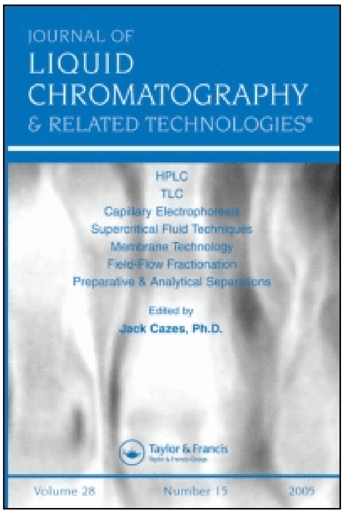

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FIELD-FLOW FRACTIONATION WITH ASYMMETRICAL ELECTROOSMOTIC FLOW. II. CHARGED PARTICLES

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ABSTRACT

The study of field-flow fractionation (FFF) with asymmetrical electroosmotically driven flow is extended to the case of charged sample particles. In this case, field-flow fractionation is combined with capillary zone electrophoresis in the same flat FFF channel.

Retention, dispersion, and resolution for sedimentation and gravitational FFF of charged particles with asymmetrical electroosmotic flow are studied by mathematical modeling.

INTRODUCTION

The new variant of field-flow fractionation was proposed[1] and studied.[2] It was suggested to use the electroosmotically driven flow instead of the Poiseuille flow. Electoroosmotic flow of conducting fluid (buffer) is generated by applying an electric field along the channel with charged (having the nonzero zeta-potentials) walls.

The usual electroosmotic flow can't be used for FFF, because its profile is too close to uniform. In order to realize the FFF type of separation, one neads to have nonuniformity of the longitudinal flow. So, it was proposed to realize the asymmetrical electroosmotic flow by making the channel walls of different materials or by chemically modifying them in order to have the different (nonequal) values of the zeta-potentials of the walls. As was shown, in[1,2] different kinds of asymmetrical electroosmotic flow profiles in FFF channels can be generated. Some of these profiles were shown to be very promising as far as high efficiency and resolution are concerned.

Yet, in Refs. 1 and 2, only the case of the uncharged sample particles was studied. In this paper, we extend the study to the case of the charged sample particles. In this case the particles are not only moving with the longitudinal flow (here asymmetrical electroosmotic flow) but are also forced by the longitudinal electric field to move along the channel electrophoretically. The case is similar to the usual capillary zone electrophoresis (CZE) where the total velocity of the particle is the sum of the electroosmotic velocity of the flow and the electrophoretic velocity of the particle.

The difference from the CZE case is that, in our situation, electroosmotic flow is nonuniform and that we also have some kind of transversal field (as usually in FFF). So, the two processes and two types of separation are combined here: FFF and CZE.

A great variety of variants of FFF and CZE combination could be imagined,[3] depending on the various factors such as: the ratio of the zeta-potentials of the channel walls, the sign and the value of the ratio of the electrophoretic and electroosmotic velocities, the type of the transversal field, and so on.

In this paper, we will restrict ourselves to the case, where the charges of the particles have the same sign as the zeta-potentials of the walls, so that the particles are electrostatically subtracted by the walls. It also means that the particles are moving electrophoretically in the direction opposite to the electroosmotic flow of the fluid. This case is easier for theoretical studies and experimental realization because the problem of particle sorption on the walls is reduced.

Retention, efficiency and resolution for FFF with asymmetrical electroosmotic flow of charged particles are studied in this paper for the cases of different functional dependences of particle charge versus particle radius. Very high values of resolution are predicted for some cases.

