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DEVELOPMENT OF CONTINUOUSLY OPERATING TWO-DIMENSIONAL THERMAL FIELD-FLOW FRACTIONATION EQUIPMENT

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ABSTRACT

A new type of the thermal field-flow fractionation apparatus designed and constructed in the Laboratory of Analytical Chemistry provides continuous fractionation of polymers according to their molecular masses. The principle is to separate sample components into filaments from the continuous sample flow in a disk-shaped channel and to collect a sample species at the circumference of the channel. The upper wall of the channel is stationary, and the lower one rotates slowly. The method is based on a two-dimensional fractionation mechanism and demonstrates the collection of 2 polystyrene samples in a cyclohexane carrier. The method also provides a gentle fractionation environment for high polymers with low shear forces, flexible run conditions, and numerous industrial applications from special products to process quality control. The construction procedure of the complete apparatus is described with technical details.

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Key Words: Field-flow fractionation; Continuous fractionation; Two-dimensional; Separation; Thermal gradient; Polystyrene; Cyclohexane; Tetrahydrofuran

INTRODUCTION

Field-flow fractionation (FFF) is a method for fractionation of soluble macromolecules and particles that was initially developed by Professor J. Calvin Giddings (1). The basic principle of the method is to force the sample components into different velocity streams of a laminar-flowing carrier in a thin rectangular channel by applying an external force field across the channel (2). The samples are separated according to their molecular mass or particle size by applying a suitable field, such as force of gravity, thermal gradient, cross-flow force, or electrical field (3). In addition to the external force, the ordinary diffusion of the sample components affects the separation.

In the conventional thermal FFF (ThFFF) method, the field that drives separation is a temperature gradient generated by heating the upper channel wall and cooling the lower wall. The thermal gradient induces thermal diffusion of the sample components, and this diffusion shifts the components toward the cooler wall through a selective mechanism that is not well understood. The distance of the sample molecule to the accumulation wall depends on the ratio of thermal diffusion to the ordinary diffusion that acts as an opposing force. When the sample zones are at different average distances from the wall, separation takes place (4, 5). In ThFFF the highest thermal gradients that can be applied across the thin channel are typically 1.0×10^4 K/cm, and this acts as a limiting factor for the separations (6). However, by varying the carrier flow parameters by programming the flow rate or by changing the shape of the flow profile, the versatility of the method can be expanded (7,8,9).

In FFF, the open channel configuration allows unlimited variations of flow profile and therefore, can be also employed in a continuous two-dimensional mode. The method used here is based on the combination of parabolic radial flow and linear tangential flow in a disk-shaped fractionation channel in which the lower channel wall rotates. The radial flow inlet is in the center of the channel and the outlets are symmetrically located along the circumference edge. The sample mixture is introduced continuously at a certain point along the channel. Under the influence of the field, the molecules are forced toward the rotating accumulation wall and separated according to their molecular mass. With combined tangential flow, the separated sample components at different distances from the wall split into component filaments and each is continuously collected at the edge of the channel.

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This paper describes the construction of a prototype of the fractionation apparatus based on the above concept. A demonstration of the separation procedure for 2 polystyrene samples was also performed, and this paper presents the fractograms from the conventional ThFFF runs.

EXPERIMENTAL

Principle

The concept of this continuous separation method conforms to that reported by Giddings (10) and is based on fluid dynamics and FFF (11,12,13). By introducing the carrier from the center of the disk-shaped channel, the result is an outwardly directed, symmetric, radial flow with a parabolic flow profile. In an ideal case, the circumference edge of the channel acts as a sink for the radial flow, but in our application a number of outlets along the edge served as a sink. A sample port at a certain distance from the center is an additional source of flow. When introducing the sample mixture into radial flow with the field applied across the channel, the sample components are separated in the parabolic flow according to a FFF separation mechanism. Because the method is continuous, stopping the carrier flow for relaxation is not possible. By rotating the lower channel wall parallel to the stationary upper wall at a constant velocity, tangential flow is generated resulting in a linear, asymmetrical flow-profile component that is at right angles to the radial flow. The samples at different distances from the accumulation wall have different trajectories and can be collected at the edge of the channel at different tangential positions (14). Figure 1 illustrates schematically the flow and the separation of the samples in the disk-shaped channel.

