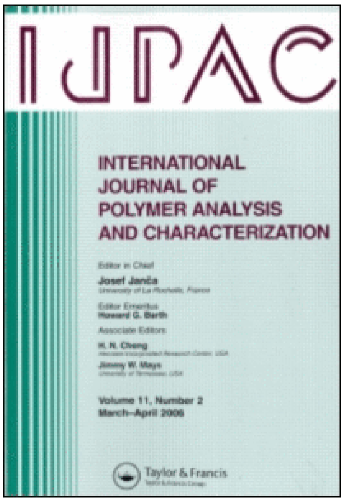

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Micro-Thermal Field-Flow Fractionation in the Analysis of Polymers and Particles: A Review

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Micro-Thermal Field-Flow Fractionation in the Analysis of Polymers and Particles: A Review

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Abstract: The benefits of the miniaturization of thermal field-flow fractionation (TFFF) channel are reviewed in order to demonstrate that high-performance separations can be achieved with a micro-channel of optimized construction working under carefully chosen experimental conditions and operational variables. Micro-TFFF is highly competitive in comparison with size exclusion chromatography (SEC) of macromolecules for molar masses up to approximately one million g/mol. However, the versatility of micro-TFFF is superior to SEC for macromolecules of ultra high molar masses not only because there is not an inherent highest molar mass limit of the macromolecules that can be separated by micro-TFFF in contrast to SEC but also, as proved experimentally, due to the fact that shear degradation of the macromolecular coils, a well-known phenomenon in SEC, does not occur in micro-TFFF. Such mild conditions permit the analysis of macromolecular aggregates, micro-gels, and similar associative structures without any destruction. Moreover, colloidal submicron and micron-sized particles of synthetic, natural, or biological origin can also be separated and characterized by micro-TFFF without any modification of the separation system. The free choice of the carrier liquids affords an another advantage to micro-TFFF.

It has been predicted theoretically and proven experimentally that high resolution is achieved more efficiently by increasing the temperature drop across the separation channel than with a decrease in channel thickness. This is due to the fact that the total heat flow between the hot and cold walls is substantially reduced in micro-TFFF. This makes the operation of the micro-TFFF system

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not only 30 times more economical from the viewpoint of electrical energy consumption but also safer. The experimental implementation and application to polymer and particle analysis confirmed the potential of micro-TFFF.

Keywords: Polymers; Colloidal particles; Micrometer-sized particles; Chromatographic beads analysis; Micro-thermal field-flow fractionation

INTRODUCTION

Thermal field-flow fractionation (TFFF) was invented and applied to the separation of polystyrenes in 1967.[1] It is the oldest of all field-flow fractionation (FFF) methods, conceptually proposed by Giddings.[2] Its universal applicability for the fractionation of various polymers was demonstrated in 1979.[3] Although several applications of TFFF to the analysis of polymers and colloidal particles were published and reviewed[4,5] and TFFF became a high-performance method currently used for the determination of the molar mass distribution (MMD) of polymers, nowadays commercially available TFFF channels,[6] as well as customi-built units,[7] have essentially the same size as those constructed at the very beginning[1]: approximately 50 × 2 × 0.01 cm.

The first successful attempt to reduce the size of the TFFF channel was published in 1978 by Giddings et al.,[8] who demonstrated that reducing the channel thickness from 0.0127 to 0.0051 cm resulted in efficient separation of polystyrenes in a limited range of molar masses. Their results are shown in Figure 1.

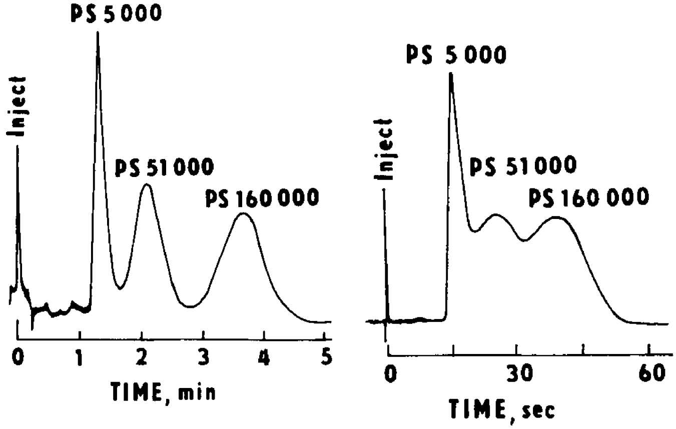

Figure 1. Fractograms of a mixture of three PS standards obtained at various flow rates in a TFFF channel of reduced thickness, w = 0.051 cm. Reproduced with permission from Giddings et al.[8]