THEORY

The analytical solution for the electroosmotic flow profile V(Y) in the flat FFF channel with the arbitrary (nonequal) values of the zeta-potentials of the walls was derived in Ref. 2:

$$V(Y) = A[(\zeta_R - 1)\frac{\sinh kY}{\sinh k} + (\zeta_R + 1)\frac{\cosh kY}{\cosh k}$$

$$+ (1 - \zeta_R)Y - (1 + \zeta_R)] \qquad (1)$$

where

$$A = \frac{\zeta_2 \varepsilon\varepsilon_0 E_Z}{2\eta}, \quad k = \varpi w / 2, \quad \zeta_R = \zeta_1 / \zeta_2,$$

$\varepsilon, \varepsilon_0$ - dielectric constant of the buffer and permitivity of the free space, η - viscosity of the buffer, E_z - external longitudinal electric field, ζ_1 - the zeta-potential of the accumulating wall, ζ_2 - the zeta-potential of the opposite wall, w - the thickness of the FFF channel, Y = 1-2y/w - dimensionless transversal coordinate (0<y<w), $\varpi^{-1} = \left(\frac{2ne^2}{\varepsilon\varepsilon_0 k_B T}\right)^{-\frac{1}{2}}$ - Debye length, n - number of ions per init volume of the buffer, e - proton charge, k_B - Boltzman constant, T - temperature. For large values of k, the first two terms in the square brackets are substantially non-zero only in the immeadiate vicinity of the walls (in the Debye layer distance) while everywhere else the electroosmotic flow profile is dominated by the last two terms in the square brackets. Even for the very diluted buffer with concentration $C_0=10^{-5}$M the Debye length is equal to $\varpi^{-1} = 0.1 \mu m$, and so, for usual FFF channels, the values of k are rather high (k>5000).

When the charged sample particle is placed in the FFF channel with such asymmetric electroosmotic flow, it is moving with the electroosmotic flow with velocity V(Y) and is moving electrophoretically relatively to the flow, so that longitudinal velocity of the charged particle is equal to

$$V_p(Y) = V(Y) + \mu_{ep} E_Z \qquad (2)$$

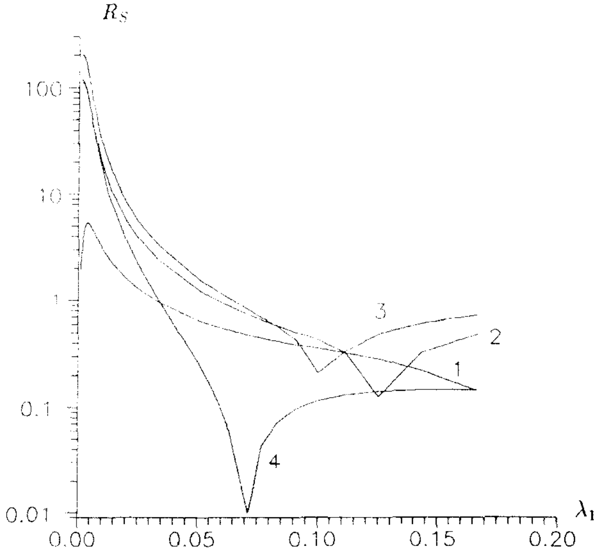

Figure 1. FFF with linear electroosmotic flow $(\zeta_R = 0)$. Resolution of charged particles.

$r_1 / r_2 = 1.2 \Rightarrow \lambda_1 / \lambda_2 = 0.58$

$k = 10000, \quad |V_{ep_1}| = 0.5|A|, \quad V_{ep} \uparrow\downarrow V_{osm}, \quad L = 0.5m$

$1 - \mu_1 / \mu_2 = 1 \qquad z \sim r$

$2 - \mu_1 / \mu_2 = 1.2 \qquad z \sim r^2$

$3 - \mu_1 / \mu_2 = 1.44 \qquad z \sim r^3$

$4 - \mu_1 / \mu_2 = 0.832 \qquad z \sim const$

μ_{ep} - electrophoretic mobility of the particle, that is considered here to be independent of coordinate (this assumption is usually used in CZE when the transversal temperature gradients are not too high).

In addition to these two types of motion, there is, as usually, in FFF, the movement of the particles in the transversal direction. Particles are forced by the external transversal field of some kind to drift towards the accumulating wall with velocity - u, and take part in the Brownian motion, characterized by the diffusivity - D.

