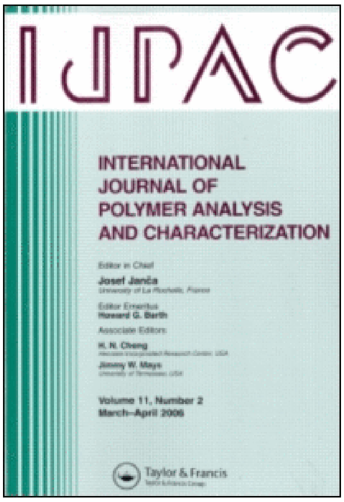

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Optimization of Retention in Micro-Thermal Field-Flow Fractionation for the Determination of True Particle Size Distribution

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Abstract: Determination of true particle size distribution of colloidal samples by field-flow fractionation requires the suppression or measurement of all contributions to the zone broadening. In order to achieve high-performance separations, experiments are often carried out at the optimal velocity of the carrier fluid at which the zone broadening due to the nonequilibrium processes is minimal. This method is traditionally used in chromatography where it was justified by classical theories. It was also applied in field-flow fractionation without raising the question on its efficiency. In this article, it is predicted theoretically and proven experimentally that high-performance separation and thus accurate determination of particle size distribution can be performed more efficiently by optimizing the experimental conditions with regard to the retention rather than to the optimal velocity of the carrier liquid.

Keywords: Colloidal particles; Micro-thermal field-flow fractionation; Precise particle size distribution; Polystyrene latex; Retention optimization

INTRODUCTION

Micro-thermal field-flow fractionation (micro-TFFF) was originally proposed and a miniaturized channel constructed and applied to the separation of polymers and colloidal particles in 2002.[1] Several repeatable

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and reproducible results obtained with the use of micro-TFFF were reviewed recently by Janča.[2,3]

An important conclusion found theoretically[1] and proven experimentally[1] was that an increase in resolution can be achieved more efficiently by increasing the temperature drop across the micro-TFFF channel than with a decrease in channel thickness. A 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 polystyrene latex (PSL) particles demonstrated the highest performance with micro-TFFF.[4] The relatively low number of the particles that can be counted in TEM should not represent the true PSD of the whole sample, DLS does not provide correct information on PSD at all, and only HC and micro-TFFF are methods apt to separate the colloidal samples according to size and provide correct information on PSD. However, the advantage of micro-TFFF over HC is that high-resolution separations can be performed in practically unlimited particle size ranges and in various solvents without any modification of the separation channel, simply by adjusting the temperature drop across the channel. This flexibility cannot be obtained with HC.

The repeatability and reproducibility of the determination of the mean particle size and PSD by micro-TFFF was recently studied by two different laboratories.[5] It has been found that the precision of the mean particle size measured in one laboratory was better than 1% relative. Average repeatability of the determination of the mean particle size in two participating laboratories was better than 3% relative when using identical experimental protocols. No other method of particle size analysis can provide results of comparable precision. Average repeatability of the width of the raw fractograms that contain the information on PSD was of the order of 5% relative. However, this value cannot be considered as the ultimate limit because the experiments were not carried out under optimal experimental conditions that would permit reaching much higher resolution. Whereas the classical method to obtain the highest resolution (adopted from chromatography) is to find an optimal flow rate at which the theoretical plate height reaches its minimum, it will be demonstrated in this article that a more suitable experimental approach is to optimize the retention. Since the retention is related to the temperature drop across the channel, an increase in temperature drop generates an increase in retention and, consequently, an increase in resolution. Although the approach described in this article was experimentally verified with the use of micro-TFFF, it is generally applicable to any field-flow fractionation (FFF) method.

