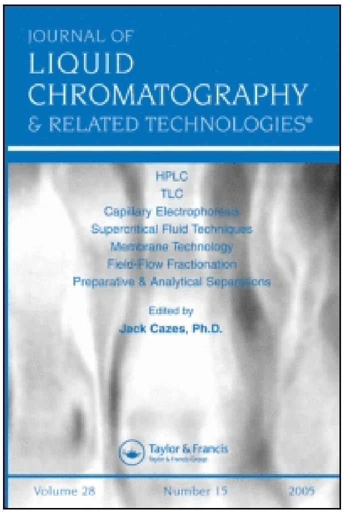

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PRELIMINARY REPORT ON FOCUSING FIELD-FLOW FRACTIONATION BASED ON COUPLING OF GRADIENT GENERATED BY PRIMARY FIELD WITH SECONDARY FIELD ACTION

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ABSTRACT

Coupling of the spatially oriented gradient of the effective property (anisotropy) of a fluid generated by a primary field with the action of a secondary field of different nature can be exploited to form the focused zones of particulate species dispersed in continuum fluid and to separate them according to differences responding to the property gradient of the continuum. This concept applied in focusing field-flow fractionation was developed theoretically. Preliminary experiments were performed to demonstrate the establishment of the density gradient by the effect of primary electric field and the formation of the focused zones by the effect of secondary gravitational field under static (non-flow) as well as under dynamic (elution due to fluid flow) conditions. The use of the proposed method is intended for analytical and preparative separations of particles of the biological and synthetic origin.

INTRODUCTION

Focusing field-flow fractionation (focusing FFF) [1] lies on simultaneous action of the field forces and of the carrier liquid flow on the sample components to be separated inside an open channel. The flow of the carrier liquid and the forces act in mutually perpendicular directions. The sample components are eluted at different times depending on the positions of their focused zones in the established flow velocity profile.

The spatially oriented gradient of the effective property (anisotropy) of the carrier liquid combined with the field action can lead to the formation of the focused zones of particulate dispersed species [2]. Either the proper force of the primary field generating the gradient or a secondary field of different nature coupled with the established gradient can produce the focusing effect [3].

This general concept is developed theoretically for a special case of density gradient established due to the action of the primary electric field on a binary carrier liquid and coupled with secondary gravitational field to form the focused zones of the large diameter dispersed particles. The appropriate experiments are designed to demonstrate the formation of the focused zones of particle standard under static conditions realized in a separation cell and under dynamic (elution) conditions in focusing FFF channel.

THEORY

The spatially oriented gradient of the effective property of a fluid can be generated in a pseudo-continuous binary carrier liquid whose at least one constituent is affected by the primary field. The density gradient appears, e.g., when charged colloid particles move relative to the suspending liquid due to the electric field effect. The mass flux n_m of this constituent in the direction of the field action along the x-axis is given by:

$$n_m = -D_m \frac{dc_m}{dx} + U_{m,e}\, c_m \qquad (1)$$

where D_m is the mass diffusivity, c_m is the concentration, and $U_{m,e}$ is the velocity of the motion of the constituent species (charged density modifier colloid particles) due to the primary electric field. The direction of the electrophoretic migration of the constituent species with respect to the electric field orientation depends on the sign of surface charge of the colloid particles. In order to simplify the mathematical treatment, D_m and $U_{m,e}$ values are considered to be constant for all constituent species, or in other words, the affected constituent is regarded as monodisperse. In the steady state it holds:

$$D_m \frac{dc_m}{dx} = U_{m,e}\, c_m \qquad (2)$$

The solution of Eqn. (2) is:

$$c_m(x) = c_m(0) \exp\left(\frac{-x\, |U_{m,e}|}{D_m} \right) \qquad (3)$$

Since it holds that $\rho_m = c_m/\phi_m$, where ρ_m is the density, and ϕ_m is the volume fraction, Eqn.(3) can be rewritten by using $\phi_{m,ave}$, the average volume fraction of the density modifier colloid particles, calculated by integrating the appropriate relationship for volume fraction distribution across the separation channel or cell to obtain [3]:

$$\rho(x) = \rho_l + \frac{\phi_{m,ave}\, \Delta\rho_m \left(\frac{w\, |U_{m,e}|}{D_m} \right)}{\left[1 - \exp\left(\frac{-w\, |U_{m,e}|}{D_m} \right) \right]} \exp\left(\frac{-x\, |U_{m,e}|}{D_m} \right) \qquad (4)$$

where ρ_l is the density of the suspending liquid, w is the distance between the electrodes in the separation cell or channel, and $\Delta\rho_m = \rho_m - \rho_l$. Eqn.(4) describes the steady-state bulk density distribution in the carrier liquid established by the effect of the primary electric field.

Secondary gravitational field coupled with the established density gradient is applied to focus the dispersed sample components at the isopycnic positions where their densities are equal to the local density of the carrier liquid. The mass flux n_i, of a single focused component of the fractionated sample can be written as [1]:

$$n_i = - D_i \frac{dc_i}{dx} + U_i(x)\, c_i \qquad (5)$$

where D_i is the diffusion coefficient of a single sample component, c_i is the component concentration, and $U_i(x)$ is the x-coordinate dependent velocity of the component motion due to focusing forces. The focused zones are established if the conditions: $U_i(x) > 0$ for $x < x_{max}$, $U_i(x) < 0$ for $x > x_{max}$, and $U_i(x) = 0$ for $x = x_{max}$, are satisfied, where x_{max} corresponds to the isopycnic position at which the concentration of the focused sample component is maximal. It holds at equilibrium:

$$D_i \frac{dc_i}{dx} = U_i(x)\, c_i \qquad (6)$$

The velocity $U_i(x)$ can be written as:

$$U_i(x) = \frac{\left(\rho(x) - \rho_i\right) D_i v_i\, g_f}{kT} \qquad (7)$$

where ρ_i, v_i, are the density and the volume, respectively, of the particle of the sample component undergoing the focusing, g_f is the gravitational acceleration, k is Boltzmann constant, and T is the absolute temperature. By substituting from

Eqns.(4) and (7) into Eqn.(6), the solution obtained by integration within the limits x, x_{max}, and $c_i(x)$, $c_i(x_{max})$ is [3]:

$$c_i(x) = c_i(x_{max}) \exp\left\{ -\left(\frac{v_i\, g_f\, \phi_{m,ave}\, \Delta\rho_m\, w}{kT \left[1 - \exp\left(\frac{-w\, |U_{m,e}|}{D_m} \right) \right]} \right) \right.$$

$$\left. \times \left(\exp\left(\frac{-x\, |U_{m,e}|}{D_m} \right) - \exp\left(\frac{-x_{max}\, |U_{m,e}|}{D_m} \right) \left(1 + \frac{|U_{m,e}|}{D_m} (x_{max} - x) \right) \right) \right\} \qquad (8)$$

which is the concentration distribution function describing the steady-state focused zone of one component of the fractionated sample.

The presented theoretical approach was used for computer simulation of the formation of the focused zones of multicomponent samples under various operational conditions in our recent study [3]. These conditions can be chosen to exploit the natural gravitation as the effective secondary field in focusing FFF.

The experiments described in the following text were intended to demonstrate the establishment of the density gradient due to the effect of the electric field and of the focused zones of model polymer latex particles under static conditions without flow and under dynamic elution conditions in focusing FFF channel.

EXPERIMENTAL

Instruments and methods

Circular cell for static thin layer isopycnic focusing (TLIF) [4] had the internal diameter of 11 mm and the thickness of 5 mm determined by the distance between the metallic electrodes. The axis of the cell was oriented vertically. The electric potential of 300 mV was applied between the electrodes across TLIF cell.