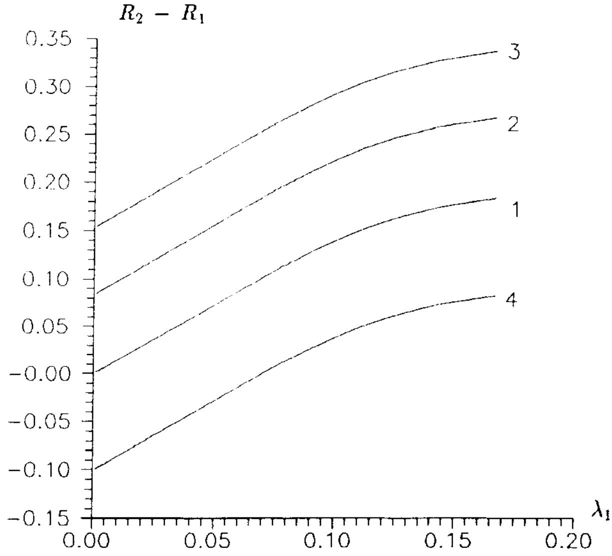

Figure 2. FFF with linear electroosmotic flow $(\zeta_R = 0)$. Retention difference $R_2 - R_1$. Conditions the same as in Figure 1.

We will consider the relaxation processes to be completed and exponential transversal distribution of the particles to be established. So we use the usual FFF equations[4] to calculate mean zone velocity $\bar{U}$, retention R, and height equivalent to theoretical plate, H:

$$\bar{U} = \int_{-1}^{1} V_p(Y)\exp(Y/2\lambda)dY \Big/ \int_{-1}^{1} \exp(Y/2\lambda)\,dY = V_{FFF} + V_{ep}$$

where (3)

$$V_{FFF} = [4\lambda \sinh(1/2\lambda)]^{-1} \Big[\int_{-1}^{1} V(Y)\exp(Y/2\lambda)dY \Big],$$

$$V_{ep} = \mu_{ep}E_Z,$$

$\lambda = uw/D$ - the basic FFF parameter, characterizing the transversal distribution of the particles.

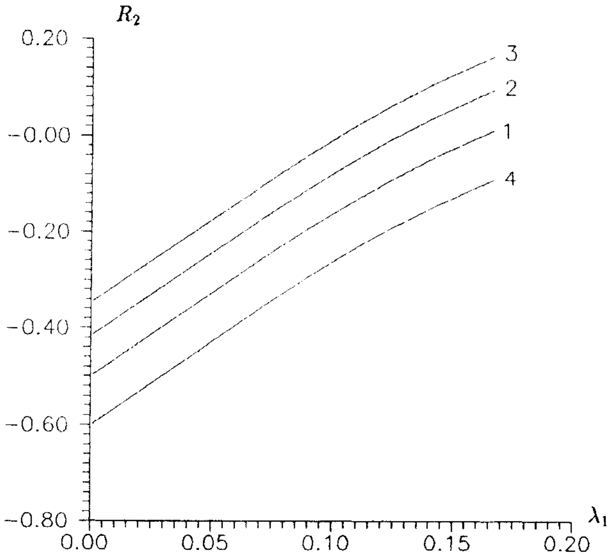

Figure 3. FFF with linear electroosmotic flow $(\zeta_R = 0)$. Retention for the second sort of particles. Conditions the same as in Figure 1.