Instrumentation

The field applied in this experimental study was a thermal gradient. It was generated by heating the upper wall element with an electric resistance heater and by cooling the lower element using cold tap water. The true value of the temperature gradient between channel walls was not measured, but it was estimated by measuring the temperatures outside of both channel blocks. The lower wall was rotated slowly with constant speed using a synchronous stepping motor (The Superior Electric Co, Bristol, Conn, USA). The disk-shaped channel was cut into a 250 μm-thick Mylar sheet that also acted as a spacer between the wall elements. The symmetric radial flow was established by suction of the pressurized carrier into fraction collection syringes (Discardit II, Becton Dickinson, S.A., Fraga, Spain).

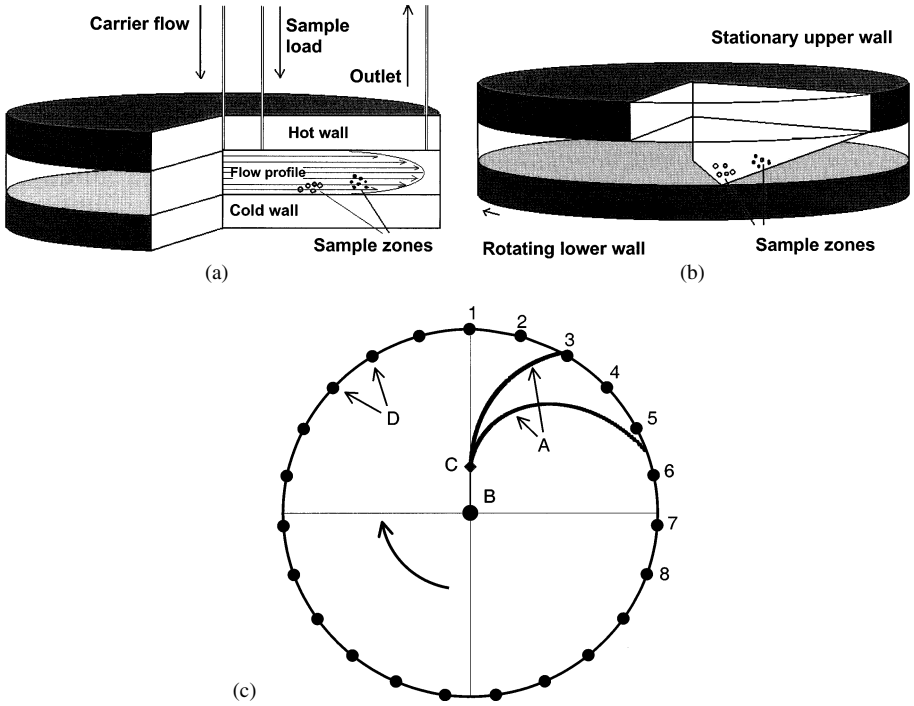

Figure 1. Schematic drawings of the flow between 2 parallel disk-shaped walls and the separation of 2 samples: a) sample zones separated in a parabolic radial flow by influence of a force field; b) 2 sample zones separated in tangential flow established by the rotation of the lower channel wall; and c) hypothetical sample trajectories in which (A) is view from the top of the instrument; (B) is the channel axis; (C) is the sample-introduction port; and (D) represents the outlet ports.

The collected fractions were analyzed using a conventional ThFFF-fractionation channel made for determining the sample concentrations in each fraction.

