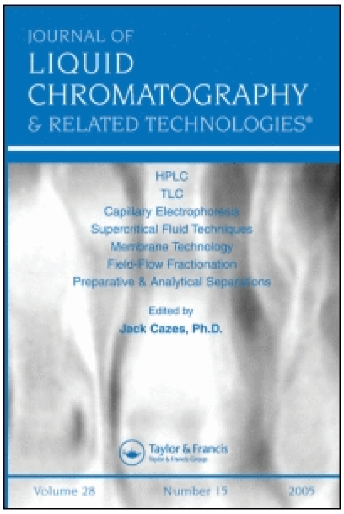


Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Characterization of Chromatographic Silica Beads by Micro-Thermal Field-Flow Fractionation

Irina A. Ananieva[a][b]; Milan Minárik[c]; René Boutin[a]; Oleg A. Shpigun[b]; Josef Janča[a]
[a] Université de La Rochelle, Pôle Sciences et Technologie, La Rochelle Cedex 01, France [b] Chemistry Department, Lomonosov Moscow State University, Moscow, Russia [c] Watrex Praha, Prague, Czech Republic

To cite this Article Ananieva, Irina A. , Minárik, Milan , Boutin, René , Shpigun, Oleg A. and Janča, Josef(2004) 'Characterization of Chromatographic Silica Beads by Micro-Thermal Field-Flow Fractionation', Journal of Liquid Chromatography & Related Technologies, 27: 15, 2313 — 2327
To link to this Article: DOI: 10.1081/JLC-200028129
URL: http://dx.doi.org/10.1081/JLC-200028129

JOURNAL OF LIQUID CHROMATOGRAPHY & RELATED TECHNOLOGIES®
Vol. 27, No. 15, pp. 2313–2327, 2004

Characterization of Chromatographic Silica Beads by Micro-Thermal Field-Flow Fractionation

Irina A. Ananieva,[1,2] Milan Minárik,[3] René Boutin,[1]
Oleg A. Shpigun,[2] and Josef Janča[1,*]

[1]Université de La Rochelle, Pôle Sciences et Technologie,
La Rochelle, France
[2]Chemistry Department, Lomonosov Moscow State University,
Moscow, Russia
[3]Watrex Praha, Prague, Czech Republic

ABSTRACT

Micro-thermal field-flow fractionation (micro-TFFF) was used to characterize silica based chromatographic beads within the diameter range from 3 to 10 μm. The main goal of this study was to demonstrate that silica particles that sediment in water suspensions and are usually separated by gravitational field-flow fractionation (GFFF), can be retained more effectively under the conditions of micro-TFFF. It has been found, that the retentions of all studied particles substantially increased when the

---

[*] Correspondence: Josef Janča, Université de La Rochelle, Pôle Sciences et Technologie, Avenue Michel Crépeau, 17042 La Rochelle Cedex 01, France; E-mail: jjanca@univ-lr.fr.

DOI: 10.1081/JLC-200028129 1082-6076 (Print); 1520-572X (Online)
www.dekker.com

temperature drop, ΔT, across the micro-TFFF channel was applied, in comparison with the retentions when only the gravitation was acting as an effective field at $\Delta T = 0\,\mathrm{K}$. This finding confirmed that micrometer size and high-density silica particles exhibit a positive thermal diffusion coefficient. Various operational conditions were tested up to extreme temperature drop, $\Delta T = 80\,\mathrm{K}$, at the temperature of the cold wall $T_c = 300\,\mathrm{K}$. As a result, the time of micro-TFFF analysis can be as short as 10 min. The samples were also characterized by scanning electron microscopy (SEM). The comparison of the results of micro-TFFF and SEM showed good agreement of the mean particle sizes and of the particle size distributions (PSD) estimated from the SEM pictures with the peak retentions, widths, and shapes of the micro-TFFF fractograms. Micro-TFFF was found as a rapid and easily applicable method for the characterization of the mean particle size and PSD of the chromatographic beads. This is an important conclusion because micro-TFFF can, thus, become a convenient and inexpensive analytical tool for the quality control of the uniformity of chromatographic beads, which determines the efficiency of the packed columns.

Key Words: Micro-thermal field-flow fractionation; Scanning electron microscopy; Chromatographic silica beads.