THEORY

The width of the fractogram in micro-TFFF contains information on the zone broadening and PSD in the case of particle separation and on the molar mass distribution if the macromolecules are concerned. As a matter of fact, the zone broadening and PSD are convoluted, and this convolution is quantitatively described by the height equivalent to the theoretical plate H[6]:

$$H = \frac{2D}{R\langle v\rangle} + \frac{\chi w^2\langle v\rangle}{D} + \sum H_i + H_{PSD} = L\left(\frac{\sigma_{V_R}}{V_R}\right)^2 \tag{1}$$

where D is diffusion coefficient, R is retention ratio, $\langle v\rangle$ is the mean linear velocity of the carrier liquid inside the channel, w is the thickness of the channel, the sum of H_i represents the extra-channel contributions to the plate height (which can be reduced to a negligible minimum by a suitable construction of the separation system), H_{PSD} is the contribution due to the PSD, L is the length of the channel, V_R is the retention volume of the retained particles, and σ_{V_R} is standard deviation of the experimental fractogram expressed in retention volume units. It has to be stressed that H_{PSD} has not a usual meaning of the band broadening due to *dispersion processes* because the zone is in fact "broadened" by a *separation process*, and thus this contribution reflects the true PSD. The dimensionless parameter χ can be approximated for highly retained species by:

$$\lim_{R\to 0}\chi = \frac{R^3}{9} \tag{2}$$

Equation (2) results from the approximations,[7] namely from the relationship[6]:

$$\lim_{\lambda\to 0}\chi = 24\lambda^3 \tag{3}$$

in which the dimensionless retention parameter λ is related to the retention ratio R by:

$$\lim_{\lambda\to 0}R = 6\lambda \tag{4}$$

Retention ratio $R = V_0/V_R$ is available from the experimental retention volume of the unretained marker molecules, V_0, and the retention volume of the retained species, V_R.

The first term in Equation (1) describes the longitudinal diffusion and the second one corresponds to the nonequilibrium (mass transfer) processes. When Equations (1) and (2) are combined, the result is:

$$H = \frac{2D}{R\langle v\rangle} + \frac{R^3 w^2\langle v\rangle}{9D} + \sum H_i + H_{PSD} \tag{5}$$

Equation (5) thus describes the dependence of the plate height H on the two experimental variables, R and $\langle v \rangle$. A minimum of the function $H(R, \langle v \rangle)$ exists if:

$$\frac{\partial H(R, \langle v \rangle)}{\partial R} = 0 \qquad \text{and} \qquad \frac{\partial H(R, \langle v \rangle)}{\partial \langle v \rangle} = 0 \tag{6}$$

and

$$AC - B^2 > 0 \qquad A > 0 \tag{7}$$

where

$$A = \frac{\partial^2 H(R, \langle v \rangle)}{\partial R^2} \quad B = \frac{\partial^2 H(R, \langle v \rangle)}{\partial R \partial \langle v \rangle} \qquad C = \frac{\partial^2 H(R, \langle v \rangle)}{\partial \langle v \rangle^2} \tag{8}$$

Whereas the condition $A > 0$ is immediately obvious when calculating the corresponding derivatives, the condition $AC - B > 0$ is not so simply evident. However, graphical representation of the function $H(R, \langle v \rangle)$ in Figure 1 shows clearly the range of optimum experimental variables R and $\langle v \rangle$. For low values of $\langle v \rangle$, an increase of the retention ratio up to $R = 0.3$ does not cause a dramatic increase in H.

The minimum of the function $H(\langle v \rangle)$ and thus the optimal $\langle v \rangle_{opt}$, calculated from Equation (5), is[1]:

$$\frac{\partial H}{\partial \langle v \rangle} = 0 \quad \text{which results in} \qquad \langle v \rangle_{opt} = \frac{3D}{Rw^2}\sqrt{2} \tag{9}$$