The continuous fractionation channel and fraction collector were constructed in-house. In the disk-shaped fractionation channel the heated upper wall is stationary and the cooled lower wall rotates. The lower wall was made of aluminum, which was electroplated by nickel to avoid corrosion and mechanical failures. The lower element was cooled by tap water introduced through the holes drilled into the element axis. A schematic cross section of the apparatus is presented in Fig. 2.

The upper wall block was also made of aluminum with 5 mm-thick copper plate covering the channel side. Copper was used because the steel inlet and outlet capillaries can be easily soldered on it. The diameter of the channel was 135

mm and the calculated volume 3.6 mL, using 250 μm as the spacer thickness. The distance between the outlet steel capillaries (i.d. 0.5 mm) on the circumference edge was 17 mm. The sample introduction port was aligned with the first fraction-collecting syringe 17 mm from the center of the channel. Finally, both channel walls were machined and highly polished to minimize turbulent and nonuniform flows during fractionation. The channel circumference edge was sealed with 2 fluoroelastomer (Viton™) O-rings located between the polished hot-wall surface and the aluminum O-ring housing fitted onto the surface of the lower block.

The carrier was introduced into the fractionation channel from a reservoir pressurized by air (150 kPa). The sample liquid was fed using a microflow pump (Micro Feeder Model MF-2, Azumadenkikogyo Co, Ltd, Japan) with a 500-μL

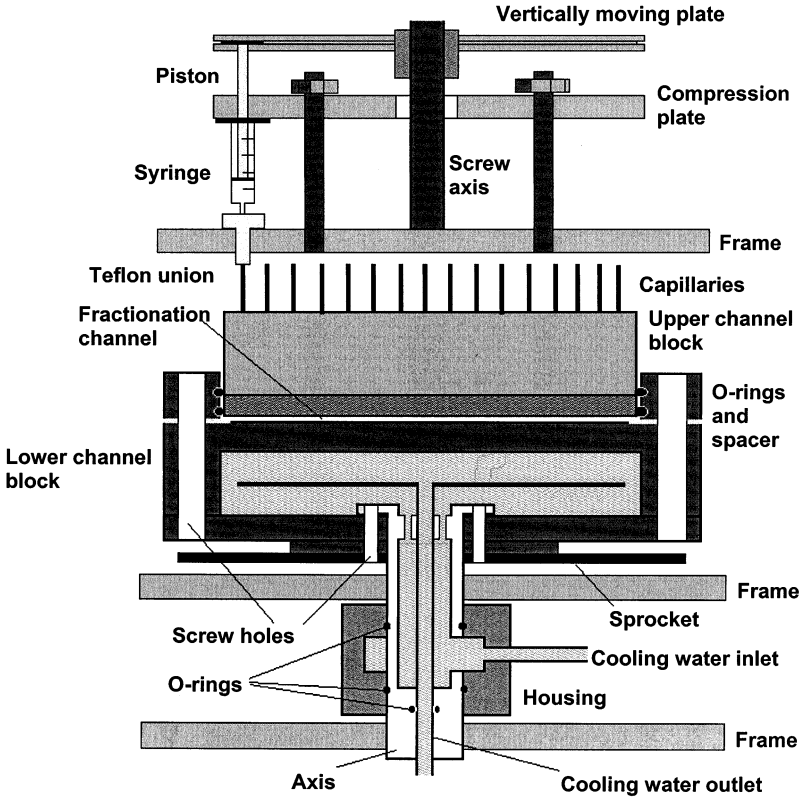

Figure 2. Cross section of the continuous ThFFF apparatus with rotating wall axis, cooling water conduits, and fraction collection unit. Only 1 of the 23 fraction collection syringes is shown.

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high-performance liquid chromatography (HPLC) syringe installed on a clip. The flow rate of the sample liquid was adjusted to 5 μL/min.