In 1993, Giddings[9] concluded theoretically that miniaturization of the FFF channels can provide only limited advantages. Nevertheless, as pointed out later,[10] closer look at the theory of FFF reveals that although most FFF systems do not benefit significantly from miniaturization, a few FFF techniques could benefit. Indeed, the first micro-electrical FFF was introduced in 2002 for the separation of latex particles[11] and the first micro-dielectrophoretic FFF was reported in 1999.[12]

Finally, a problematic micro-TFFF construction has been proposed by Edwards et al.,[13] and, simultaneous, an actually functioning micro-TFFF has been constructed and verified by Janča.[14] Whereas only one not-convincing separation was described by Edwards et al.,[13] a series of repeatable and reproducible results reviewed in this report were published with the use of the channel described by Janča.[14]

The crucial problem of the fractogram published by Edwards et al.[13] is that whereas the theory for the normal, polarization FFF mechanism predicts larger peaks for the retained species than the peak of an unretained solute, just the opposite behavior can be seen in Figure 2, representing the fractogram of Edwards et al.[13]. The experimental plate height, H, calculated for the first, unretained peak in Figure 2 is 53 times

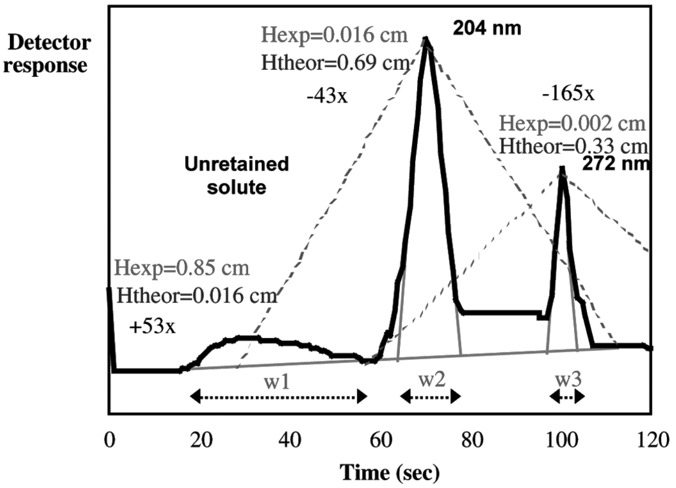

Figure 2. Fractogram of a mixture of two polystyrene latexes (PSL) published in Edwards et al.[13] and superimposed by a demonstration of the band widths w_1, w_2, and w_3 of the unretained solute and of the "retained" PSL samples of particle diameters 204 nm and 272 nm, respectively. The plate heights H_{theor} and H_{exp} are calculated from the general theory of FFF and from the concerned experimental peaks, which are 53× higher, 43× lower, and 165× lower, than values of the theory for the corresponding peaks w_1, w_2, and w_3, respectively.[33]

higher than that in the theory, thus showing very low efficiency of the micro-TFFF channel. On the other hand, the experimental plate heights calculated for the two peaks supposed to correspond to the separation of 204 nm and 274 nm diameter polystyrene latex (PSL) particles are 43 times and 165 times (!) lower than the theoretical values. Moreover, the effective temperature drop inside the channel was 4.8 K, with the real temperature difference of 46 K measured on the outer sides of the channel.

It is unrealistic to establish inside such a channel a high temperature drop of the order of 40 K and higher necessary for most separations already achieved in standard-size TFFF channels. The reduced thickness, 0.0027 cm, of the channel described by Edwards et al.[13] does not represent a good solution, as will be discussed further in this review. As a result, it is not impossible to imagine that in the future more sophisticated technologies will be used than the mechanical micromachining used in constructing our channel,[14] but it will certainly be not the solution proposed by Edwards et al.[13] Our experimental results discussed below demonstrate firmly the impossibility or reproducing the experiment described by Edwards et al.[13] and shown in Figure 2. The fractogram in Figure 2 is probably an artifact.