It has to be noted that in Janča[1] some typographical errors occurred. The translational diffusion coefficient D of the hard spherical particles is given by the well-known Stokes-Einstein relationship:

$$D = \frac{k_B T}{6\pi \mu r} \tag{10}$$

where k_B is Boltzmann constant, T is the temperature, μ is dynamic viscosity of the suspending liquid, and r is the radius of a rigid spherical particle. Diffusion coefficients of the colloidal particles in the nanometer and micrometer size range are low. Consequently, the $\langle v \rangle_{opt}$ calculated from Equation (9) is very low, of the order of $\langle v \rangle = 10^{-2}$ to 10^{-3} cm/s. Although such low linear velocities of the carrier liquid can be realized in micro-TFFF experiments, the resulting separation time is impractically long. Whenever $\sum H_i$, corresponding to extra-channel contributions to zone broadening, is minimized to a negligible level by a suitable construction of the separation system, the value of H of the experimental

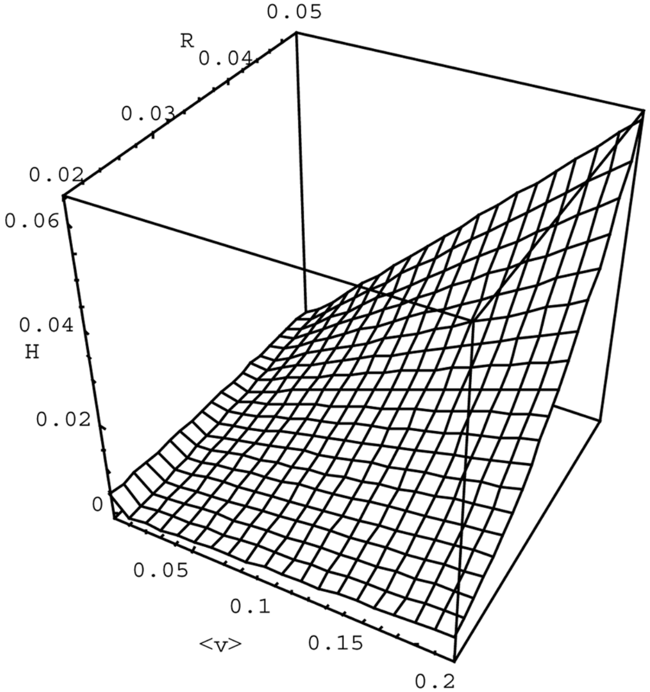

Figure 1. Three-dimensional representation of the dependence of the height equivalent to the theoretical plate H on the retention ratio R and the linear velocity of the carrier liquid $\langle v \rangle$, calculated from Equations (5) and (10) for PSL 155 and $\sum H_i + H_{PSD} = 0$.

dependence of H on $\langle v \rangle$ extrapolated to $\langle v \rangle = 0$ can be considered, with a good approximation, as corresponding to sample PSD contribution H_{PSD}.

The optimization of a separation whose goal is to achieve the highest efficiency (minimum H) by performing the experiments at $\langle v \rangle_{opt}$ is known from chromatography where it was justified by classical theories of the nonequilibrium processes and zone dispersion (see, for example, Giddings).[8] This approach was also applied in FFF, particularly in TFFF, to evaluate the polydispersity of the separated polymer samples.[6,9,10] Recently, the same procedure was confirmed as useful in micro-TFFF.[4] The experiments were not carried out at impractical $\langle v \rangle_{opt}$ but at velocities low enough to eliminate the zone broadening contribution due to relaxation processes.[4] However, the determination of the true H_{PSD} from the series of data obtained at low linear velocities of the carrier liquid is a time-consuming procedure.

The minimum of the function $H(R)$ and thus the optimal R_{opt} calculated from Equation (5) is:

$$R_{opt} = \sqrt{\frac{D}{\langle v \rangle w}} \sqrt[4]{6} \tag{11}$$

Thus for given operational parameters $\langle v \rangle$, w, and D, the force of the external field should be tuned so as to obtain the required R_{opt}. The 3-D representation of the function $H(R, \langle v \rangle)$ in Figure 1 shows clearly that for highly retained species (low values of R), the slope of the function $H(\langle v \rangle)$ is lower than for weakly retained species (high values of R). In other words, the contribution to the zone broadening due to dispersion processes inside the channel will be less important for highly retained species independently of the operational parameters.