Rectangular cross-section channel for focusing FFF experiments was constructed by inserting the polymer foil in which the channel proper was cut between two metallic foils forming the electrodes and the walls of the channel. The channel was equipped by inlet and outlet capillaries connected to the six port injection valve (with the injection loop of 50 μl in volume) and to the Waters model 440 (USA) absorbance detector operating at 546 nm. The distance between the tapered ends of the channel was of 200 mm, the thickness of the channel was of 0.508 mm, and the width of 15 mm. Hydrostatic pressure produced the constant flowrate of 0.03 ml/min of the carrier liquid. Servotrace line recorder, Sefram (France), was used to record the fractograms.

Relative viscosity of Percoll solutions as a function of concentration was measured by using Viscomatic MS, Fica (France) automatic capillary viscometer. The determination of this dependence was necessary for the estimate of the influence of the viscosity gradient on the flow velocity profile formed inside the separation channel for focusing FFF. This viscosity gradient is due to concentration/density gradient generated by the primary electric field.

Materials

Commercial product Percoll, Pharmacia Fine Chemicals AB (Sweden), consisting of colloidal silica particles coated with polyvinylpyrrolidone was used as density gradient forming liquid. Original Percoll, having the density of 1.130 g/ml, was diluted with deionized water to obtain the required average density of the carrier liquid.

Colored particles of Density Marker Beads, Pharmacia Fine Chemicals AB (Sweden), possessing well-defined buoyant densities, were used to visualize the formation of the density gradient and to demonstrate the focusing effect in TLIF cell.

Sample of polystyrene (PS) latex microspheres used, Duke Scientific Corporation (USA), was the narrow particle size standard having the density of 1.050 g/ml and the average particle diameter of 19.58 μm.

RESULTS AND DISCUSSION

TLIF

The formation of the density gradient by the effect of electric field on Percoll was evidenced by the appearance of distinct focused zones of Density Marker Beads in TLIF cell. Two Density Marker Beads of nominal densities of $\rho_1 = 1.033$ g/ml and $\rho_2 = 1.051$ g/ml were suspended in diluted Percoll of the initial average density of 1.028 g/ml. The steady-state focused zones were situated at the distances of 2,0 and 3,0 mm, respectively, from the bottom of TLIF cell after 2 hours of electric and gravitational fields action.

Similar experiment in TLIF cell was performed by using PS latex standard particles suspended in Percoll of the initial density of 1.050 g/ml. Narrow steady-state focused zone was formed at the middle height position in TLIF cell thus indicating that focusing FFF of these particles should appear in the separation channel as well.

More extensive study of the effect of various operational variables on the results of TLIF was performed, the results will appear soon [4]. The general conclusion is that experimental results are in good agreement with the theoretical assumptions.

Focusing FFF

The fractograms obtained under conditions of focusing FFF of PS standard latex particles with Percoll of initial density $\rho = 1.050$ g/ml used as carrier liquid are shown in Fig.1. The elution performed without the electric field applied resulted in a single, relatively broad peak with the front eluting at the volume corresponding approximately to 0.68 of V_0, the void volume of the channel (see Fig.1A). Unretained, low molecular weight species having higher diffusion coefficients should elute under given experimental conditions at the elution volume approximately equal to the void volume of the channel. This was confirmed by injecting a low molecular weight dye dissolved in identical Percoll solution as used in previous experiment. The result is shown in Fig.2. The *elution*