APPLICATIONS OF MICRO-TFFF

Contrary to the conclusions of a previous study,[9] it has been found[14] that the miniaturization of the TFFF channel is useful, resulting in reduced carrier liquid and energy consumption and, more importantly, in an extended range of the operational conditions that can easily be utilized. It has been found theoretically[14] and proven experimentally[15] that an increase in resolution can be achieved more efficiently by increasing the temperature drop across the channel than with a decrease in channel thickness. Such performance can be obtained because the total heat flow between the hot and cold walls is substantially reduced in the micro-TFFF channel.

Besides the normal, polarization mechanism, the focusing mechanism can actively be used in micro-TFFF. The result is, for example, very good separation of micron-sized PSL particles[16,17] represented in Figure 3, which proves that a substantial increase in the flow rate results in high-speed separation without an important decrease in resolution.

Fractionation of Synthetic Polymers

Polystyrenes (PS) were used as model samples to demonstrate the performance of micro-TFFF in the most usual range of molar masses and

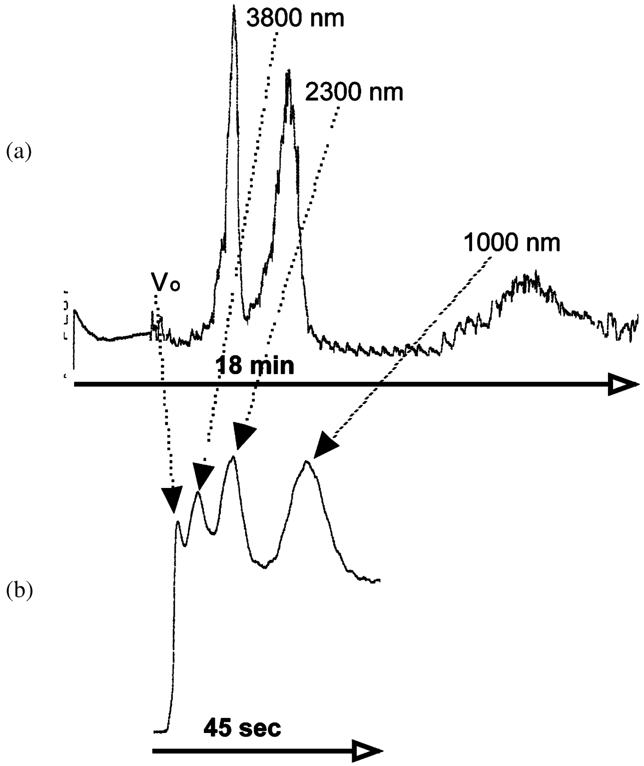

Figure 3. High-speed separation of a mixture of three polystyrene latexes obtained at various flow rates in micro-TFFF channel under the conditions of effective focusing mechanism. Reproduced with permission from Ananieva et al. and Janča et al.[16,17]

to compare the results with size exclusion chromatography (SEC).[14,18] The fractograms of three PS of different molar masses in Figure 4 represent high-resolution separation achieved by micro-TFFF. This resolution is comparable with that obtained in SEC. However, higher resolution is achieved in micro-TFFF, than with SEC for a separation of ultra high molar mass (UHMM) PS.[18] In this case, a programmed decrease in ΔT was applied to shorten the time of analysis.[19]

A detailed study of the behavior of UHMM PS fractionated initially by frontal TFFF under the conditions of high shear stress and then analyzed by micro-TFFF under mild conditions did not reveal any noticeable shear degradation of polymer chains observed frequently when separated by SEC.[20]

A challenging but not yet sufficiently explored application of micro-TFFF seems to be the characterization of the branched polymers.

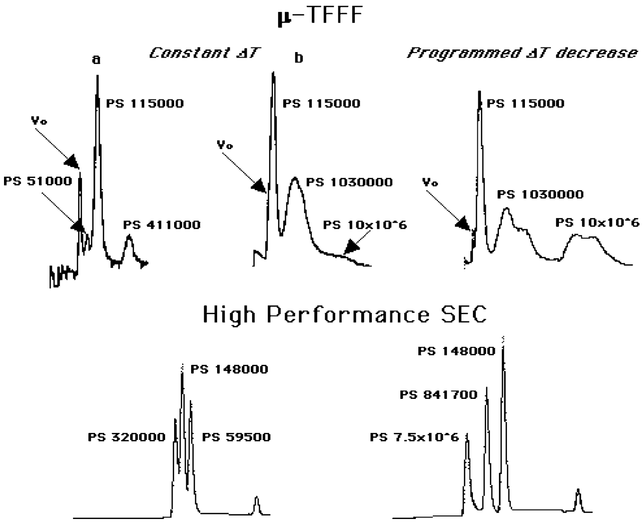

Figure 4. Comparison of the separations of polystyrenes carried out by micro-TFFF at constant and programmed decrease in temperature drop with the chromatograms from high-performance SEC. Reproduced with permission from Janča.[18,19]

