increases with increasing electrolyte concentration. With the neutral surfactant Brij 35 on the other hand, the retention hardly changes with the ionic strength of the solution. A possible explanation for these differences between SDS and Brij 35 may be that there is an adsorption competition between the phosphate ions and the SDS ions on the surface of the particle, while with the neutral Brij surfactant such competition is

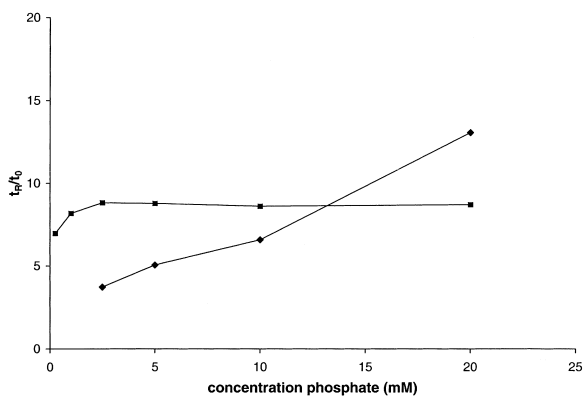


Fig. 5. Particle (PS latex 202 nm) retention time as function of ionic strength in the presence of (◆) SDS (1 mM; $\Delta T=40$ K); (■) Brij 35 (0.2 mM; $\Delta T=30$ K); flow-rate=0.45 ml/min; carrier: phosphate buffer (pH 7.4).

absent. Although SDS mainly adsorbs to the PS latex surface by hydrophobic interaction, the presence of a substantial cloud of counter ions is likely to induce ionic interaction as well.

From the results obtained it appears that Brij is a more suitable surfactant than SDS for size or composition analysis of latex particles by ThFFF, since the particle retention is less dependent on the composition of the carrier composition with Brij.

The effect of the presence of ACN as an organic modifier in the carrier solution was studied. In Fig. 6 the fractograms are depicted of PS latex 67, 107, 155 and 202 nm in a carrier containing 0.5 mM TBAP, 1 mM SDS and different amounts of ACN. Overall, a high retention was found at a relatively low temperature drop with ACN in the carrier. Furthermore, the presence of ACN considerably influences retention time, and therefore, the thermal diffusion. The calculated thermal diffusion coefficients are depicted in Fig. 7. It can be seen that the particle D_T values in these carriers are indeed exceptionally high and even approach the D_T values found for polymers [36].

If we confine ourselves to the carrier containing 0.5 mM TBAP and 1 mM SDS and compare the D_T value of, for example, PS 107 nm ($4.8 \cdot 10^{-12}$ m²/s K) with the D_T values found in 1 mM phosphate + 1 mM SDS ($0.92 \cdot 10^{-12}$ m²/s K) and 1 mM Tris + 1 mM SDS ($0.8 \cdot 10^{-12}$ m²/s K) we see that the type of salt added can have a very strong influence on the thermal diffusion coefficient.