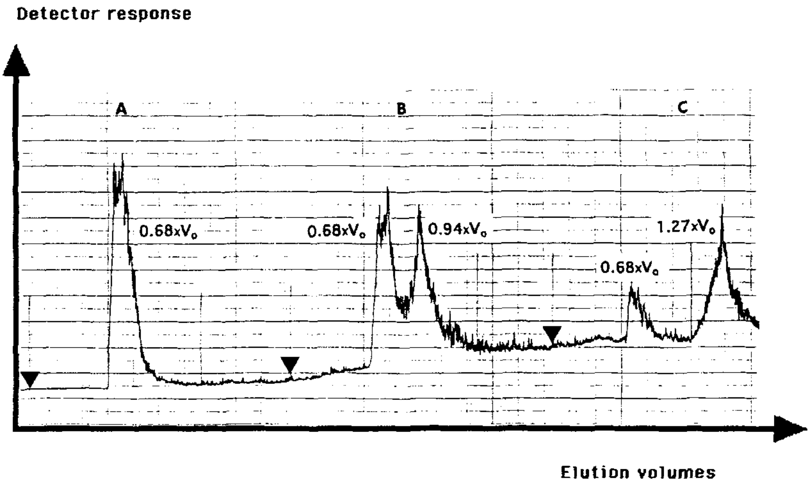

Figure 1.

Fractograms of polystyrene latex standard of the given density obtained for different intensities of electric field under conditions of focusing FFF. Arrows indicate the injection points.

Electric potential across the separation channel:

A) 0 mV
B) 100 mV
C) 150 mV

volume of the dye is equal to the void volume of the channel within the limits of experimental errors. The described experiments confirm the known fact that if the time of the elution is not long enough regarding the diffusion coefficient of the eluted species the important fronting on the elution curve appears because the part of the sample is carried at fastest streamline and has not enough time to diffuse in lower velocity region. Accordingly, in rectangular cross-section channel, the maximal streamline velocity at the centerline of the channel is 1.5x of the average velocity of the isoviscous flow which is in perfect agreement with the observed elution volume of the front of the unfocused peak.

The flow behavior in the following experiments with the electric field applied is obviously not isoviscous. The viscometric measurements allowed to

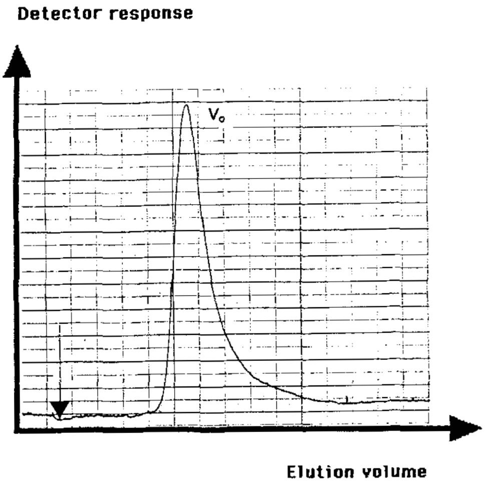

Figure 2.

Fractogram of unretained low molecular weight dye indicating the void volume of the separation channel for focusing FFF.

determine the dependence of the viscosity on the density of Percoll solutions of different concentrations. The results of viscometry are shown in Fig.3. The experimental points were approximated by 4th degree polynomial function:

$$\eta_{rel} = 5.7179\text{x}10^4 - 2.1818\text{x}10^5\,\rho + 3.1214\text{x}10^5\,\rho^2 - 1.9845\text{x}10^5\,\rho^3 + 4.7312\text{x}10^4\,\rho^4 \qquad (9)$$

where η_{rel} is the relative viscosity of Percoll solution in water. This dependence, measured at 25°C, was used to calculate the shape of the non-isoviscous flow velocity profile established in separation channel under conditions of the experiment. Numerical calculations were performed by using the above presented theoretical relationships for density distribution due to the electric field effect on suspended colloid silica particles by assuming their zeta potential to be of the

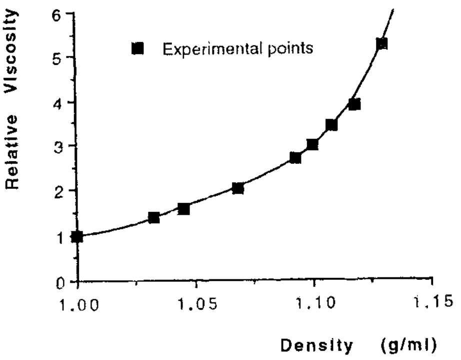

Figure 3.

