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THEORETICAL EXAMINATION OF FOCUSING FIELD-FLOW FRACTIONATION

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SUMMARY

Theoretical aspects of focusing field-flow fractionation are considered. Selecting the channel shape for sedimentation–flotation focusing fractionation in a transverse density gradient is discussed. The possibility of isoelectric focusing field-flow fractionation in a transverse pH gradient in a flat channel is examined. Separation in such systems is shown to be possible.

Focusing field-flow fractionation in a rotating cylindrical column with a medium of constant density intermediate between those of the separated particles is considered. The separation of particles in such a column is shown to be possible with hardly any change in the initial sample width.

Focusing field-flow fractionation in a transverse high-gradient magnetic field of thin ferromagnetic wires, magnetized across the axis, has been assessed and possible separations considered.

INTRODUCTION

A modification of field-flow fractionation characterized by a transverse force able to concentrate particles, in a pre-assigned laminar region of flow, whose position depends on their properties, was first proposed by Janča[1] and called focusing field-flow fractionation (FFFF). The FFFF method, even using a transverse force of the same nature, can make it possible to separate particles in other ways than in conventional field-flow fractionation in a transverse field (FFF). The possibility of eliminating the interaction of particles with the channel walls, which often interferes with the FFF process and reduces its efficiency, may prove to be another advantage of FFFF. FFFF undoubtedly has an important advantage over direct methods, *i.e.*, those without field-flow, using the focusing principles: a small cross-section of the channel makes it possible to reduce substantially the time needed for equilibrium to be reached in the system, and hence the separation time.

Theoretical evaluation of sedimentation–flotation focusing field-flow fractionation (SFFFFF)

The possibilities of FFFF with a transverse centrifugal force have been con-

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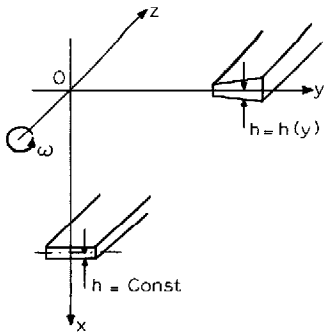

Fig. 1. SFFFFF in a channel with a cross-section whose width varies across the channel along the y-axis and in a flat channel.

sidered[1,2]. The problem of focusing was discussed later[3]. When the channel is situated on the centrifuge rotor a gradient of the density of the medium can be created in it by adding heavy metal salts to the carrier liquid. A technique of this kind is used in biological investigations[4]. Under the action of the centrifugal force the particles collect in those places where the density of the medium is equal to their density. As the laminar flow velocities are different in these regions, the particles with different densities must form fractions with different velocities.

SFFFFF has been considered[2] in a channel of cross-section in the shape of a long narrow slit, whose width $h(y)$ varied across the channel along the y-axis (Fig. 1). For the separation of particles in different sections of y = constant it was proposed to use the non-uniformity of the velocity profile along the y-axis.

It has been proposed[5] to use for SFFFFF a flat channel (h = constant) where the focusing is achieved at x = constant. In this instance the hydrodynamic dispersion of a fraction can be substantially decreased because the particles are focused in a comparatively narrow velocity range $u_z(x)$ around the point $x = x_0$.

The centrifugal force, F_g, acting on a particle is

$$F_g(x) = \Delta\rho(x) v\, \omega^2 R \tag{1}$$

where $\rho(x) = \rho - \rho_0(x)$, ρ being the particle density, $\rho_0(x)$ the density of the medium, v the particle volume, ω the angular velocity of centrifuge rotor rotation and x the distance from the axis of ratation to the centre of the channel cross-section (Fig. 1).

In FFF, usually $R \gg h$. Close to $x = x_0$,

$$F_g \approx v\, \omega^2\, R\, \nabla\rho_0(x_0)\, (x - x_0) \tag{2}$$

If a sufficiently long time has passed after the sample was introduced into the channel, the transverse distribution of the concentration of particles differs only slightly from its equilibrium distribution established under the action of the force given by eqn. 2 and the thermal motion in the absence of flow. This allows the calculation of the average velocity $\bar{u}$ of the fraction and its root-mean-square width σ with the help of the non-equilibrium theory[6,7] or similar methods[8,9] that make it possible to describe the outlet curve shape in greater detail. For the times $t \gg \tau_0$, where τ_0 is the time

needed for equilibrium to be reached, all the methods yield similar results:

$$\bar{u} = u_0 \left(1 - \delta^2 - \frac{1}{2\,\varepsilon_0}\right) \tag{3}$$

$$\delta^2 = 2 \cdot \frac{u_0^2 h^2}{D\varepsilon_0^2}\left(\delta^2 + \frac{1}{8\,\varepsilon_0}\right)t \tag{4}$$