INTRODUCTION

Porous silica based or cross-linked polymer particles are the most popular supports for the packed columns used in contemporary high performance liquid chromatography. It is a well-known fact that the highest efficiency of the columns is achieved if the spherical particles of sufficiently uniform size are used. Their diameter lies usually in the range from 1 to 10 μm for micro-bore or packed capillary columns, and up to 20 μm for standard size columns for various techniques of liquid chromatography. The determination of the particle size distribution (PSD) and of the mean particle diameter of the chromatographic support particles is, thus, of primary importance.

The application of newly developed micro-thermal field-flow fractionation[1] (micro-TFFF) seemed to be an interesting challenge, promising to open new ways in exploring the potential of this separation method. We have already shown,[2–4] that micro-TFFF can successfully be applied for the separation of large size polystyrene latex particles, whose diameters were comparable with the diameter of the chromatographic beads. The focusing was the dominating separation mechanism, while steric mechanism was found to be a rather exceptional case.[2–4] On the other hand, the high density and large size of the silica beads concerned in this study cause their rapid sedimentation in the gravitational field. This is a completely different situation with respect to the dominating separation mechanism. Consequently, the operational conditions of micro-TFFF have to be optimized with respect to these facts, in order to achieve an effective separation and characterization of the silica beads, which is competitive in comparison with the separations by other methods.

Silica beads were separated and characterized until now with variable success by gravitational field flow fractionation (GFFF),[5–7] sedimentation FFF,[8,9] and flow FFF.[10] The GFFF[6,7] was of special interest, because the home-made channel of standard size, built up in the late eighties, was quite inexpensive. The application of micro-TFFF could then be challenging from several points of view: (i) simultaneous exploitation of the gravitational force and thermophoresis can lead to higher performance of the fractionation; (ii) versatile micro-TFFF channel represents a suitable technique, which seems to be very universal, as concerns its already proven applications for the separation and characterization of macromolecules and colloidal particles; (iii) miniaturization opens more extended possibilities of variation of the operational parameters, due to substantially lower heat flux across the channel; (iv) samples to be characterized can be as small as a few nanograms; (v) consumption of the carrier liquid for one analysis is minimized, thus, allowing the use of special solvents.

EXPERIMENTAL

The apparatus for micro-TFFF used in this work was a prototype, which consisted of an intelligent pump model PU-980 (Jasco, Japan), an injection valve model 7410 (Rheodyne, USA) with a 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 model 2003 was designed in our laboratory and fabricated by Lascialfari, SARL (La Rochelle, France). The details were described previously,[1] its thickness chosen for this work was 0.250 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 the 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% of detergent Brij 78 (Fluka, Germany) and 3 mM NaCl was used as the carrier liquid. The silica beads, whose data are given in Table 1, were supplied by Watrex-Praha.

A scanning electron microscopy (SEM) apparatus (Model Jeol 5410 LV, Japan) was used to visualize the form, size, and PSD of the individual silica samples.

RESULTS AND DISCUSSION

Retention of Silica Particles in Gravitational Field with a Superimposed Temperature Gradient

It has previously been demonstrated,[6,7] that silica particles of the size comparable with the samples studied in this work sediment under the effect of gravitation in GFFF. A recent study[11] proved that the silica particles of such a size exhibit measurable thermophoresis in various simple liquids and, thus, are retained under the conditions of standard TFFF. Three simple carrier liquids were tested,[11] water, acetonitrile, and heptane. The highest thermophoretic mobility was found for silica, as well as for octadecylsilane modified silica, suspended in water based carrier liquid. On the other hand, taking into account the density and viscosity of the mentioned carrier liquids, sedimentation velocity of the particles should be the lowest in water.

Table 1. Designation of studied silica beads.

Silica bead sample	Nominal average particle diameter (μm)	Supplier	Name of commercial product
Silica 3	3	Dr. Maisch, HPLC GmbH, Germany	ReproSil-Pur ODS-3, 3 μm
Silica 5a	5	Lachema, Brno, Czech Republic	Silasorb C2 (LC)
Silica 5b	5	Dr. Maisch, HPLC GmbH, Germany	ReproSil-Pur ODS-3, 5 μm
Silica 5c	5	Macherey Nagel, Germany	Nucleosil 100-5
Silica 5d	5	Beckman, USA	Ultrasphere ODS 5 μm
Silica 10	10	Dr. Maisch, HPLC GmbH, Germany	ReproSil-Pur C-18 AQ, 10 μm
Silica X	Not given	Phase separations, UK	Spherisorb ODS-2 5U

First measurements were, thus, aimed to check whether water based carrier liquid can be used under extreme temperature drop, ΔT conditions, to take advantage of the synergy of sedimentation and thermodiffusion, and maximize the retention. The composition of the carrier liquid was optimized to attenuate the electrostatic charges of the particles and, thus, to avoid their excessive repulsive interactions, and to minimize their adsorption on the accumulation wall.