The main conclusion that results from the theoretical analysis is that within the practical range of the linear velocities $\langle v \rangle$ and retention ratios R, above their corresponding optimal values, the first term in Equation (5) is negligible in comparison with the second one. Consequently, it is obvious from Equation (5) that a decrease in linear velocity $\langle v \rangle$ of the carrier liquid has much less important impact on the efficiency H than a decrease in retention ratio R, both resulting in the same prolongation of the separation time. A detailed study of various contributions to the plate height H in Equation (5) and their relative proportion with regard to H_{PSD} and thus with regard to precise PSD, evaluated from the experimental data by the proposed method of *retention optimization*, is presented in this article.

EXPERIMENTAL SECTION

The apparatus for micro-TFFF consisted of a syringe pump model IPC 2050 (Linet Compact, Czech Republic) equipped with a special stainless-steel syringe (Institute of Scientific Instruments, Academy of Sciences of the Czech Republic), a commercial micro-TFFF channel unit (MicroFrac Laboratory, microfrac@atlas.cz, www.watrex.cz, Czech Republic), equipped with an electronic device (MicroFrac Laboratory, Czech Republic) regulating the electric power for heating the cartridge and controlling the temperature of the hot wall. The dimensions of the micro-TFFF channel used in this work were 0.1 × 3.2 × 76 mm. The compact micro-TFFF unit was further equipped with an injection valve model 7410 (Rheodyne, USA) with a 1 μL loop and with a system of a graduated micro-splitter valve, model P 470, and a micro-metering valve, model P 446 (Upchurch Scientific, USA) allowing the splitting of the carrier liquid flow into two separated entries of the channel and also the casual splitting of the outgoing liquid between the detector and the waste.[2,3]

A variable wavelength detector (Jasco UV-975, Japan) equipped with a 1 μL measuring cell and a recorder-integrator (Hewlett-Packard 3395, USA) were used to record the fractograms. A low-temperature thermostat, model RML 6 B (Lauda, Germany), was used to control the temperature of the cold wall of the channel. The temperatures at very close proximities of the cold and hot walls were also measured independently by a digital thermometer (Hanna Instruments, Portugal) equipped with two thermocouples.

An aqueous solution of 0.1% detergent Brij 78 (Fluka, Germany) and 3 mM/L NaCl was used as a carrier liquid. The experimental conditions of micro-TFFF experiments were: flow rate of the carrier liquid was 0.2 mL/h, corresponding to $\langle v \rangle = 0.0174\,\mathrm{cm/s}$, temperature of the cold wall was always 303 K, and temperature drop across the channel varied from $\Delta T = 10$ to $\Delta T = 48\,\mathrm{K}$.

Spherical PSL particles (Polymer Laboratories, UK) of narrow PSD and of nominal mean particle diameter of 155 nm (PSL 155) and pure acetone (non-retained marker molecules) were used in this study. The particle size of the PSL sample provided by the manufacturer was confirmed by the DLS measurement.

RESULTS AND DISCUSSION

A rationalized manipulation of the retention ratio R is possible under the condition that the relationship between R and the force of the applied field is known. This relationship was well described from the very beginning of the theory of FFF,[11] which predicts the linear dependence of the retention parameter λ on the inverse value of the field force. The theoretical prediction was verified experimentally for sedimentation FFF[12] ($\lambda = \mathrm{f}(1/G)$, where G is the gravitational or centrifugal acceleration), electrical FFF[13] ($\lambda = \mathrm{f}(1/\mathrm{E})$, where E is the potential of the electrical field), flow FFF[14] ($\lambda = \mathrm{f}(1/V^{\circ})$, where V° is the cross-flow rate), and thermal FFF[15] ($\lambda = \mathrm{f}(1/\Delta T)$.