The fraction collection unit was installed above the channel to minimize the length of the capillaries between the channel outlet and the collection syringes. Twenty-three 10-mL syringes were installed on the aluminum plate along the circumference of a circle with diameter 18 cm. For each of the syringes, a Luer fitting made of Teflon was installed in holes on the aluminum plate. The inlet capillaries were fitted from below into the Teflon fittings. Plungers of the syringes were installed into a circular holder plate that was moved vertically along an electric-motor driven, slowly rotating screw axis, resulting in equal carrier suction in each syringe. All components were installed into a rigid aluminum frame.

The conventional ThFFF system, presented in detail in our previous work (15), consisted of an in-house constructed ThFFF channel, an HPLC pump (PU-980; Jasco Corp, Tokyo, Japan), injection and bypass valves (C6W; Valco Instrument Co, Inc, Houston, Tex, USA), and a UV detector (SP8450; Spectra Physics Inc, San Jose, Calif, USA) with a detection wavelength of 254 nm. The channel dimensions were 45 cm × 2 cm × 125 μm. The temperature difference ΔT was set to 100°C, and the flow rate was 100 μL/min. The volume of the injected sample was 10 μL. The system was controlled and data collected by a computer (M24; Ing. C. Olivetti & C. S.p.A., Italy).

Materials

Two polystyrene standards were used in this study. The molecular masses (M_w) of the standards were 19.85 kg/mol (PS 19.8) (Waters Associates, Framingham, Mass, USA) and 96.0 kg/mol (PS 96.0) (Polymer Laboratories Ltd, UK) with polydispersities (M_w/M_n) of 1.01 and 1.03, respectively. The samples were dissolved in analytical-grade cyclohexane (Merck KGaS, Darmstadt, Germany) for continuous fractionation. After evaporating the cyclohexane from the collected fractions, the samples were dissolved in HPLC-grade tetrahydrofuran (Labscan Ltd, Dublin, Ireland) for conventional ThFFF. The concentrations of the samples in the sample mixture were 0.35% (w/w) for PS19.8 and 0.51% (w/w) for PS96.0.

RESULTS AND DISCUSSION

Two polystyrene samples with different molecular masses were used to study the performance of the experimental continuous ThFFF equipment. The samples were dissolved in a cyclohexane carrier for the continuous method and in tetrahydrofuran for conventional ThFFF. The conventional method was used to determine the relative amounts of the samples in fraction collecting syringes after each continuous fractionation run.

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At first, the zone-broadening effect was studied without the force field. The runs were carried out at a radial flow rate of 100 μL/min for each fraction collector, resulting in a total flow rate of 2.3 mL/min into the channel inlet. The continuous flow of the PS96.0 sample solution was set to 5 μL/min through the sample inlet. Without the lower channel wall in rotation, the sample stream collected only into the first collection syringe aligned with the injection port. At a lower-channel rotation speed of 0.96 rph, 85% of the total collected amount was found in syringe number 3 and about 10% was found in syringe 4 (Fig. 3a).