The dependence of the retention of Silica 3 sample on the temperature drop, ΔT, is demonstrated in Fig. 1. The retention volume is practically doubled for $\Delta T = 40\,\mathrm{K}$ in comparison with $\Delta T = 0\,\mathrm{K}$, and tripled for $\Delta T = 80\,\mathrm{K}$. It has to be stressed that the temperature of the cold wall, T_c, was 294 K for the experiments at $\Delta T = 0\,\mathrm{K}$ and $\Delta T = 40\,\mathrm{K}$, while lower cold wall temperature, $T_c = 280\,\mathrm{K}$ had to be fixed for the experiments at $\Delta T = 80\,\mathrm{K}$. Consequently, the contribution of the sedimentation to the retention should be somewhat lower at lower T_c due to higher viscosity and density of water. A substantial increase of the retention when applying the temperature drop in comparison with the action of only gravitational driving force, indicates that either the relaxation processes after the injection of the sample are not fully accomplished in GFFF, or a weaker gravitational force is not able to eliminate the opposite action of the lift forces at the given flow rate. Both effects can, of course, be present simultaneously and lead to lower retention. Obviously, the application of a stop flow procedure after the injection of the sample to accomplish the relaxation processes, as well as a decrease of the flow rate to reduce the lift forces, result in longer separation times.

The ratio of the thermophoretic mobility of the silica particles in water to that in acetonitrile was found previously[11] to be roughly 2, while the ratio of sedimentation velocity of the particles for the same couple of solvents was roughly $1/3$. Contrary to the gravitational force, ΔT can be manipulated in order to increase the total force, driving the retained particles to the accumulation wall. The simultaneous action of the gravitation and thermophoresis, thus, opens a possibility to accelerate the relaxation processes at the very beginning of the separation and, also, to compensate lift forces appearing at higher flow rates of the carrier liquid. As a result, the separation time could be substantially shortened when applying a temperature drop in comparison with the separation due to single gravitational driving force in GFFF.

Consequently, with respect to the above described experimental findings, the water seems to be the preferred carrier liquid for the separation of large silica particles by micro-TFFF from the viewpoint of higher achievable retentions at higher ΔT that can be reached in water in comparison with acetonitrile (by taking into account the freezing and boiling points of both liquids).