$$R = \bar{U} / <V> \tag{4}$$

where $<V> = \frac{1}{2}\int_{-1}^{1} V(Y)dY$ - mean flow velocity, for the case when V(Y) is given by equation (1) $<V>$:

$$<V> = -A(1+\zeta_R)(1-\frac{1}{k}tanhk) \tag{5}$$

Height equivalent to theoretical plate is determined by two terms: molecular diffusion and Taylor dispersion terms

$$H = 2D / \bar{U} + \chi\frac{w^2 <V>}{D} \tag{6}$$

Coefficient χ is characterizing the role of Taylor dispersion and can be calculated for the case of arbitrary flow profile according to Refs. 5,6:

$$\chi = D_\upsilon / (2 < V >^2 R) \tag{7}$$

where

$$D_\upsilon = \int_{-1}^{1} B^2(Y) \exp(-Y / 2\lambda) dY / (4\lambda \sinh(1/2\lambda))$$

and finally

$$B(Y) = \int_{-1}^{Y} (\bar{U} - V(Y')) \exp(Y' / 2\lambda) dY'$$

We also studied resolution and described it in the usual chromatographic way:

$$R_S = \frac{(\bar{U}_1 - \bar{U}_2)L}{2(\sigma_1 + \sigma_2)\bar{v}_2} \tag{8}$$

where $\bar{U}_1, \bar{U}_2$ are the mean zone velocities for the particles of two different sorts and $\sigma_1 = \sqrt{H_1 L}, \sigma_2 = \sqrt{H_2 L}, H_1, H_2$ - are the peak widths and HETP's for these two sorts of particles correspondingly, L - length of the FFF channel.

It is generally accepted in the theory of FFF to study R, χ and H dependences versus λ. In our case, we have also another parameter characterizing the particles and the separation process - electrophoretic mobility μ_{ep}. So, we need to make some assumptions about the possible correlations between lambdas and electrophoretic mobilities. Let us consider the cases of Gravitational FFF and Sedimentation FFF. As it is well known for these types of FFF, λ is inversely proportional to the cube of particle radius. Let us consider the case when we are analyzing the sample that is the mixture of particles of various sizes but equal density. Electrophoretic mobility of the particle can be written as:

$$\mu_{ep} = z / 6\pi\eta r \tag{9}$$

where z - effective electric charge of the particle, r - radius of the particle.

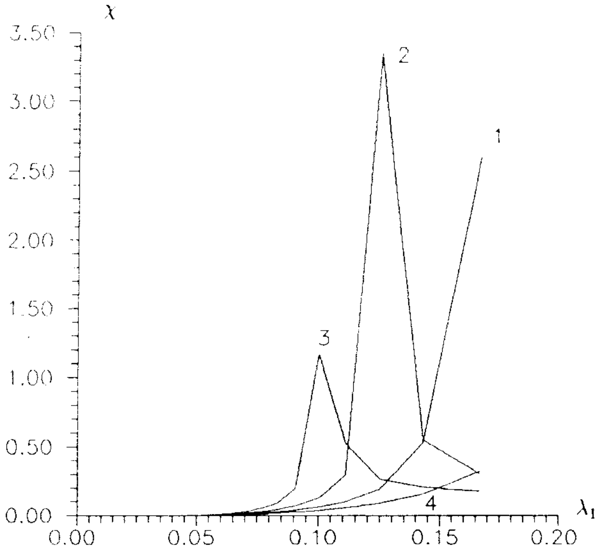

Figure 4. FFF with linear electroosmotic flow $(\zeta_R = 0)$. χ - coefficient for the second sort of particles. Conditions the same as in Figure 1.

Different situations may take place. Charge of the particles can be proportional to radius; then electrophoretic mobilities are independent of radius. This is the case of DNA fragments, for example. In this case there will be no CZE type of separation and all resolution will be only due to FFF mechanism.

Another situation takes place if charges are sorbed by the surface of the particle. Then, electrical charge is proportional to r^2 and electrophoretic mobility is proportional to r. Charge of the particle can also be proportional to the volume of the particle or r^3; then, electrophoretic mobilities are proportional to r^2. It is also possible that all of the particles have equal charges, (for example, different macromolecules that have the same charged group); then their electrophoretic mobilities will be inversely proportional to r. The results for these four cases are presented in the following section.