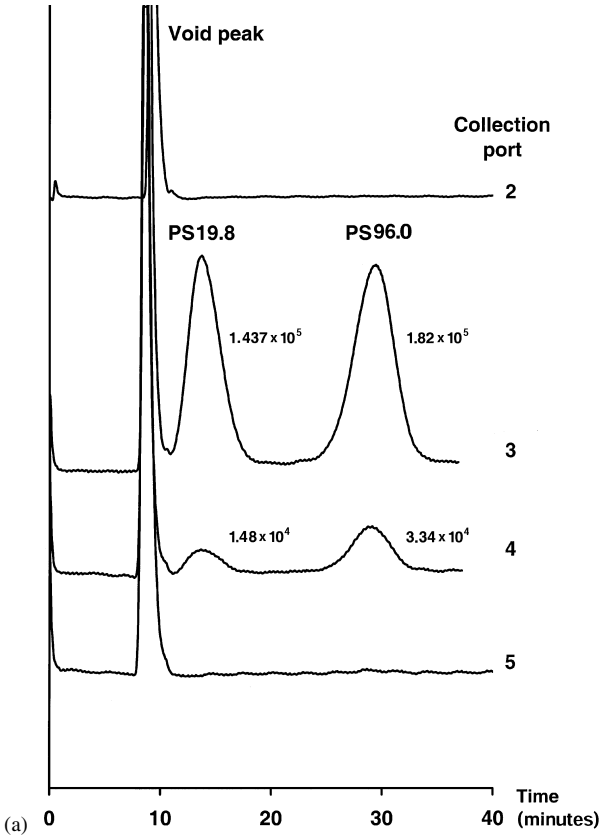

Figure 3. Fractograms and peak areas obtained with conventional ThFFF ($\Delta T = 100°C$) for fractions collected from continuous fractionation. The continuous runs were performed without (a) and with (b) thermal gradient. The rotation speed was 0.96 rph and the radial flow rate was 100 μL/min.

(continued)

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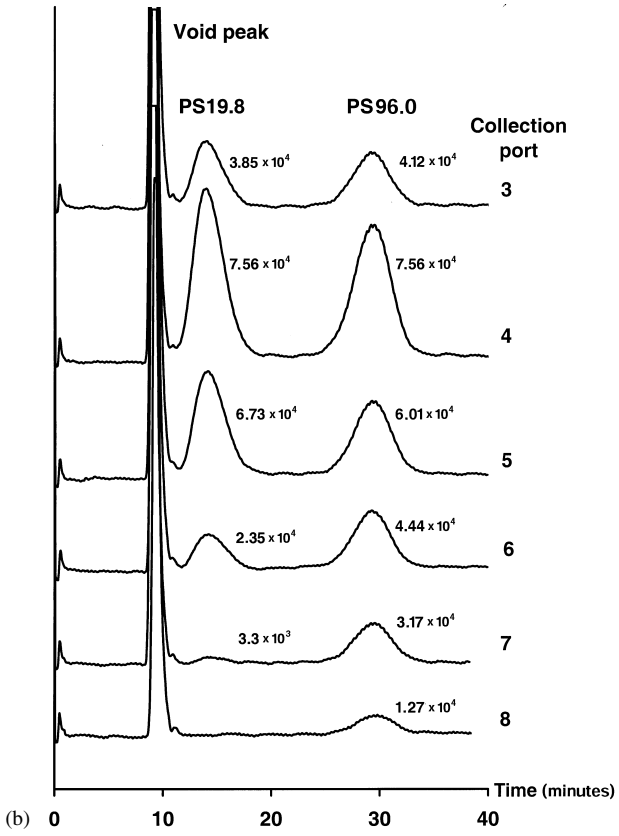

Figure 3. Continued.

The next experiment was done with a mixture of the 2 polystyrene samples (PS19.8 and PS96.0). A constant thermal gradient was applied across the channel and by rotating the lower channel wall at different speeds with all the other parameters unchanged. Under stabilized conditions, the temperature of the upper block was 55°C and the lower block was at 13°C. The sample mixture was introduced at a flow rate of 5 μL/min for 30 min after which the fractionation was continued for 15 min to collect all of the sample from the channel. Figure 3 illustrates the ThFFF analyses of the fractions collected from the outlet ports of the continuous separation channel. The fractionation results from conventional ThFFF conducted without applied thermal gradient are shown in Fig. 3a. Figure 3b shows the fraction peaks determined from experiments in which the gradient was applied. Figure 4 presents the effect of rotation speed of the lower channel wall on the continuous sample collection.

The experiment was continued at different radial flow rates and constant rotation speed (1.92 rph) and thermal gradient. Figure 5 represents the effect of radial flow rate on the separation of 2 polystyrene samples. At slower radial flow rates, prolonged sample introduction and collection times were used to collect enough sample for conventional ThFFF runs.