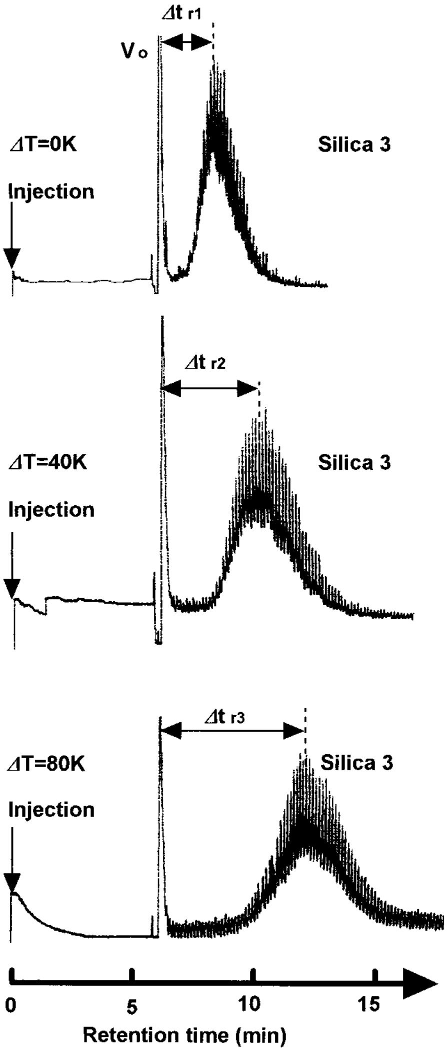


Figure 1. Fractograms of Silica 3 by micro-TFFF at different temperature drops ΔT. Experimental conditions: temperature of the cold wall, $T_c = 294\,\mathrm{K}$ for $\Delta T = 0\,\mathrm{K}$ and $\Delta T = 40\,\mathrm{K}$, and $T_c = 280\,\mathrm{K}$ for $\Delta T = 80\,\mathrm{K}$; injected volume $5\,\mu\mathrm{L}$; flow rate during 40 sec of the injection $25\,\mu\mathrm{L/min}$; stop-flow time 6 min; flow rate during the separation $250\,\mu\mathrm{L/min}$.

Retention of Silica Particles of Different Sizes and the Effect of the Injection-Stop-Flow Procedure

Figure 2 shows the important differences in retentions of three silica samples of different mean diameters. These data allowed the determination of the calibration function, i.e., of the dependence of retention volume vs. particle diameter.

All experiments whose results are shown in Figs. 1 and 2, were carried out by applying an optimized protocol, which was as follows. The injection of the sample was carried out during 40 sec at the low flow rate (0.025 mL/min). This allowed a complete introduction of the sample from the injection loop into the separation channel without an excessive broadening of the zone. The injection was followed by a stop-flow period of 6 min for the relaxation. Thereafter, higher flow rate (0.250 mL/min) was applied until the end of the separation. By applying this optimized protocol, the typical times of separation were 12–15 min (for the applied $\Delta T = 40\,\mathrm{K}$ and $\Delta T = 80\,\mathrm{K}$). However, Fig. 3 shows that at the highest applied $\Delta T = 80\,\mathrm{K}$, the relaxation period can simply be struck out without a major increase of the band broadening, but leading to an important shortening of the separation time. Thus, the above hypothesis concerning the positive and advantageous effect of the application of a temperature drop in micro-TFFF, instead of the only action of the gravitational force in GFFF, is confirmed.

Separation Mechanism

Although, obviously, the order of retentions as a function of particle size of the investigated silica beads obtained under the adopted experimental conditions corresponds to the steric mode of separation, it was interesting to check whether lift forces intervene in the separation mechanism. A comparison of the theoretical dependence of inverse retention ratio $1/R$ on the particle radius r, calculated recently[3] by using the thermal diffusion coefficient measured previously[2] for micrometer size polystyrene latex particles, with the experimental data obtained in this study for the investigated silica beads retained at the highest applied temperature drop $\Delta T = 80\,\mathrm{K}$, is shown in Fig. 4. A very close agreement of the experimental data with the theory can be seen in a part of the theoretical curve corresponding to the steric exclusion mechanism of separation. This agreement confirms the dominating mechanism of steric exclusion. Once the driving force is high enough to compress all particles to the accumulation wall, the steric mechanism controls the separation and the retention is independent on the value of the thermal diffusion coefficient. On the other hand, whenever