RESULTS AND DISCUSSION

Figures 1-4 present the results for the case of linear electroosmotic flow with $\zeta_R = 0$ (the zeta-potential of the accumulating wall is equal to zero). As

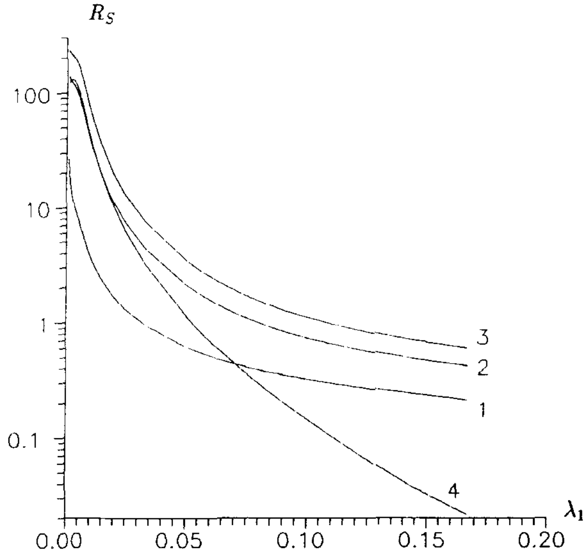

Figure 5. FFF with trapezoidal electroosmotic flow $(\zeta_R = 0.5)$. Resolution of charged particles. Conditions the same as in Figure 1.

was previously mentioned, we considered only the case of the particles moving electrophoretically in the direction opposite to the direction of the electroosmotic flow. The resolution of two sorts of particles with different by 20% radii is presented in Figure 1. Other functions are presented in Figures 2-4 to explain some anomalies in the dependence of resolution versus λ. Electrophoretic velocity of the first sort of particles was taken equal to 1/4 of the maximum of V(Y) ($|V_{ep_1}| = 0.5\,|A|$).

Curve 1 is for the case of equal electrophoretic mobilities of the particles; curve 2 for the case where the ratio of electrophoretic mobilities is proportional to the ratio of radii; curve 3 for the ratio of mobilities proportional to the square of the ratio of radii; and curve 4 for mobilities inversely proportional to radii.

The same designation of the curves was used throughout the paper. Through the entire paper, parameter λ for the first sort the particles - λ_1 was taken as the independent variable.

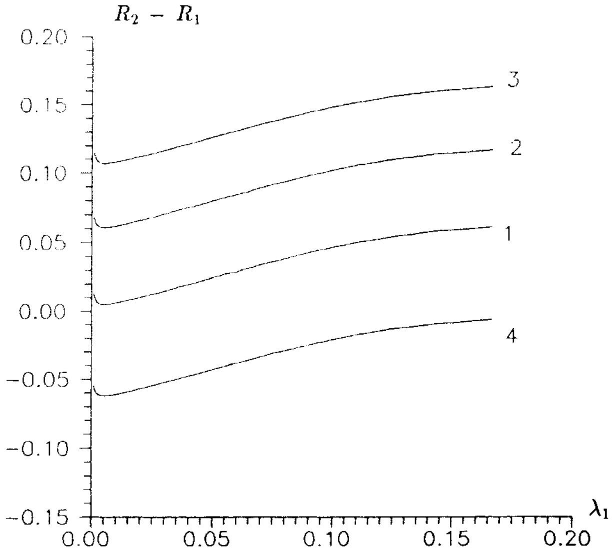

Figure 6. FFF with trapezoidal electroosmotic flow $(\zeta_R = 0.5)$. Retention difference R_2 - R_1. Conditions the same as in Figure 1.

Parameter λ_2 for the second sort of particles was calculated as $\lambda_2 = \lambda_1 \left(\frac{r_1}{r_2}\right)^3$ (this is right for Sedimentation FFF and Gravitational FFF).