The fractograms of the PSL 155 sample obtained at different ΔT in our micro-TFFF experiments are shown in Figure 2. The dependence of λ on $1/\Delta T$ is demonstrated in Figure 3, which confirms the linearity of this relationship for micro-TFFF. The retention parameter λ was numerically calculated from the known transcendental equation relating the experimental data with the retention[6]:

$$R = 6\lambda\left[\coth\left(\frac{1}{2\lambda}\right) - 2\lambda\right] = \frac{V_0}{V_R} \tag{12}$$

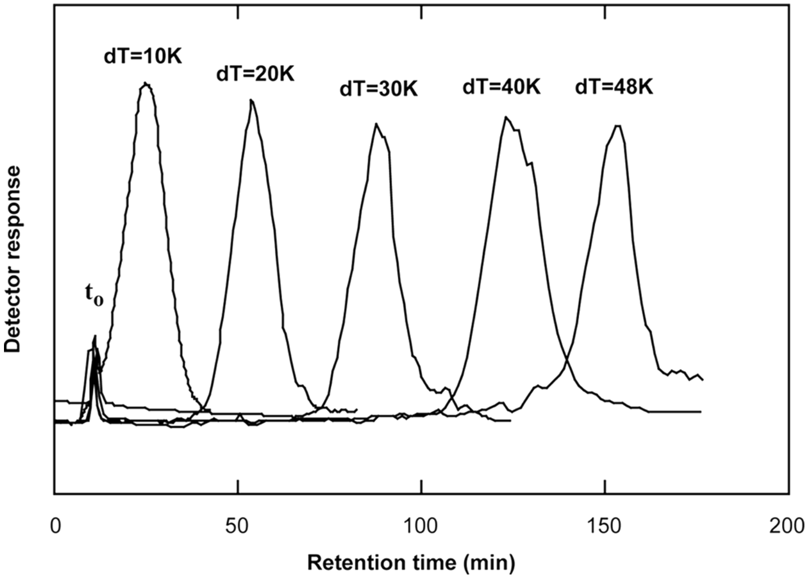

Figure 2. Fractograms of the PSL 155 sample obtained at different temperature drops $\mathrm{d}T = \Delta T$ across the channel and thus at different retention ratios R.

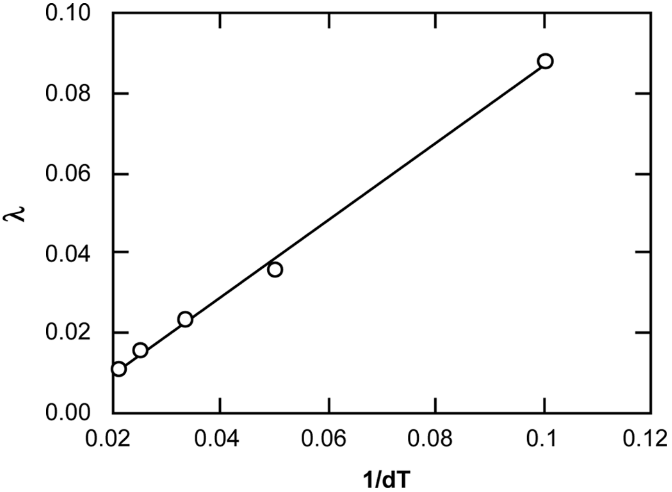

Figure 3. Experimental dependence of the retention parameter λ on the inverse value of temperature drop $\mathrm{d}T = \Delta T$, calculated from the fractograms in Figure 2.

No correction for non-parabolic flow velocity profile was applied because more than 90% of highly retained particles are concentrated at the accumulation wall in a layer that is less than 5% of the channel thickness w, and, as a result, such a correction is negligible.