Podzimek et al.[21] have shown recently that the elution order of highly branched polystyrenes in SEC does not follow a monotonous dependence of the elution volume as a function of the molar mass; see Figure 5. This unusual behavior has been found by using a molar mass sensitive multi-angle laser light-scattering (MALS) detector with the SEC column. On the other hand, the elution volume versus molar mass dependence obtained by using the MALS detector coupled with the TFFF channel was monotonous. This finding indicates that the retention in TFFF is the function of the molar mass regardless, as a first approximation, of the structure of the polymer chains. Consequently, the use of SEC seems to be problematic, at least for highly branched polymers. However, more extensive investigation of this peculiar behavior is necessary.

Fractionation of Ultrahigh Molar Mass Polymers

As mentioned above, an important goal was to elucidate the problem of polymer degradation under the conditions of TFFF. An unidentified peak emerging at the retention volume equal to two void volumes of the channel observed in experiments with the use of the stop-flow procedure was attributed by Chubarova and Nesterov[22,23] to low molar

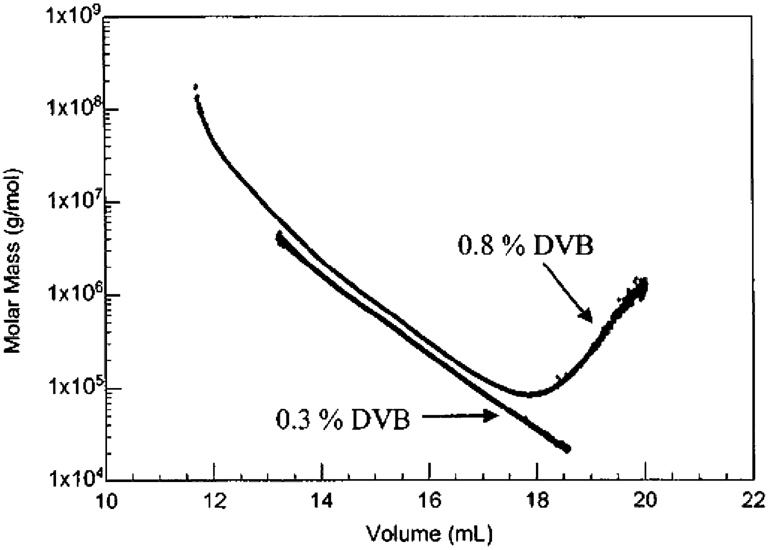

Figure 5. Separations of differently branched polystyrenes carried out by SEC coupled with MALS detector. Reproduced with permission from Podzimek et al.[21]

mass products of polymer degradation. Contrary to this conclusion, we have found[20] that the above-mentioned peak can clearly be identified as an artifact related to the use of tetrahydrofurane as carrier liquid. As a result, the concerned experimental findings[22,23] cannot seriously be considered as a proof of polymer degradation. The frontal TFFF with the use of a polymer solution as a carrier liquid and the refractionation of the collected fractions by micro-TFFF confirmed that the probability of degradation of polymers in TFFF experiments, performed under optimum conditions, is practically zero. All our results proved that ultra high molar mass polymers can correctly be analyzed by TFFF or micro-TFFF.[20]

Fractionation of Nanometer-Sized Particles

The comparison of transmission electron microscopy (TEM), dynamic light scattering (DLS), hydrodynamic chromatography (HC), and micro-TFFF applied to the determination of the particle size distribution (PSD) of PS latex particles demonstrated that micro-TFFF had the highest performance.[15] Some of the results of this comparison are shown in Figure 6. Extensive studies of the effect of various operational parameters on the separation of nanometer-sized particles confirmed the high