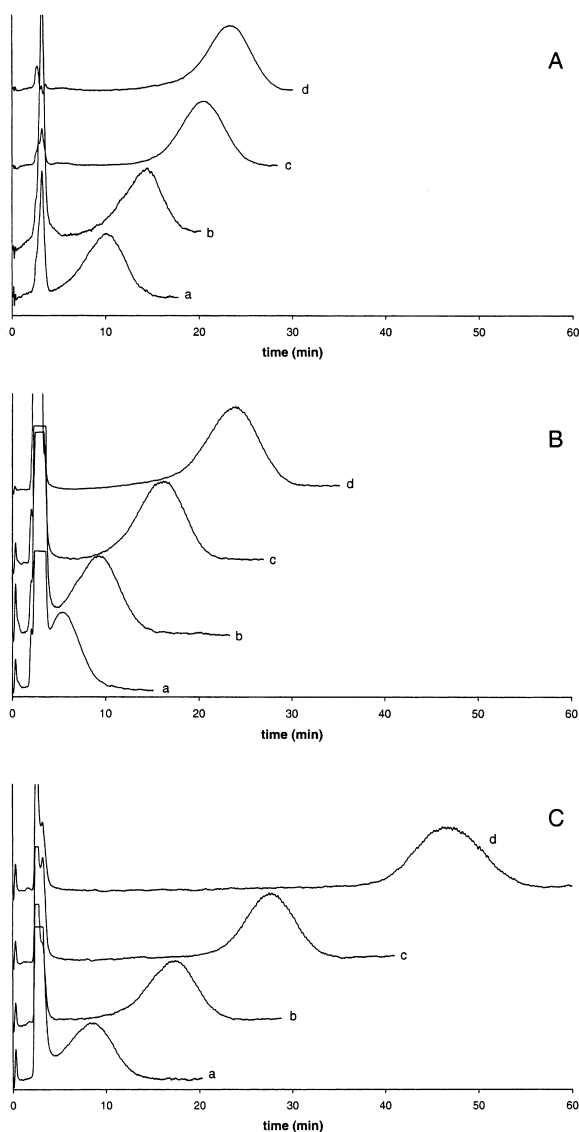


Fig. 6. Fractograms of PS latex particles; (a) 64 nm, (b) 107 nm, (c) 155 nm and (d) 202 nm in various ACN–water carrier mixtures with $\Delta T = 20$ K and flow-rate = 0.25 ml/min. (A) 0 mol% ACN, $T_c = 297$, (B) 5 mol% ACN, $T_c = 296$, (C) 10 mol% ACN, $T_c = 296$. To all carriers 0.5 mM TBAP and 1 mM SDS were added.

4.3. Size dependence of the thermal diffusion

A striking feature of the plots depicted in Fig. 7 is that D_T decreases with particle size in water and increases in the ACN–water mixtures. Apparently,

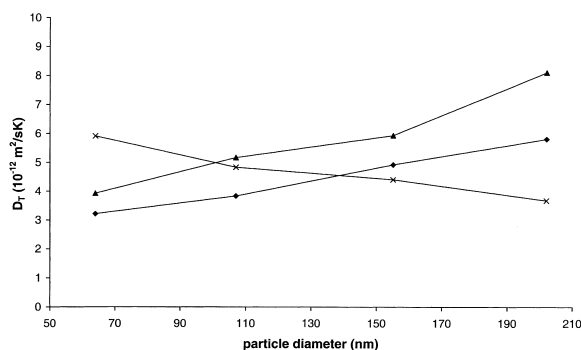


Fig. 7. Thermal diffusion coefficients versus particle diameter. (X) Water, (♦) 5 mol% ACN, (▲) 10 mol% ACN.

the addition of ACN to the carrier not only influences the retention but also the selectivity of the method.

The size dependence can be expressed by the so-called size-based selectivity, S_r , defined as [37]:

$$S_r = \frac{\partial \log \left(\frac{t_R}{t_0} \right)}{\partial \log r} \quad (5)$$

where r is the particle radius. When the thermal diffusion is independent of the particle size, an S_r value of unity is obtained. In Table 2, S_r values for the different carriers used in this study are given. For both the phosphate and the Tris buffer, the thermal diffusion coefficient appears to be almost independent of particle size, irrespective of the type of surfactant used. A strikingly low selectivity ($S_r = 0.54$), however, was found for water with TBAP as electrolyte, indicating that the D_T decreases with particle size. Much higher selectivities were found for the ACN-containing carriers; S_r values of 1.30 and 1.45 for carriers with 5 and 10% (mol/mol)

Table 2
Size-based selectivity values for PS particles in several carriers

Carrier composition	S_r
1 mM Phosphate + 1 mM Brij 35	0.99
0.5 mM Tris + 1 mM Brij 35	0.90
1 mM Phosphate + 1 mM SDS	0.94
1 mM Tris + 1 mM SDS	1.04
0.5 mM TBAP + 1 mM SDS	0.54
0.5 mM TBAP + 1 mM SDS + 5% (mol/mol) ACN	1.30
0.5 mM TBAP + 1 mM SDS + 10% (mol/mol) ACN	1.45

ACN, were found, respectively. Different size dependencies of thermal diffusion in aqueous and organic carriers were also observed in another study; no satisfactory explanation for this phenomenon has been put forward yet [17].