Figures 3 and 4 indicate that if thermal gradient was applied and the lower channel wall was rotated during the run, the proportions of the sample components gradually changed with the syringe number. For experiments conducted without applied thermal gradient, such a change was not found (Fig. 3a). At rotation speed of 0.96 rph, the completely separated sample component PS96.0 was found in syringe number 9, and at a rotation speed of 1.92 rph, it was found in syringe 13 (Fig. 4). PS96.0 was found to make a full revolution at the rotation speed of 1.92 rph and radial flow rate of 67 μL/min, which did not occur with higher radial flow rates (Fig. 5). Zone broadening was observed in the runs performed with thermal gradient. The factors affecting the efficiency are similar to those in conventional FFF. However, in this instrument, the divergence of the radial flow contributed to the zone broadening as well. The limited number of outlet capillaries may have also caused broadening by decreasing the uniformity of the radial flow streamlines near the circumference.

The quality of apparatus materials was critical because of the pressure and high temperature of the organic solvent. The O-ring and channel materials showed

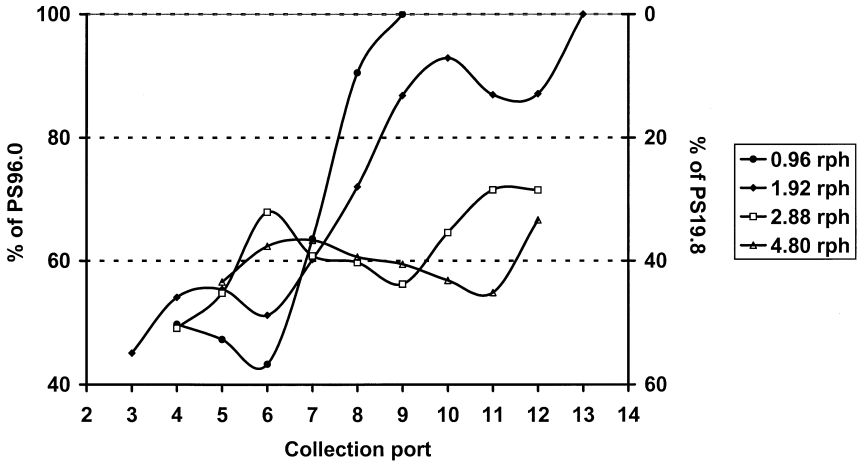

Figure 4. Effect of the rotation speed on the percentages of the 2 sample components (calculated from the peak areas of PS19.8 and PS96.0) in the collected fractions. The percentages of PS19.8 and PS96.0 in the original sample mixture were 40.4% and 59.6%, respectively. The radial flow rate was 100 μL/min (measured at the sample collection port).

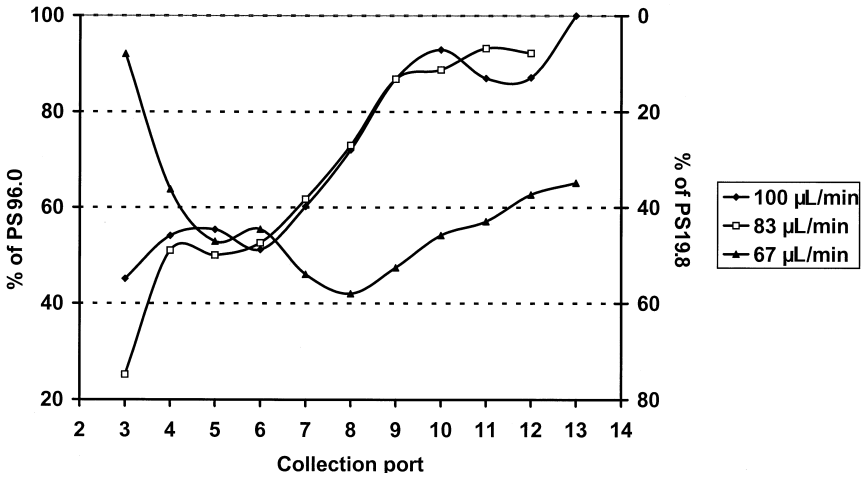

Figure 5. Effect of the radial flow rate on the percentages of the 2 sample components (PS19.8 and PS96.0) in the collected fractions. The percentages of PS19.8 and PS96.0 in the original sample mixture were 40.4% and 59.6%, respectively. The rotation speed of the lower wall was 1.92 rph.