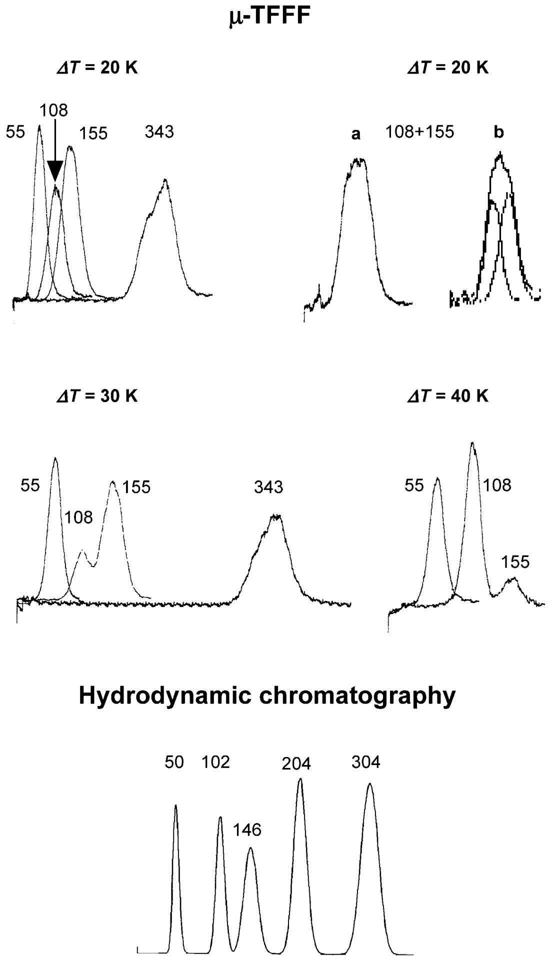

Figure 6. Comparison of micro-TFFF and HC applied to particle size analysis. Reproduced with permission from Janča et al.[15]

performance of micro-TFFF applied to the analysis and characterization of these species.[24–26]

Fractionation of Micrometer-Sized Particles

Besides the high-performance and high-speed separations of micrometer-sized particles performed by the focusing mechanism,[27,28] micro-TFFF was used to characterize silica-based chromatographic beads in the size range 3–10 μm.[29] It has been shown that silica particles, which sediment in water suspensions and are usually separated by gravitational field-flow fractionation (GFFF), are retained more when exposed to the temperature gradient. Micro-TFFF was found to be a fast and easily applicable method for the determination of the PSD of chromatographic beads, and thus it can become a convenient and inexpensive analytical tool for quality control of the uniformity of chromatographic beads. Figure 7 shows some results of this study. Interesting but not yet fully understood results

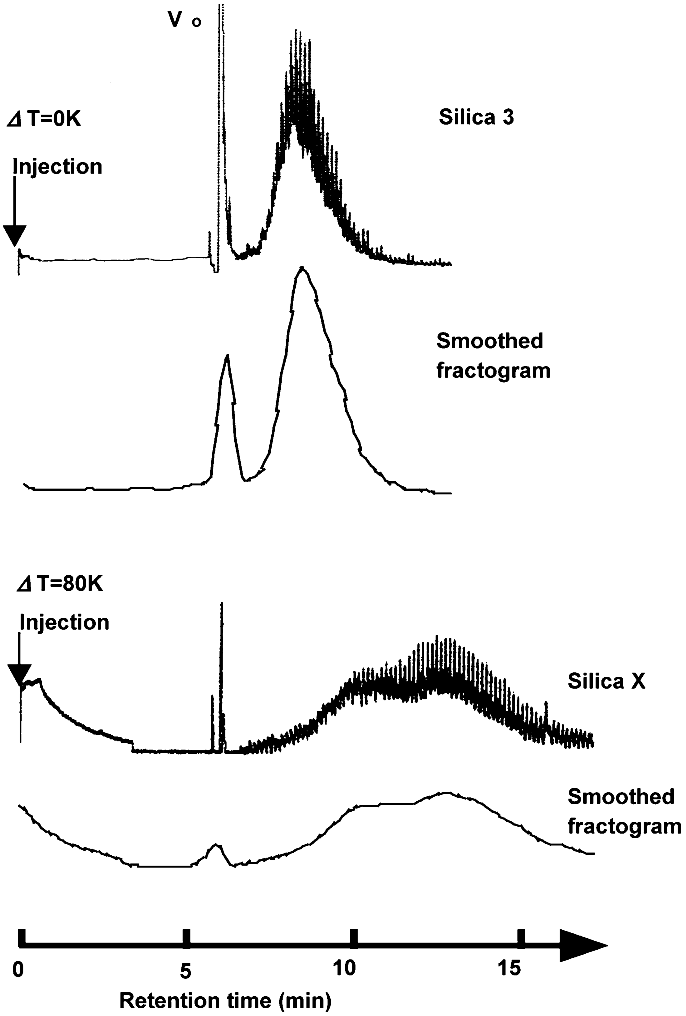

Figure 7. Comparison of the raw and smoothed fractograms of two silica chromatographic beads. Reproduced with permission from Ananieva et al.[29]