It is necessary to evaluate the extra-channel contributions $\sum H_i$, which are caused by the injector, detector, and connecting capillaries. Since the variances of all zone broadening contributions are additive:

$$\sigma_T^2 = \sum \sigma_i^2 = \sigma_{Channel}^2 + \sigma_{Injector+Detector+Capillaries}^2 \tag{13}$$

The σ_T^2 values can be measured for the whole separation system, and $\sigma_{Injector+Detector+Capillaries}^2$ can be measured when the injector and the inlet capillary are connected without the channel directly to the entry of outlet capillary and the detector. This measurement has been performed with the use of a 0.1% (v/v) solution of acetone and of the suspension of PSL 155 sample at $\Delta T = 0\,\mathrm{K}$ and at different flow rates within the large range from 0.3 to 10 mL/h. The difference between the values σ_T^2 and $\sigma_{Injector+Detector+Capillaries}^2$ gives the corrected variance $\sigma_{Channel}^2$, which is due to the channel alone. As can be seen in Figure 4, showing the results of the measurements, the contribution of extra-channel elements of the tested micro-TFFF separation unit to the zone broadening is not very important, independently of the unretained solutes, acetone, or PSL 155 sample. Low molar mass acetone and PSL 155 latex were used because of their important difference in diffusion coefficients. Whereas zone broadening of the acetone is produced under the conditions of Taylor's dispersion regime, zone dispersion of low diffusion coefficient latex particles belongs to the segregated flow regime (see Janča[16] and the references cited therein). Consequently, it seemed reasonable to check the validity of Equation (13) in both regimes, under the conditions of our experiments.

The dependence of $H = \mathrm{f}(R)$ in Figure 1 clearly indicates that the second term in Equation (5) dominates over the first one for all practical linear velocities $\langle v \rangle$ of the carrier liquid. The comparison of the $H(R)$ function, calculated from Equation (5) by neglecting the first term as well as $\sum H_i$ and H_{PSD} contributions, with the experimental data for PSL 155 obtained under the experimental conditions specified above and at different ΔT is shown in Figure 5. Obviously, the experimental $H_{exp} = \sum H_i + H_{PSD}$ values are only slightly corrected by deducing $\sum H_i$, thus by deducing the contribution of extra-channel elements. When deducing the theoretical value of H_{theor}, calculated from Equation (5) by retaining only the second term, corresponding to the nonequilibrium processes, an almost constant value of $H_{exp,corr}$-H_{theor} was obtained (linear regression straight line of very low slope). At the lowest retention ratio R, the values of $H_{exp,corr}$ and $H_{exp,corr}$-H_{theor} are almost indistinguishable.