no defects under such conditions. The preliminary results were promising and support future development. More studies will be carried out to optimize the numerous run conditions, such as channel thickness, thermal gradient strength, and the distance of the sample-introduction port from the channel center. Because the sample dilution during the fractionation may result in high consumption of the carrier, solvent recycling will be incorporated into the instrument. A great challenge in the future is to apply different kinds of force fields across the channel to allow for preparative fractionation of biotechnological and other type of the samples.

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REFERENCES

1. Giddings, J.C. A New Separation Concept Based on a Coupling of Concentration and Flow Nonuniformities. Sep. Sci. **1966,** *1,* 123–125.

2. Myers, M.N. Overview of Field-Flow Fractionation. J. Microcol. Sep. **1997,** *9* (3), 151–162.
3. Wahlund, K.G. Field-Flow Fractionation. European Chromatography News **1988,** *2*, 12–15.
4. Myers, M.N.; Caldwell, K.D.; Giddings, J.C. A Study of Retention in Thermal Field-Flow Fractionation. Sep. Sci. **1974,** *9,* 47–70.
5. Gunderson, J.J.; Caldwell, K.D.; Giddings, J.C. Influence of Temperature Gradients on Velocity Profiles and Separation Parameters in Thermal Field-Flow Fractionation. Sep. Sci. Technol. **1984,** *19,* 667–683.
6. Giddings, J.C. Micro-FFF: Theoretical and Practical Aspects of Reducing the Dimensions of Field-Flow Fractionation Channels. J. Microcol. Sep. **1993,** *5,* 497–503.
7. Giddings, J.C.; Caldwell, K.D.; Moellmer, J.F.; Dickinson, T.H.; Myers, M.N.; Martin, M. Flow Programmed Field-Flow Fractionation. Anal. Chem. **1979,** *51*, 30–33.
8. Janca, J.; Chmelik, J. Focusing in Field-Flow Fractionation. Anal. Chem. **1984,** *56,* 2481–2484.
9. Andreev, V.P.; Stepanov, Y.V.; Giddings, J.C. Field-Flow Fractionation with Asymmetrical Electroosmotic Flow I. Uncharged Particles. J. Microcol. Sep. **1997,** *9* (3), 163–168.
10. Giddings, J.C. Theoretical Basis for a Continuous, Large-Capacity Gas Chromatographic Apparatus. Anal. Chem. **1962,** *34,* 37–39.
11. Milne-Thomson, L.M. *Theoretical Hydrodynamics*, 5th ed.; Macmillan & Co Ltd: London, 1968; 209–211, 362–363.
12. Hovingh, M.E.; Thompson, G.E.; Giddings, J.C. Column Parameters in Thermal Field-Flow Fractionation. Anal. Chem. **1970,** *42,* 195–203.
13. Gunderson, J.J.; Giddings, J.C. Chemical Composition and Molecular-Size Factors in Polymer Analysis by Thermal Field-Flow Fractionation and Size Exclusion Chromatography. Macromolecules, **1986,** *19*, 2618–2621.
14. Pao, R.H.F. *Fluid Dynamics*; Charles E. Merrill Books, Inc: Columbus, Ohio, 1967; 178.
15. Dammert, R.; Jussila, M.; Vastamäki, P.; Riekkola, M.L.; Sundholm, F. Determination and Comparison of Molar Mass Distributions of Substituted Polystyrenes and Block Copolymers by Using Thermal Field-Flow Fractionation, Size Exclusion Chromatography, and Light Scattering. Polymer **1997,** *38* (26), 6237–6280.

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