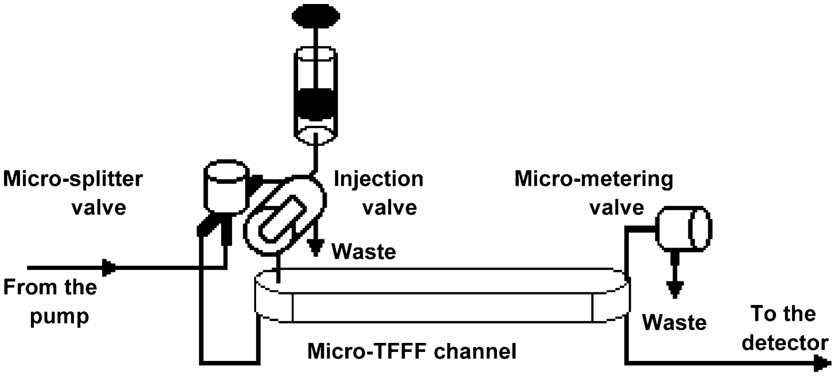

Figure 8. Schematic representation of the fluid circuit of the micro-TFFF channel unit.

were obtained by micro-TFFF of electrically conductive copolymer colloidal particles.[30]

EXPERIMENTAL SETUP FOR MICRO-TFFF

The apparatus for micro-TFFF can be assembled of standard liquid chromatography components,[31] with only the column substituted by the versatile micro-TFFF channel unit shown in Figures 8 and 9. Nevertheless, the construction of the commercially available versatile and compact micro-TFFF unit is an assembly of sophisticated components.[32]

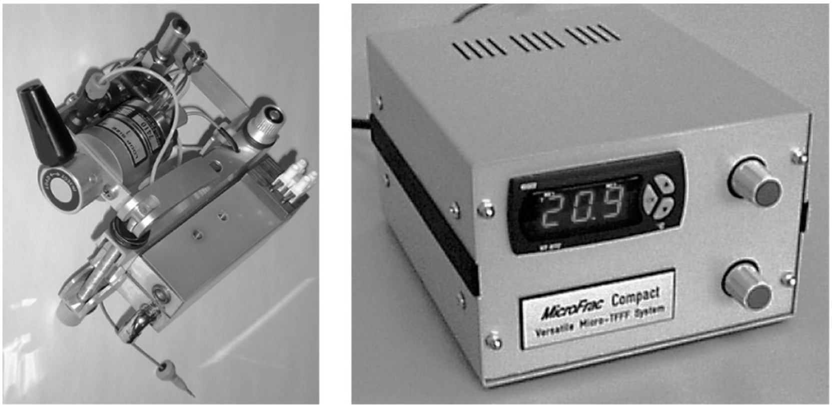

Figure 9. Commercial micro-TFFF versatile channel unit MicroFrac Compact and hot wall temperature controller MicroFrac.

Some of them represent original solutions, necessary to avoid the problems analyzed by Janča.[33] As a result, the length of the channel can be reduced without losing resolution if the linear velocity of the carrier liquid is reduced correspondingly.[14] A decrease of the thickness of the channel is counter productive[28] due to the existence of the inversion point at which the polarization mechanism is balanced by the steric exclusion mechanism and, consequently, the selectivity is zero. The decrease in breadth is accompanied by a positive consequence: reduced total heat flow across the channel. Nevertheless, the reduced aspect ratio breadth/thickness could have a negative consequence: higher band broadening[32] caused by the increased contribution of the dispersive phenomena at the side walls of the channel. However, the new principle of hydrodynamic splitting[32] eliminates perfectly the effect of the sidewalls.

CONCLUSION

With regard to the applications of micro-TFFF already performed, published, and reviewed here, it is obvious that this new technique is the most universal of all techniques of FFF. It is well suited for analytical separation and characterization of polymers in solution without an a priori imposed molar mass limit. The separations are carried out under mild conditions, thus preventing shear degradation of the macromolecular chains. Colloidal nanometer-size particles in suspension as well as large micrometer-size particles can also be separated and characterized from various viewpoints without any modification of the channel. The chemical resistance of the channel allows one to use organic as well as water-based solvents in a large range of pH values and temperatures.