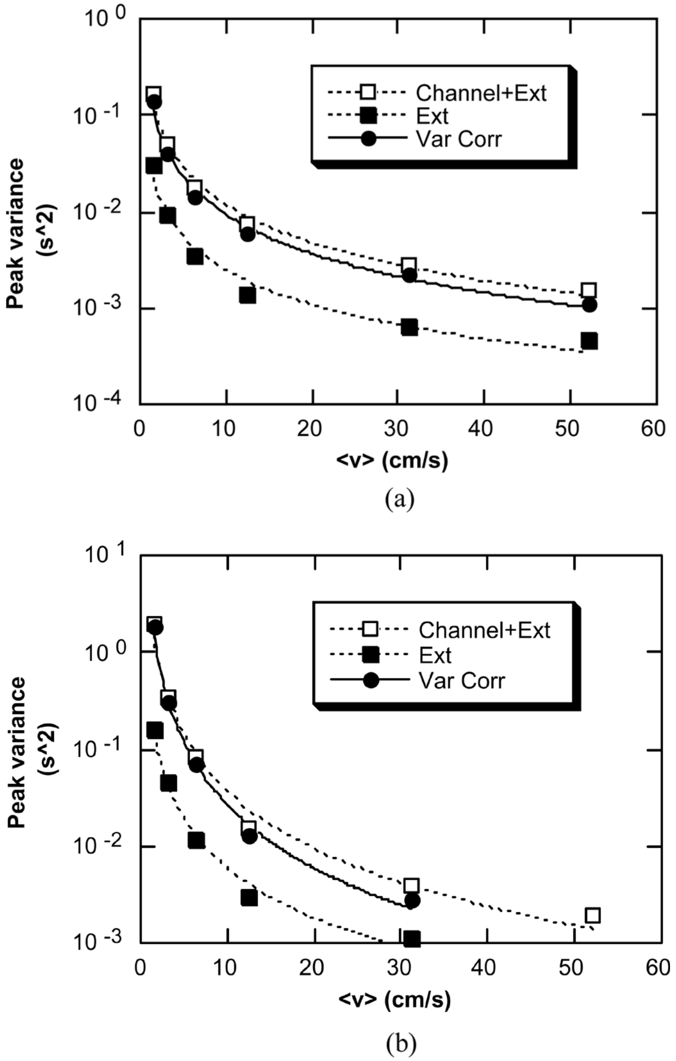

Figure 4. Peak variance $s^2 = \sigma^2$ as a function of the linear velocity of the carrier liquid $\langle v \rangle$. (a) Experimental data obtained for the unretained acetone with the whole micro-TFFF separation system (Channel + Ext), with the injection valve, detector, and connecting capillaries (Ext), and the corrected variance value (Var Corr), which is the difference between $(\sigma_{\mathrm{Channel+Ext}})^2$ and $(\sigma_{\mathrm{Ext}})^2$; (b) experimental data obtained for the PSL 155 sample ($\Delta T = 0$) with the whole micro-TFFF separation system (Channel + Ext), with the injection valve, detector, and connecting capillaries (Ext), and the corrected variance value (Var Corr), which is the difference between $(\sigma_{\mathrm{Channel+Ext}})^2$ and $(\sigma_{\mathrm{Ext}})^2$.

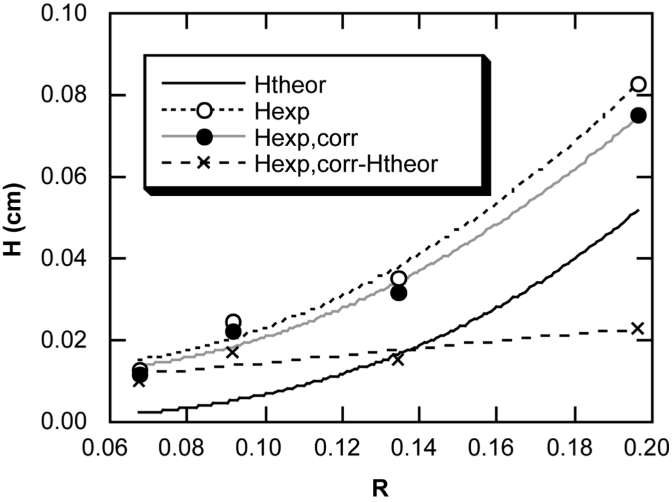

Figure 5. Dependence of the height equivalent to the theoretical plate H on the retention ratio R. H_{theor} is calculated from Equation (5) by taking into account only the second term. H_{exp} values are calculated from the experimental fractograms in Figure 2, $H_{exp,corr}$ are the experimental plate heights corrected for the band broadening due to the external elements of the channel, and $H_{exp,corr}$ are the values that should correspond to the true PSD.

CONCLUSIONS

Theoretical analysis of the efficiency in micro-TFFF indicated that it should be much more effective to optimize the operational conditions with regard to the retention rather than to the mean linear velocity of the carrier liquid (the approach frequently used in chromatography). The experiments performed with one typical particulate sample confirmed the theoretical conclusions. If the micro-TFFF is correctly constructed, the corrections for the band-broadening contributions of the elements external to the separation channel can be almost negligible. As a result, the true PSD of the analyzed samples can be determined from a single experiment carried out at relatively low but acceptable (from the viewpoint of the time of analysis) linear velocity of the carrier liquid provided that the retention is high enough.

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