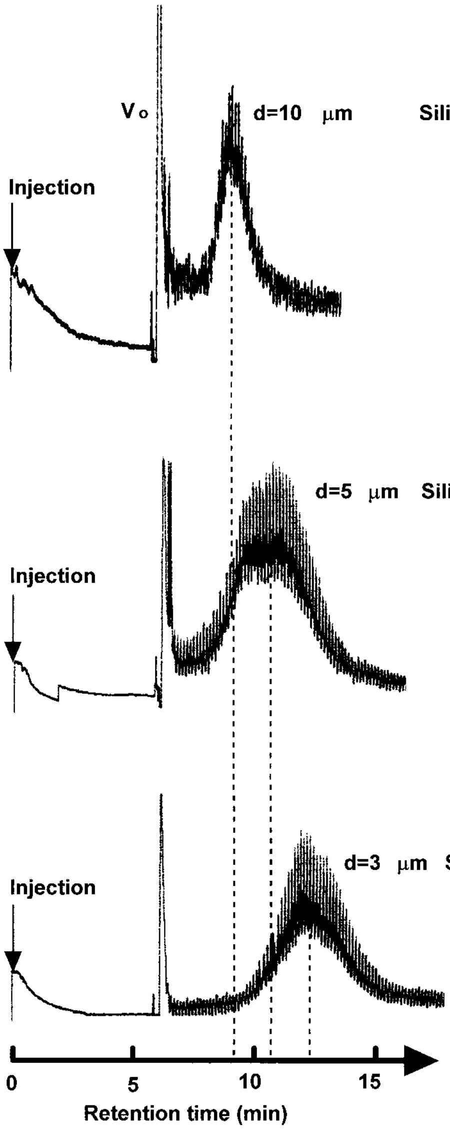


Figure 2. Fractograms of different size silica samples by micro-TFFF. Experimental conditions: temperature of the cold wall, $T_c = 280\,\mathrm{K}$, $\Delta T = 80\,\mathrm{K}$; injected volume 5 μL; flow rate during 40 sec of the injection 25 μL/min; stop-flow time 6 min; flow rate during the separation 250 μL/min.

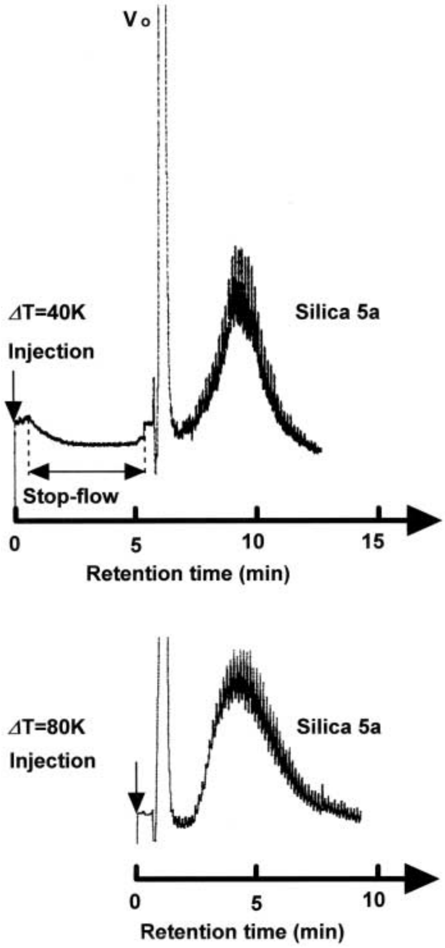


Figure 3. Fractograms of Silica 5a sample by micro-TFFF obtained under the conditions of different operational protocols. Experimental conditions: temperature of the cold wall, $T_c = 294\,\text{K}$ for $\Delta T = 40\,\text{K}$, and $T_c = 280\,\text{K}$ for $\Delta T = 80\,\text{K}$; injected volume $5\,\mu\text{L}$; flow rate during 40 sec of the injection $25\,\mu\text{L/min}$; stop-flow time 6 min at $\Delta T = 40\,\text{K}$ and 0 min at $\Delta T = 80\,\text{K}$; flow rate during the separation $250\,\mu\text{L/min}$.

the driving force was not high enough (in the experiments with $\Delta T = 0\,\text{K}$ and $\Delta T = 40\,\text{K}$) the lift forces appeared at the applied flow rate and resulted in lower retentions.

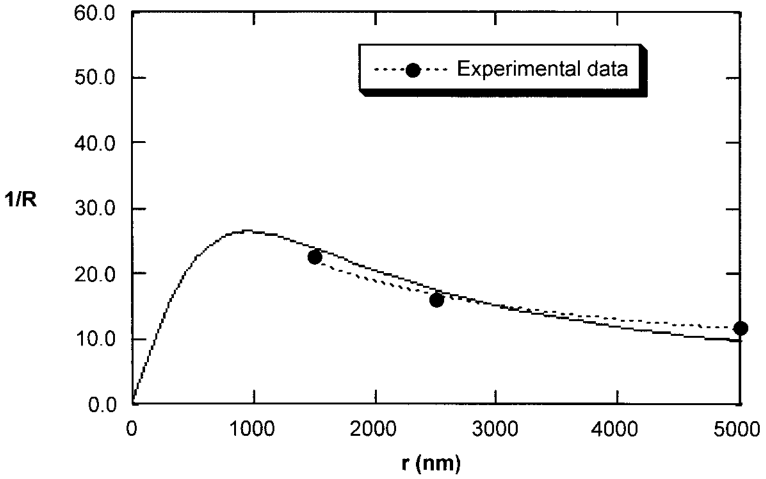


Figure 4. Comparison of the theoretical dependence of the inverse retention ratio $1/R$ on the particle radius r with the experimental data obtained from fractograms in Fig. 2. The theoretical curve is taken from Ref.[3].