Table I. Main characteristics of the micro-TFFF channel

1. Length $L = 76/71$ mm; breadth $b = 3.2$ mm; thickness $w = 0.100$ mm
2. Total volume 31 μl; injected volume 1 μl
3. Volume of connecting capillaries: injector-channel 1.2 μl, channel-detector 1.2 μl
4. Maximum electrical (heat) power: 150 W
5. Temperature operating range (tested): –15 to 100°C
6. pH operating range (tested): 3 to 11
7. Carrier liquids used: organic solvents, water-based solvents
8. Tested applications:
    - Macromolecules in MM range 20 000 to 30 000 000 g/mol
    - Nano-particles in size range 30 nm to 1 μm
    - Micron-size particles in size range 1 to 10 μm

The separations already achieved were not all optimized in order to achieve maximum reasonable resolution. Consequently, some particular separations published in recent articles are obviously far from the best possible results. The separation of large and heavy silica particles[29] is such an example of a non-optimized but challenging separation. Future experimental studies should thus be oriented towards optimization of the experimental conditions in all domains where micro-TFFF has already proven to be a highly competitive method.

The miniaturized TFFF channel is commercially available. Its main characteristics are summarized in Table I. Detailed information concerning the commercial versatile micro-TFFF channel can be obtained at microfrac@atlas.cz.

REFERENCES

[1] Thompson, G. H., M. N. Myers, and J. C. Giddings. (1967). An observation of a field-flow fractionation effect with polystyrene samples. *Sep. Sci.* **2**, 797.

[2] Giddings, J. C. (1966). A new separation concept based on a coupling of concentration and flow nonuniformities. *Sep. Sci.* **1**, 123.

[3] Giddings, J. C., M. N. Myers, and J. Janča. (1979). Retention characteristics of various polymers in thermal field-flow fractionation. *J. Chromatogr.* **186**, 37.

[4] Janča, J. (1988). *Field-Flow Fractionation: Analysis of Macromolecules and Particles*. New York: Marcel Dekker.

[5] Schimpf, M. E., K. D. Caldwell, and J. C. Giddings. (2000). *Field-Flow Fractionation Handbook*. New York: John Wiley.

[6] Shiundu, P. M. and J. C. Giddings. (1995). Influence of bulk and surface composition on the retention of colloidal particles in thermal field-flow fractionation. *J. Chromatogr. A* **715**, 117.

[7] Mes, E. P. C., R. Tijssen, and W. Th. Kok. (2001). Influence of the carrier composition on thermal field-flow fractionation for the characterisation of sub-micron polystyrene latex particles. *J. Chromatogr. A* **907**, 201.

[8] Giddings, J. C., M. Martin, and M. N. Myers. (1978). High speed polymer separations by thermal field-flow fractionation. *J. Chromatogr.* **158**, 419.

[9] Giddings, J. C. (1993). Micro-FFF: Theoretical and practical aspects of reducing the dimensions of field-flow fractionation channels. *J. Microcolumn Sep.* **5**, 497.

[10] Gale, B. K., K. D. Caldwell, and A. B. Frazier. (2001). Geometric scaling effects in electrical field flow fractionation. 1: Theoretical analysis. *Anal. Chem.* **73**, 2345.

[11] Gale, B. K., K. D. Caldwell, and A. B. Frazier. (2002). Geometric scaling effects in electrical field flow fractionation. 2: Experimental results. *Anal. Chem.* **74**, 1024.

[12] Yang, J., Y. Huang, X.-B. Wang, F. F. Becker, and P. R. C. Gascoyne. (1999). Cell separation on microfabricated electrodes using dielectrophoretic/gravitational field-flow fractionation. *Anal. Chem.* **71**, 911.

[13] Edwards, T. L., B. K. Gale, and A. B. Frazier. (2002). A microfabricated thermal field-flow fractionation system. *Anal. Chem.* **74**, 1211.

[14] Janča, J. (2002). Micro-channel thermal field-flow fractionation: New challenge in analysis of macromolecules and particles. *J. Liq. Chromatogr. Relat. Technol.* **25**, 683.

[15] Janča, J., J.-F. Berneron, and R. Boutin. (2003). Micro-thermal field-flow fractionation: New high-performance method for particle size distribution analysis. *J. Colloid Interface Sci.* **260**, 317.