Although particle retention in the carrier containing only TBAP and SDS was quite high, the separation was still insufficient due to a decrease of D_T with size (see Fig. 6A). From Fig. 6 it is apparent that the best resolution is obtained with the highest ACN content (10%, mol/mol). Due to the remarkably high size selectivity, all particles could be baseline separated at a ΔT value as low as 20 K. Unfortunately, it was not possible to use higher concentrations of ACN as, in that case, broad and irregular peaks eluted, indicating that some particle–wall interaction occurred.

5. Conclusions

Particle thermal diffusion and, consequently, particle retention in ThFFF are highly sensitive to small changes in the carrier composition. Accurate particle analysis requires, therefore, careful tuning of the experimental conditions.

We found that the addition of an electrolyte for sufficient particle retention and a surfactant to prevent particle–wall interactions are required. Furthermore, the results of this study indicate that thermal diffusion is mainly determined by the interaction between the particle's surface and the carrier.

A study of the size selectivity showed that the thermal diffusion coefficient of particles often changes with particle size. Moreover, the measurements showed that this size dependency can change with carrier composition. By carefully selecting the carrier composition very high size selectivities can be obtained. Though the high size selectivity has a positive influence on the separation power, in general, the carrier composition-dependent size selectivity complicates particle analysis. With every change in the composition of the carrier a new calibration of the system is required. Fortunately, at least in the case of particle size analysis, this drawback can be circumvented by coupling ThFFF to an absolute size-detection technique such as multi-angle light scattering (MALS). The determination of the chemi-

cal composition of particles by ThFFF becomes, however, very difficult. More systematic studies of ThFFF retention of particles and, in particular, the thermal diffusion should be done to further improve the applicability of ThFFF for particle analysis.

6. Nomenclature

D	Diffusion coefficient, m^2/s
D_T	Thermal diffusion coefficient, $m^2/s K$
H	Plate height, m
k	Boltzmann's constant, J/K
r	Particle radius, m
S_r	Size-based selectivity
t_R	Retention time
t_0	Void time
ΔT	Temperature drop, K
T	Absolute temperature, K
T_c	Cold wall temperature, K
w	Channel height, m
η	Carrier viscosity, Pa s
$\langle v \rangle$	Mean flow velocity, m
ξ	λ -dependent function in the plate height equation
λ	Dimensionless retention parameter

Acknowledgements

We thank D. Buitengeweg for his assistance with the ThFFF measurements.

References

- [1] J.C. Giddings, *Science* 260 (1993) 1456.
- [2] J.C. Giddings, X. Chen, K.-G. Wahlund, M.N. Myers, *Anal. Chem.* 59 (1987) 1957.
- [3] S. Lee, S.P. Rao, M.H. Moon, J.C. Giddings, *Anal. Chem.* 68 (1996) 1545.
- [4] J.C. Giddings, F.-S. Yang, *J. Colloid Interface Sci.* 105 (1985) 55.
- [5] J.C. Giddings, M.H. Moon, P.S. Williams, M.N. Myers, *Anal. Chem.* 63 (1991) 2869.
- [6] G. Liu, J.C. Giddings, *Chromatographia* 34 (1992) 483.
- [7] M.E. Schimpf, L.M. Wheeler, P.F. Romeo, in: T. Provder (Ed.), *Chromatography of Polymers*, ACS Symposium Series, Vol. 521, ACS, Washington, DC, 1993, Chapter 5.