Here u_0 is the maximum velocity of laminar flow whose profile is regarded as parabolic, D is the diffusion coefficient of particles, $\delta = x_0/h$, and

$$\varepsilon_0 = \frac{\omega^2 R v \,\nabla\rho_0(x_0)h^2}{2\,K_B T} \tag{5}$$

It is not difficult to see that ε_0 is the transverse Peclet number. Expressions 3 and 4 hold at $\varepsilon_0 \gg 1$. For typical values of separation device parameters ($\nabla\rho = 10^{-1}$ g/cm^4, $\omega^2 R = 4 \cdot 10^8$ cm^2/s, $h = 10^{-2}$ cm), at room temperature ($K_B T \approx 4 \cdot 10^{-14}$ erg) and for particles with $v = 10^{-15}$ cm^3 we obtain $\varepsilon_0 \approx 45$. This means that the requirement of $\varepsilon_0 \gg 1$ is satisfactorily met.

From eqn. 3 the average velocity of the fraction is seen to depend not only on the flow velocity at $x = x_0$, where it is equal to u_0 $(1 - \delta^2)$, but also on ε_0. This is caused by the particles being distributed across the flow in a region of width *ca.* $h/\sqrt{\varepsilon_0}$ around $x = x_0$, and not concentrated at $x = x_0$. The $\bar{u}$ *vs.* ε_0 dependence makes it possible in principle to separate, in the course of SFFFFF, particles with the same density by this parameter. From eqn. 5 it can be seen that in the case under consideration particles with the same density must be separated by volumes v. The relative decrease of σ in comparison with δ must obviously be equal to *ca.* $1/\varepsilon_0^2$ *i.e.*, be fairly considerable. The separation time must, correspondingly, also decrease.

Electric focusing field-flow fractionation (EFFFF)

For a large class of substances there is a characteristic dependence of the value, and even the sign, of their electrophoretic mobility, b, on pH. This dependence serves as the basis for separation, *e.g.*, proteins in the pH gradient spontaneously created in an electric field with the help of special additives[3]. In such a pH gradient, with the appropriate choice of its direction, the charged particles move until they enter the region where their electrophoretic mobility drops to zero, *i.e.*, where the pH is equal to their isoelectric point, pI. The particle velocity, u_x in a liquid in an electric field of intensity E is

$$u_x(x) = b(x)\mathrm{E} \tag{6}$$

Here x, as in the previous section, is a coordinate across the channel. Close to $x = x_0$, where x_0 is the transverse coordinate of the region where pH = pI,

$$u_x(x) \approx \nabla\, b(x_0)\, E(x - x_0) \tag{7}$$

In a flat channel, owing to the similarity of eqns. 2 and 7, $\bar{u}$ and ε_0 values wil be given by eqns. 3 and 4, where ε_0 should be taken as

$$\varepsilon_0 = \frac{\nabla b(x_0)\, E\, h^2}{2\, K_B\, T} \tag{8}$$

With EFFFF also it is obviously possible to separate particles not only by pI but also by ε_0. From eqn. 8 it can be seen that in this instance separation by ε_0 corresponds to separation by the value of $b(x_0) \approx$ d b(pI)/d(pH), the slope of the curve of electrophoretic mobility *vs.* pH in the vicinity of pI. This value can in principle serve as a criterion of the number of dissociating groups in a molecule and their diversity: the greater the number of the same dissociating groups in the molecule, the steeper is the b(pH) dependence. If, however, the molecule contains several types of such groups, and a proportion of them dissociate only at a pH lying far from pI, the b(pH) dependence must be less steep. With "direct" isoelectric focusing, the presence of weakly sloping b(pH) dependences is more likely to produce an interfering effect leading to widening of the individual zones.

Examination of sedimentation–flotation focusing field-flow fractionation in a system with uniform density of the medium

The SFFFFF schemes considered earlier[2,4,5] have an internal limit associated with using the density gradient of the medium for focusing of the particles. On the one hand, the density gradient is established sooner in a channel with a smaller cross-section and, on the other, the separation efficiency increases with increase in the value of ε_0, all other conditions being equal, with increasing cross-section of the channel.

This limit manifests itself most strongly in the separation of particles with large ε_0, when SFFFFF should be the most effective. At a sufficiently large difference in the $\bar{u}$ values the separation time can be even shorter than the time needed for the equilibrium density gradient to be established.

Conducting SFFFFF in a cylindrical column of radius R, rotating around its axis[10] (Fig. 2), can help in overcoming this limit. By creating a density of the medium lying between those of the two given kinds of separated particles, one can collect the particles that are heavier than the medium at the column walls and the lighter particles at its axis. The light particles in this instance will move in the flow with a velocity of *ca.* u_0, and the velocity of heavy particles should be $\ll u_0$.