[16] Ananieva, I. A., A. Yu. Menshikova, T. G. Evseeva, and J. Janča. (2004). High-speed micro-thermal focusing field-flow fractionation of micron-size particles. *Collect. Czech. Chem. Commun.* **69**, 322.

[17] Janča, J., I. A. Ananieva, A. Yu. Menshikova, and T. G. Evseeva. (2004). Micro-thermal focusing field-flow fractionation. *J. Chromatogr. B* **800**, 33.

[18] Janča, J. (2002). Micro-thermal field-flow fractionation. *Collect. Czech Chem. Commun.* **67**, 1596.

[19] Janča, J. (2002). Micro-channel thermal field-flow fractionation: Analysis of ultra-high molar mass polymers and colloidal particles with constant and programmed field force operation. *J. Liq. Chromatogr. Relat. Technol.* **25**, 2173.

[20] Janča, J. and P. Strnad. (2004). Micro- and frontal thermal field-flow fractionation: On the shear degradation of ultra-high molar mass polymers. *J. Liq. Chromatogr. Relat. Technol.* **27**, 193.

[21] Podzimek, S., T. Vlcek, and C. Johann. (2001). Characterization of branched polymers by size exclusion chromatography coupled with multiangle light scattering detector. I: Size exclusion chromatography elution behavior of branched polymers. *J. Appl. Polym. Sci.* **81**, 1588.

[22] Chubarova, E. V. and V. Nesterov. (1996). Behavior of macromolecules in nonhomogeneous hydrodynamic fields: Degradation mechanism of macromolecules. In *Strategies in Size Exclusion Chromatography*, eds. M. Potschka and P. L. Dubin, p. 127. Washington, D.C.: American Chemical Society.

[23] Chubarova, E. V. (2000). Thermal field-flow fractionation of initial dilute polymer solutions as a shear degradation model: Scaling model of macromolecule degradation at concentrations exceeding the critical entaglement value. *J. Macromol. Sci. Phys. B*, **39**, 583.

[24] Janča, J. (2003). Micro-channel thermal field-flow fractionation: High speed analysis of colloidal particles. *J. Liq. Chromatogr. Relat. Technol.* **26**, 849.

[25] Janča, J. (2003). Micro-thermal field-flow fractionation of colloidal particles: Effect of temperature on retention and relaxation processes. *Collect. Czech Chem. Commun.* **68**, 672.

[26] Janča, J. (2003). Micro-thermal field-flow fractionation: New challenge in experimental studies of thermal diffusion of polymers and colloidal particles. *Philos. Mag.* **83**, 2045.

[27] Janča, J., I. A. Ananieva, A. Yu. Menshikova, T. G. Evseeva, and J. Dupák. (2004). Effect of channel width on the retention of colloidal particles in polarization, steric, and focusing micro-thermal field-flow fractionation. *J. Chromatogr. A* **1046**, 167.

[28] Janča, J. and I. A. Ananieva. (2003). Micro-thermal field-flow fractionation in the characterization of macromolecules and particles: Effect of steric-exclusion mechanism. *e-Polymers* no. 034.

[29] Ananieva, I. A., M. Minarik, R. Boutin, O. A. Shpigun, and J. Janča. (2004). Characterization of chromatographic silica beads by micro-thermal field-flow fractionation. *J. Liq. Chromatogr. Relat. Technol.* **27**, 2313.

[30] Stejskal, J., M. Trchová, I. A. Ananieva, J. Janča, J. Prokeš, S. Fedorova, and I. Sapurina. (2004). Poly(aniline-co-pyrrole): Powders, films, and colloids. Thermophoretic mobility of colloidal particles. *Synth. Metals* **146**, 29.

[31] Janča, J. (2005). Polarization, steric, and focusing micro-thermal field-flow fractionation principles, theory, instrumentation, and applications in polymers and particles analysis. *Anal. Chim. Acta* **540/1**, 187.

[32] Janča, J. and J. Dupák. (2005). Elimination of edge effects in micro-thermal field-flow fractionation channel of low aspect ratio by splitting the carrier liquid flow into the main central stream and the thin stream layers at the side channel walls. *J. Chromatogr. A* **1068**, 261.

[33] Janča, J. (2003). Comments on "A microfabricated thermal field-flow fractionation system". *Anal. Chem.* **75**, 3666.