Dependence of the relative viscosity on density of Percoll solutions in water measured at 25°C.

order of 30 mV [4]. The comparison of parabolic (isoviscous) and the resulting non-isoviscous flow velocity profiles is demonstrated in Fig.4. Without going here into more detailed quantitative discussion of this result it can clearly be seen that the shapes of the flow velocity profiles are very similar. Although the elution time can change dramatically for the species focused very closely near the channel walls, or for the species retained by the mechanism of classical (not focusing) FFF because of their accumulation at the wall, the elution time of the species focused at the intermediate positions should not be influenced substantially.

With the electric field applied, two peaks of PS latex particles appeared (see Fig.1B and C). The main peak appeared at longer elution time when the intensity of the electric field was increased, but in both cases a small peak appeared which corresponds to the unrelaxed part of PS latex particles carried in fastest streamline. The unrelaxed part and, consequently, the corresponding peak area decreased with increased intensity of the electric field. The relative elution volumes of the main peaks, which correspond to the focused zones, are 0.94 of V_0 (Fig.1B) and 1.27 of V_0 (Fig.1C). These relative elution volumes are inversely proportional to the relative velocities with respect to the average velocity of the

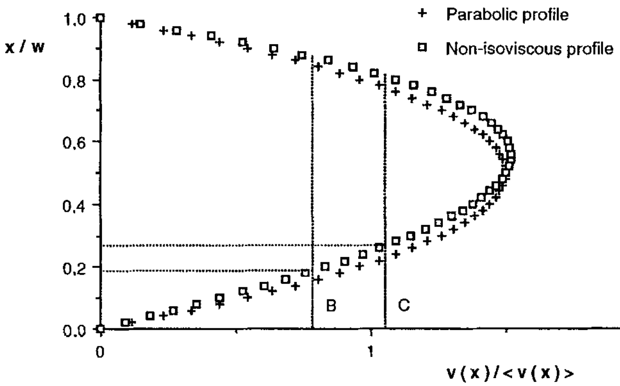

Figure 4.

Demonstration of differences between parabolic (isoviscous) and non-isoviscous flow velocity profiles formed in rectangular cross-section channel for focusing FFF. The shape of non-isoviscous flow velocity profile corresponds to the experimental conditions described in the text for electric potential of 100 mV across the channel; B and C indicate the positions of the focused zones corresponding to the appropriate experiments shown in Fig.1; <v(x)> is the average velocity of the carrier liquid, the dimensionless coordinate x/w has the origin, 0, at the lower wall of the channel.

carrier liquid and correspond to the positions of the focused zones inside the channel as indicated in Fig.4. This result obtained under dynamic (elution) conditions of focusing FFF experiment is fully consistent with the above described experiment performed under static conditions in TLIF cell.

It is evident from demonstration shown in Fig.4, that for axially symmetrical (or nearly symmetrical) flow velocity profiles such as parabolic (or nearly parabolic) ones, two focused zones positioned at the opposite sides from the central axis of the channel can be found for given relative elution volume (relative elution velocity). With respect to the increasing elution volume of the focused zones with increasing intensity of the electric field (Fig.1B and C) and to the focused zone position found in TLIF experiment, the lower positions of the focused zones indicated in Fig.4 are the most probable. However, the problem related with the accurate determination of the focused zone positions in axially symmetrical flow velocity profile actually exists and will be treated in a following paper.

The presented experimental results are of preliminary character, however, they demonstrate decisively the potentials of the new concept of focusing FFF utilizing the coupling of two fields of various nature.

REFERENCES

[1] J.Janča, Makromol.Chem., Rapid Commun., 3, 887 (1982).
[2] J.Janča, J.Appl.Polym.Sci., Appl.Polym.Symp., 51, 91 (1992).
[3] J.Janča and R.Audebert, J.Appl.Polym.Sci., Appl.Polym.Symp., in press.
[4] J.Janča and R.Audebert, Mikrochimica Acta, submitted.

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