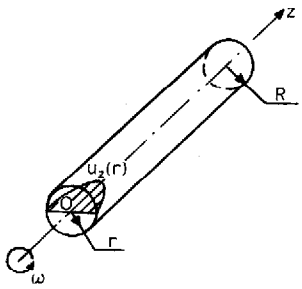

Fig. 2. SFFFFF in a cylindrical column with uniform density of the medium.

Let us consider the separation of particles with $\rho_1 = 1.51$ g/cm^3 and $\rho_2 = 1.53$ g/cm^3 in a medium with $\rho = 1.52$ g/cm^3. The volumes of the particles will be regarded as identical and equal to 10^{-16} cm^3, while the diffusion coefficient $D = 10^{-6}$ cm^2/s. This approximately corresponds to bacteriophages or DNA molecules, the principal objects of separation by sedimentation equilibrium in a density gradient. The transverse Peclet number in the system under consideration is

$$\varepsilon_0 \approx \frac{\Delta\rho \, v \, \omega^2 R^2}{K_B \, T}$$

For $\omega = 10^4$ s^{-1}, $R = 0.5$ cm and at room temperature, we obtain $\varepsilon_0 \approx \pm 2 \cdot 10^2$ for separated particles. It is to be expected that for the particles in question, because of the large difference in the average velocities of the fractions, the separation time will be short. This is especially likely when a sample containing the separated particles is at first kept in the centrifugal force field for a time of *ca.* τ_0 until equilibrium concentration distribution is established across the column, and then the flow through the column is realized.

Let us calculate the separation time, regarding the fractions as constituting layers one of which moves in the flow with velocity u_0 and the other remains stationary. In this instance the relative error of determining the velocities of the fractions must be *ca.* $1/|\varepsilon_0|$.

Let us assume that the particles have been separated if the distance along the flow between the centres of fractions is $2\sigma_0$, where σ_0 is the initial sample thickness. Then the separation time is

$$\tau = \frac{2\sigma_0}{u_0} \tag{9}$$

For typical values of σ_0 and u_0 ($\sigma_0 = 0.5$ cm, $u_0 = 0.1$ cm/s), $\tau \approx 10$ s. Dispersion of the boundaries of each fraction in the flow, caused by the presence of a velocity gradient, will be *ca.* $u_0\tau/\sqrt{\varepsilon_0}$ for light particles and $u_0\tau/\varepsilon_0$ for heavy particles, *i.e.*, $\sigma_0/\sqrt{\varepsilon_0} \ll \sigma_0$ and $\sigma_0/\varepsilon_0 \ll \sigma_0$, respectively. The diffusion component of the fraction boundary dispersion is *ca.* $\sqrt{D\tau} \ll \sigma_0$. The time needed for transverse equilibrium to be reached, τ_0, can be calculated as $\tau_0 \approx R^2/\varepsilon_0 D$. For the particle and column parameters in question, $\tau_0 \approx 40$ min. This means that the separation time almost coincides with the time needed for transverse equilibrium to be established.

For comparison we shall calculate the time needed for the density gradient to be established in such a column, τ_1. It can be calculated as R^2/D_1, where D_1 is the diffusion coefficient of heavy salt molecules ($D_1 \approx 10^{-5}$ cm^2/s); thus $\tau_1 \approx 7$ h. SFFFFF in a system with a constant density of the medium thus allows the separation time to be substantially reduced. Additional possibilities lie in combining this technique with programming the medium density. This would make it possible to extend considerably the range of the parameters of particles separated in one experiment, with a high separation efficiency being preserved.

It is of fundamental interest that the particles being focused during SFFFFF in a system with a constant medium density are separated not as a result of the

created non-homogeneity of the properties of the medium but owing to the peculiarities of the properties of the column itself and the transverse force acting in it.

The values of $\bar{u}$ and ε_0 for the above-mentioned rotating cylindrical column with a constant density of the medium have also been calculated[10]. The separation times for particles lighter and heavier than the medium, calculated with the help of the so-called 40 criterion, also proved to be smaller than ε_0.

Examination of field-flow fractionation in a transverse high-gradient magnetic field (MFFF)

We are aware of only one study where the use of a transverse magnetic force for field-flow fractionation was considered[11]. The magnetic force is known to arise in a non-uniform magnetic field. The particles whose magnetic permeability is higher than that of the medium have to be drawn into the magnetic field and, as a rule, ejects from the field those particles whose permeability is lower than that of the medium.