Practical Use of Micro-TFFF for the Characterization of Chromatographic Beads and Comparison with SEM

A practical utility of micro-TFFF was demonstrated by separating some chromatographic supports, and by a comparison of the obtained fractograms with the photographs from SEM. The results are shown in Figs. 5 and 6.

The fractogram of a commercial sample Silica 5c shows quite narrow PSD, which is confirmed also by SEM. On the other hand, the Silica X sample exhibited an obvious bimodal PSD (see the corresponding fractogram in Fig. 5). Although, such a punctual conclusion cannot be done from the SEM measurement due to a limited number of particles undergoing the SEM analysis, the SEM photograph of the sample Silica X shows definitely the presence of the particles of very different sizes.

While a perpetual question is whether few tens or hundreds of particles that can be shown and counted on the SEM pictures can accurately represent the PSD of the whole analyzed sample, this problem does not exist with the use of micro-TFFF. A simple calculation shows that if, for example, the volume fraction of the particles whose diameter is $10\,\mu m$ is of the order of 0.01, the total number of the particles contained in a volume of $5\,\mu L$ of an injected suspension of the analyzed sample is of the order of 10^5; thus, it is largely superior to the number of particles that can usually be analyzed by

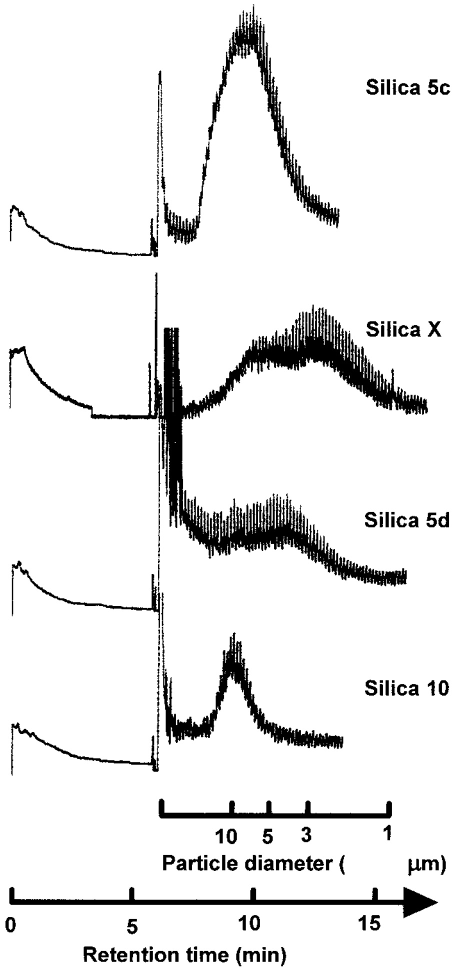
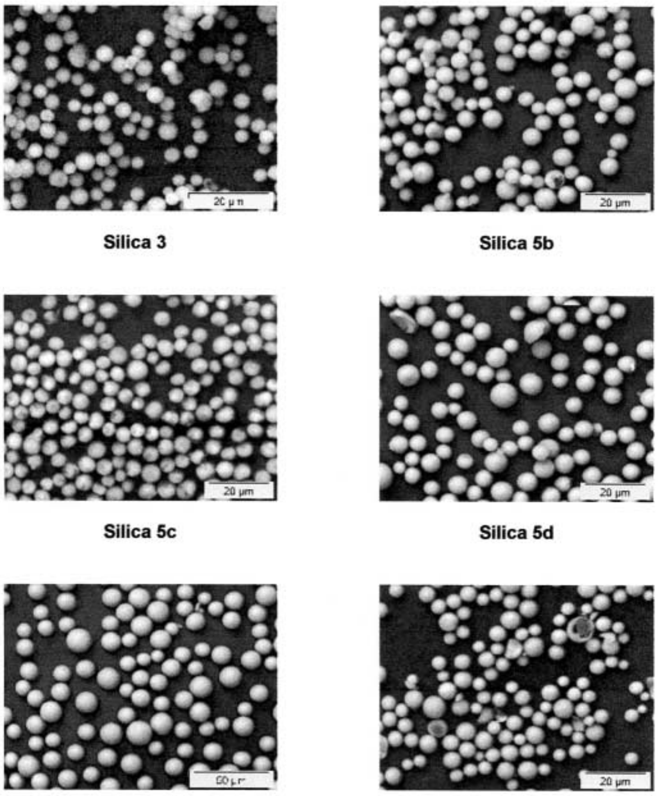