As can be seen from Figure 1, resolution is especially high for very small values of λ for cases 2, 3, and 4. There are some points in this figure that need to be explained. First, one is the decrease of resolution with the decrease of λ_1 for the curve 1. In this case, the difference in electrophoretic velocities of the particles is equal to zero and, when λ_1 is decreased, the difference of the mean zone velocities $\bar{U}_1 - \bar{U}_2$, or the difference in retentions R_2 - R_1 is also lineary decreasing with λ_1. But, the dispersion for very small λ is significantly influenced not only by Taylor dispersion, but also by molecular diffusion part of H, so that σ_1, σ_2 are decreasing more slowly than lineary with λ_1, and this leads to the decrease of resolution, R_S, for very small values of λ_1. For the rest of the curves, there is the non-zero difference in the electrophoretic mobilities of the two sorts of the particles, so that retention differences R_2 - R_1 are not going to zero with λ_1 for these cases. Peak widths σ_1 and σ_2 are decreasing with λ_1, and so, the resolution is growing. The dependences of retention difference R_2 - R_1 versus λ_1 are presented in Figure 2. Note that, for cases 2 and 3, selectivity due to FFF mechanism and CZE mechanism are acting in the same direction, that is, differences in FFF velocities $(V_{FFF_2} - V_{FFF_1})$ and electrophoretic velocities $(V_{ep_2} - V_{ep_1})$ of the particles have the same sign. While, for the fourth case, the signs of these differences are opposite and there is the point where retention difference and resolution turn to zero. Two other minimums at curve 2 and curve 3 must be explained. They can be understood from the Figure 3 that presents retention of the second sort of particles R_2. Curves 2 and 3 for R_2 cross the zero line just at the points where resolution, R_S, for the same cases 2 and 3 has minimum. This is quite understandable, because the fact that retention is crossing zero means that, for this sort of particles, both signs of velocity are equally probable. The particles, characterized by these values of λ can move with equal probabilities in one direction with electroosmotic flow and in the opposite direction electrophoretically. This means zero mean zone velocity and extremely high variance due to Taylor dispersion mechanism. The extremely high level of Taylor dispersion at these special points is presented in Figure 4, where $\chi_2(\lambda_1)$ dependences are shown. The fact that dependences of R_2 and χ_2 are presented versus parameter λ_1 may seem unusual, but it only means that λ_2 was calculated as $\lambda_2 = \lambda_1\left(\frac{r_1}{r_2}\right)^3$, and the presentation of $R_2(\lambda_1)$ and $\chi_2(\lambda_1)$ is more convinient for the explanation of $R_S(\lambda_1)$ dependences then $R_2(\lambda_2)$, $\chi_2(\lambda_2)$.

Figures 5-7 present the same functions for the same cases as Figures 1-3, but for the different flow profile. Here, the zeta-potential of accumulating wall is equal to half the zeta-potential of the upper wall $(\zeta_R = 0.5)$. So, the electroosmotic flow velocity is changing from $V_{osm} \approx |A|$ at Y=1-k to $V_{osm} \approx 2|A|$ at Y= -1+k (going to zero only in the immeadiate vicinity of the walls). Figure 5 presents the resolution of the two sorts of particles with radii differing by 20%. For very small values of λ resolution is high for all functional dependences of $\mu_{ep}(r)$ studied in the paper; for larger values of λ resolution for cases 1, 2, 3 stays rather high while, for the case 4, it decreases significantly. As can be seen from Figure 6, it is due to the closeness to zero of retention difference (R_2 - R_1) curve for the values of $\lambda_1 \approx 0.2$.