The effect exerted by a transverse high-gradient magnetic field on the average velocity of motion of a sample containing bovine albumin has been studied experimentally[11]. The carrier liquid contained Ni ions, which facilitated the observation of the effect[11]. A PTFE capillary (3 m × 0.15 cm I.D.) was used, rolled into a ball and placed on the end face of the cylindrical core of an electromagnet producing a field with intensity $H = 400$ G.

The velocity of sample motion was observed to decrease by 5%. Unfortunately, when the magnetic force was calculated, a mistake was made[11], as a result of which the value of the transverse Peclet number proved to be highly overestimated. A more detailed study[12] showed that the relative variation of the average sample velocity under the conditions specified by Semyonov[11], caused by the action of the magnetic force, should amount to 10^{-18}. However, the main shortcoming of the earlier work is an incorrect choice of the magnetic system and the objects to be separated. To obtain noticeable changes in the velocity of sample motion one has to use magnetic systems that make it possible to obtain a magnetic field with a sufficient intensity and gradient, as the magnetic force is defined by

$$F_m = \Delta\mu \, v \, \nabla \frac{H^2}{8\pi} \tag{10}$$

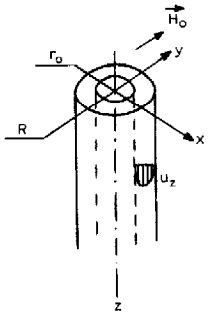

Fig. 3. Coaxial system for MFFFFF.

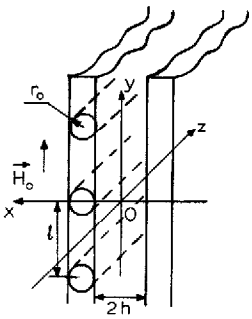

Fig. 4. Flat channel for MFFF.

where $\Delta\mu$ is the difference between the magnetic permeabilities of the particle and the medium, v is the particle volume and H is the magnetic field intensity. This means that $F_m \approx H^2/l$, where l is the characteristic variation of magnetic field intensity. Using wires made of ferromagnetics one can obtain $H \approx 2.5 \cdot 10^4$ G and $l \approx 10^{-3}$ cm. At the initial stage of the investigation one should probably try to separate particles whose size is substantially larger than that of bovine albumin molecules.

Among such particles of biological origin and interest are the particles of the microsomal fraction containing a paramagnetic enzyme such as peroxidasosome peroxidase and complexes of ferritin protein molecules containing iron.

Theoretical aspects of MFFF have been published[12,13]. The MFFF process was considered[12] for a system consisting of a cylindrical capillary and a ferromagnetic wire magnetized across the axis, placed along the axis of the capillary (Fig. 3). We studied[12] the possibilities of MFFF in a flat channel with ferromagnetic wires placed in one of its walls (Fig. 4). The latter system proved (at least theoretically) to be more efficient, owing to higher hydrodynamic characteristics of a flat channel. The study[12] showed that both of these systems have a number of common features, primarily associated with the peculiarities characterizing the magnetic field distribution of ferromagnetic wires magnetized crosswise to the axis to saturation. In such a non-homogeneous magnetic field the particles with $\Delta\mu > 0$ are always attracted to the wire surface, whereas those with $\Delta\mu > 0$ can be focused, at $M_s/2\,H_0 > 1$, in a preassigned region of the space (M_s is the saturation induction of the ferromagnetic and H_0 is the intensity of the external magnetic field magnetizing the wire).

Obviously the most advantageous technique is the focusing of particles with $\Delta\mu > 0$ in those regions where the flow velocity is maximal. In this instance the difference between the velocities of fractions with $\Delta\mu > 0$ and $\Delta\mu > 0$ will be maximal. Another common feature of the two systems in question is the dependence of the transverse Peclet number on the ratio between the radius of the wires and the channel cross-section. This dependence results from the fact that the transverse magnetic force depends on the characteristic dimensions of ferromagnetic elements.

For a system with a flat channel the optimal r_0/h ratio proved to be 0.5. It has been shown[12] that the MFFF of 1-μm particles with $\Delta\mu/4\pi = 10^{-8}$ SGSE u. and $\Delta\mu/4\pi = -10^{-7}$ SGSE u. gives a separation time that almost coincides with the time τ_0 needed for transverse equilibrium to be established in the channel, just as it was in SFFFFF in the system with a constant density of the medium. Despite their

tentative nature, the calculations performed[12,13] can serve as a basis for developing experimental devices and choosing separation methods. Further development of MFFF will probably require both a search for more effective ferromagnetic elements and optimization of the channel geometry.

CONCLUSIONS

The possibilities of FFFF are obviously not restricted to transverse forces of different physical nature and the channel geometry. The theoretical evaluations performed here can serve as a basis both for a better understanding of the processes taking place in FFFF and for creating new experimental setups.

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