Figure 5. Fractograms of various samples of commercial silica based chromatographic supports by micro-TFFF. Experimental conditions: temperature of the cold wall, $T_c = 280\,K$, $\Delta T = 80\,K$; injected volume 5 μL; flow rate during 40 sec of the injection 25 μL/min; stop-flow time 6 min; flow rate during the separation 250 μL/min.

SEM and still allow a large space in the variation of the injected volume and concentration required to optimize signal-to-noise ratio of the applied detector.

The fractogram of the sample of Silica 5d shows that a major part of the particles has a diameter around 5 μm, however, an important part of the



Silica 3 Silica 5b

Silica 5c Silica 5d

Silica 10 Silica X

Figure 6. SEM pictures of the investigated silica based particles.

particles has a diameter of 10 μm and larger. These particles are unresolved from the void volume peak at V_0. The SEM picture (see Fig. 6) confirms this observation.

Silica 10 sample has a nominal average particle diameter 10 μm. The corresponding fractogram in Fig. 5 clearly shows a maximum of the PSD near 10 μm, but also an important portion of the particles whose diameter is larger (unresolved part of the fractogram). The resulting average particle size can, of course, agree with the nominal value. Although SEM does not provide a quantitative result in terms of mean particle diameter, the corresponding picture in Fig. 6 shows many particles whose diameter is close to 10 μm, but also the presence of the particles whose diameter is almost 20 μm, thus, the result coherent with micro-TFFF fractogram.

The remaining SEM pictures in Fig. 6 are those of the samples Silica 3 and Silica 5b. The picture of Silica 3 shows quite homogeneous PSD found also by micro-TFFF (see the fractograms in Figs. 1 and 2). The SEM picture of Silica 5b sample exhibits some dispersion of the particle sizes with a major part of the particles whose diameter is close to 5 μm, but also a not negligible part of the particles whose diameter is different. The corresponding fractogram in Fig. 2 seems to indicate a bimodality of the PSD of this sample.

The raw fractograms in Figs. 1, 3, and 5 exhibit more or less important noise that seems to increase with the increasing size of the particles. Although the signal-to-noise ratio is relatively important in some cases, it does not preclude the correct interpretation of the fractograms. This is demonstrated in Fig. 7, where a simple and relatively narrow fractogram of the Silica 3 sample and a complex, bimodal fractogram of the Silica X sample are reproduced together with the smoothed fractograms, which result from the application of a powerful software.[a] It can be clearly seen from Fig. 7, that the simple and narrow smoothed fractogram, as well as a more complex bimodal smoothed fractogram, reproduce quite well their raw originals and, as a result, the estimation of the PSD from the smoothed fractograms should be accurate.

We continue our efforts in the direction to increase the stability of the signal of the detector by the appropriate modifications of some crucial technical details of the micro-TFFF apparatus, by the use of an electronic filtering of the detector signal, and by the application of the appropriate smoothing software. Nevertheless, even at the recent state-of-the-art, the results of micro-TFFF, obtained when separating and characterizing large micrometer size and high-density particles, are satisfactory.

CONCLUSIONS

Micro-TFFF has been confirmed as a convenient method for the characterization of large size particles, such as the chromatographic beads. The experimental protocol concerning the injection and stop-flow for relaxation procedure was optimized. Whenever a minor decrease in efficiency of the fractionation is acceptable, the time of the analysis can be shortened by striking out the stop-flow period. The practical application of micro-TFFF to the characterization of chromatographic supports, revealed the fact that only some of them exhibit some degree of uniformity as concerns the PSD. Quite surprisingly, some supports are rather heterogeneous from this point of view and exhibit even bimodal PSD, which might be caused by mixing

---

[a] Un-Scan-It Gel™, Silk Scientific Inc., USA.