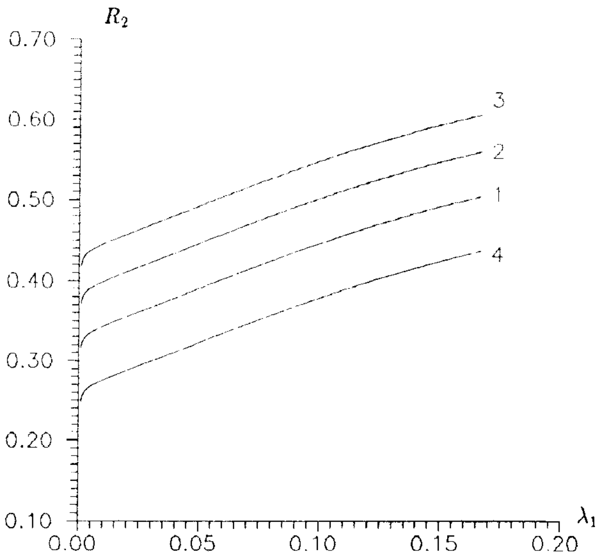

Figure 7. FFF with trapezoidal electroosmotic flow $(\zeta_R = 0.5)$. Retention for the second sort of particles. Conditions the same as in Figure 1.

Curve 1 (Figure 5) presenting the resolution of the particles with equal electrophoretic mobilities, is significantly higher in the case of the non-zero zeta-potential of the accumulating wall than in the case $\zeta_R = 0$ represented by curve 1 in Figure 6. The reason is that the mean zone velocity in the case $\zeta_R = 0.5$ is not going to zero, and so, the molecular diffusion part of HETP is not high. The curves presented in Figures 6 and 7 have a high value of curvature in the regions of very small λ. The reason for this is the presence, in the case $\zeta_R = 0.5$, of the Debye layer in the vicinity of the accumulating wall, where electroosmotic velocity is very rapidly changing from $V_{osm} \approx |A|$ to $V_{osm} = 0$. When λ is commensurable with $k^{-1} = 10^{-4}$, the nonuniformity of the flow velocity in this layer starts influencing retention.

The important difference between Figure 1 and Figure 5 is that, for the case of the trapezoidal electroosmotic flow $(\zeta_R = 0.5)$ resolution versus λ dependences are much more monotonous than in the case of linear electroosmotic flow ($\zeta_R = 0$). Monotonous $R(\lambda)$ and $R_S(\lambda)$ dependences are, naturally, more desirable for experimental realization and interpretation of experimental results, so situations when $V_{FFF} \approx -V_{ep}$ must be generally avoided.

On the other hand, in a well known paper,[7] it was predicted that the maximum resolution could be realized in CZE when electroosmotic velocities of the particles were approximetely equal and counterdirected with the electroosmotic velocity of the fluid. In this case, the extremely high resolution was predicted to be realized though, due to the very long time of analysis. This situation was not yet experimentally realized, probably because both electroosmotic and electrophoretic velocities are proportional to the external field strength and can't be changed independently during the experiment. In the case of FFF of charged particles with asymmetric electroosmotic flow V_{ep} is determined by the external field strength E_Z , while V_{FFF} is determined by E_Z and λ, so these velocities can be changed independently during the experiment and the balance $V_{FFF} = -V_{ep}$ can be established and the prediction of Ref. 7 can be experimentaly tested.

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REFERENCES

1. V. P. Andreev, M. E. Miller, J. C. Giddings, Paper 23 presented at the 5th Int. Symp. on FFF, Park City, Utah, USA, July 10-12, 1995.

2. V. P. Andreev, Y. V. Stepanov, J. C. Giddings. J. Microcol. Sepns., 1997, submitted for special J. C. Giddings issue.

3. V. P. Andreev, Y. V. Stepanov, Paper 10 presented at the 6th Int. Symp. on FFF, Ferrara, Italy, September 9-11, 1996.

4. J. C. Giddings, J. Chem.Phys., **49**, 81 (1968).

5. H. Brenner, L. J. Gajdos, J. Colloid Interface Sci., **58**, 312 (1977).

6. M. Martin, J. C. Giddings, J. Phys. Chem., **85**, 727 (1981).

7. J. M. Jorgenson, K. Lukacs, Anal. Chem., **53**, 1298 (1981).

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