- [8] E.P.C. Mes, R. Tijssen, W.Th. Kok, *Chromatographia* 50 (1999) 45.
- [9] P.M. Shiundu, J.C. Giddings, *J. Chromatogr. A* 715 (1995) 117.
- [10] S.J. Jeon, M.E. Schimpf, *Polym. Mater. Eng. Sci.* 75 (1996) 4.
- [11] S.J. Jeon, M.E. Schimpf, A. Nyborg, *Anal. Chem.* 69 (1997) 3442.
- [12] M. Martin, P.S. Williams, in: F. Dondi, G. Guiochon (Eds.), *Theoretical Advancement in Chromatography and Related Separation Techniques*, NATO ASI Series C, Kluwer, Dordrecht, 1992, p. 513.
- [13] M. Martin, *J. Chromatogr. A* 831 (1999) 73.
- [14] M.E. Schimpf, J.C. Giddings, *J. Polym. Sci., Part B: Polym. Phys.* 27 (1989) 1317.
- [15] P.M. Shiundu, E.E. Remsen, J.C. Giddings, *J. Appl. Polym. Sci.* 60 (1996) 1695.
- [16] S.J. Jeon, M.E. Schimpf, in: T. Provder (Ed.), *Particle Size Distribution III: Assessment and Characterization*, ACS, Washington, DC, 1998, Chapter 13.
- [17] P.M. Shiundu, G. Liu, J.C. Giddings, *Anal. Chem.* 67 (1995) 2705.
- [18] M.E. Schimpf, J.C. Giddings, *Macromolecules* 20 (1987) 1561.
- [19] M.E. Hovingh, G.H. Thomson, J.C. Giddings, *Anal. Chem.* 42 (1970) 195.
- [20] J.C. Giddings, *Sep. Sci. Technol.* 13 (1978) 241.
- [21] M.R. Schure, M.E. Schimpf, P.D. Schettler, in: M.E. Schimpf, K. Caldwell, J.C. Giddings (Eds.), *Field-Flow Fractionation Handbook*, Wiley-Interscience, New York, 2000, Chapter I.
- [22] M.E. Hansen, J.C. Giddings, *Anal. Chem.* 61 (1989) 811.
- [23] Y. Mori, K. Kimura, M. Tanigaki, *Anal. Chem.* 62 (1990) 2668.
- [24] P.S. Williams, Y. Xu, P. Reschiglian, J.C. Giddings, *Anal. Chem.* 69 (1997) 349.
- [25] A.C. van Asten, W.Th. Kok, R. Tijssen, H. Poppe, *J. Polym. Sci., Part B: Polym. Phys.* 34 (1996) 283.
- [26] C.A. Rue, M.E. Schimpf, *Anal. Chem.* 66 (1994) 4054.
- [27] D.S. Viswanath, G. Natarajan, *Data Book on the Viscosity of Liquids*, Hemisphere Publishing, New York, 1989.
- [28] R.C. Reid, J.M. Prausnitz, B.E. Poling, *The Properties of Gases and Liquids*, 4th ed., McGraw-Hill, New York, 1987.
- [29] J. Li, P.W. Carr, *Anal. Chem.* 69 (1997) 2530.
- [30] A.C. van Asten, H.F.M. Boelens, W.Th. Kok, H. Poppe, P.S. Williams, J.C. Giddings, *Sep. Sci. Technol.* 29 (1994) 513.
- [31] J.C. Giddings, Y.H. Yoon, K.D. Caldwell, M.N. Myers, M.E. Hovingh, *Sep. Sci.* 10 (1975) 447.
- [32] M.E. Hansen, J.C. Giddings, *Anal. Chem.* 61 (1989) 811.
- [33] R.J. Hunter, *Foundations of Colloid Science*, Vol. 1, Oxford University Press, New York, 1987.
- [34] W. Brown, J. Zhao, *Macromolecules* 26 (1993) 2711.
- [35] J. Zhao, W. Brown, *J. Phys. Chem.* 100 (1996) 3775.
- [36] A.C. van Asten, W.Th. Kok, R. Tijssen, H. Poppe, *J. Polym. Sci., Part B: Polym. Phys.* 34 (1996) 297.
- [37] M.N. Myers, J.C. Giddings, *Anal. Chem.* 54 (1982) 2284.