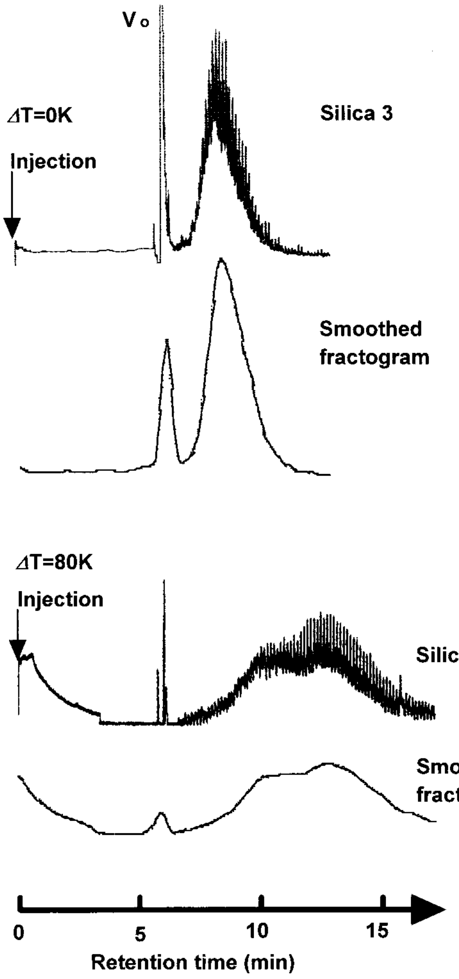


Figure 7. Comparison of the raw fractograms of Silica 3 sample exhibiting monomodal PSD and of Silica X sample exhibiting bimodal PSD with their smoothed versions.

the particles of close but different sizes. The comparison of micro-TFFF with SEM revealed a good coherency of results, however, micro-TFFF seems to be able to provide more detailed and quantitative results in less time.

ACKNOWLEDGMENT

This work was supported by the Regional Council of Poitou-Charentes.

REFERENCES

1. Janča, J. Micro-channel thermal field-flow fractionation: new challenge in analysis of macromolecules and particles. J. Liq. Chromatogr. Rel. Technol. **2002**, *25*, 683–704.
2. Janča, J.; Ananieva, I.A. Micro-thermal field-flow fractionation in the characterization of macromolecules and particles: effect of the steric exclusion mechanism. e-Polymers **2003**, (34).
3. Janča, J.; Ananieva, I.A.; Menshikova, A.Yu.; Evseeva, T.G. Micro-thermal focusing field-flow fractionation. J. Chromatogr. B **2004**, *800*, 33–44.
4. Ananieva, I.A.; Menshikova, A.Yu.; Evseeva, T.G.; Janča, J. High-speed micro-thermal focusing field-flow fractionation of micron-size particles. Coll. Czech. Chem. Comm. **2004**, *69*, 322–329.
5. Giddings, J.C.; Myers, M.N.; Caldwell, K.D.; Pav, J.W. Steric FFF as a tool for the size characterization of chromatographic supports. J. Chromatogr. **1979**, *185*, 261–271.
6. Pazourek, J.; Urbankova, E.; Chmelik, J. Experimental study on the separation of silica gel supports by gravitational field-flow fractionation. J. Chromatogr. A **1994**, *660*, 113–118.
7. Pazourek, J.; Chmelik, J. Experimental study on the separation of silica gel supports by gravitational field-flow fractionation. II. Sample preparation, stop-flow procedure and overloading effect. J. Chromatogr. A **1995**, *715*, 259–265.
8. Giddings, J.C.; Moon, M.H.; Williams, P.S.; Myers, M.N. Particle size distribution by sedimentation/steric FFF: development of a calibration procedure based on density compensation. Anal. Chem. **1991**, *63*, 1366–1372.
9. Giddings, J.C.; Moon, M.H. Measurement of particle density, porosity, and size distributions by sedimentation/steric field-flow fractionation. Application to chromatographic supports. Anal. Chem. **1991**, *63*, 2869–2877.
10. Ratanathanawongs, S.K.; Giddings, J.C. High-speed size characterization of chromatographic silica by flow/hyperlayer field-flow fractionation. J. Chromatogr. **1989**, *467*, 341–356.
11. Regazetti, A. Thermophorèse de particules microniques de silice par la méthode de fractionnement par couplage flux-force avec gradient thermique. Université d'Aix-Marseille III, May 2002; Ph.D. Thesis, No. O2AIX30019.

Received April 5, 2004
Accepted May 1, 2004
Manuscript 6363