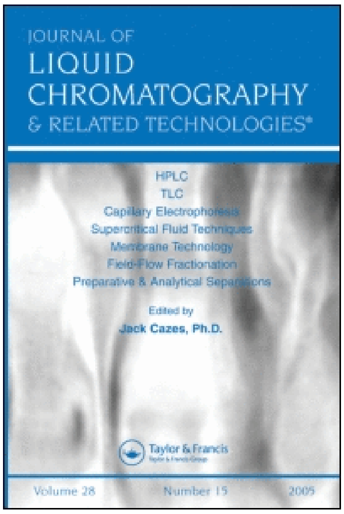


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# SEDIMENTATION-FLOTATION FOCUSING FIELD-FLOW FRACTIONATION IN CHANNELS WITH MODULATED CROSS-SECTIONAL PERMEABILITY. III. APPLICATION OF STEP DENSITY GRADIENT

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## ABSTRACT

The forming of step density gradient in channels of different shape was demonstrated experimentally. A model separation of particles of various density standards was performed. A suitable channel design together with adequate experimental conditions permits both discontinuous analytical and continuous preparative fractionations.

## INTRODUCTION

Sedimentation-Flotation Focusing Field-Flow Fractionation (SFFFFF) was proposed as a new separation method, suitable for the characterization of macromolecules and particles[1]. Separation is due to the differences in densities of macromolecules or particles migrating through a density gradient in the direction of the external gravitational or centrifugal field and being focused at isopycnic points. The focused zones are carried by flow along the channel in the direction perpendicular to the direction of the focusing external field. The shape of the cross-section of the channel determines the shape of the velocity profile formed in the flowing liquid. The effective separation of the focused zones can be achieved by choosing a suitable shape of the channel. The channels with modulated cross-sectional permeability were proposed to realize different shapes of the velocity profile[2]. The usefulness of this concept was already proven when particles of density standards and different latexes were separated in natural gravitational field[3].



Attention has not yet been devoted to the density gradient forming in SFFFFF. Essentially two possibilities can be expected. The continuous density profile can be formed due to the influence of the external gravitational or stronger centrifugal field on the density gradient medium resulting in the sedimentation of its components. The continuous density profile formed in this way has usually the exponential density distribution in the direction of the external field, however, even other shapes are possible, depending on experimental conditions. The density profile in this case is established also in the direction of the flow. Its shape depends on the ratio of the sedimentation velocity of density medium components to the velocity of the longitudinal flow. The higher this ratio the nearer to the channel inlet will be the point from which the shape of the density gradient in transversal direction (i.e., in the direction of the action of the field) can be considered to be stabilized.

The other possibility consists in forming the step density gradient providing the density gradient media of various densities flow into the channel through several separate inlets placed at the channel head. The density gradient preformed in this way is stabilized by external gravitational or centrifugal forces.

While the former situation of the density gradient formation took place (or, at least, was involved) in the successful separation of particles of different densities described in our previous paper[3], the latter way of preforming the density gradient in SFFFFF will be demonstrated experimentally in the present work.

The injection of the sample to be separated can be performed either as a short pulse or as a continuous introduction into the channel. SFFFFF can be used in the former case (with the application of a suitable velocity profile) for analytical purposes in such a way that sample components, having been separated longitudinally, leave the channel via the only outlet connected to a suitable detector. In the latter case, individual focused zones leave the channel through several outlets and SFFFFF can be used not only for analytical purposes but also as a continuous preparative technique. We mentioned both these possibilities in theoretical generalization of the focusing principle in FFF[4]. For illustration, both these alternatives are shown schematically in Figure 1.

The present work is aimed at the experimental verification of the idea of utilizing the step density gradient preforming for the separation of particles of different densities by SFFFFF.

## EXPERIMENTAL

Several channels of rectangular as well as trapezoidal cross-sections were designed and tested experi-

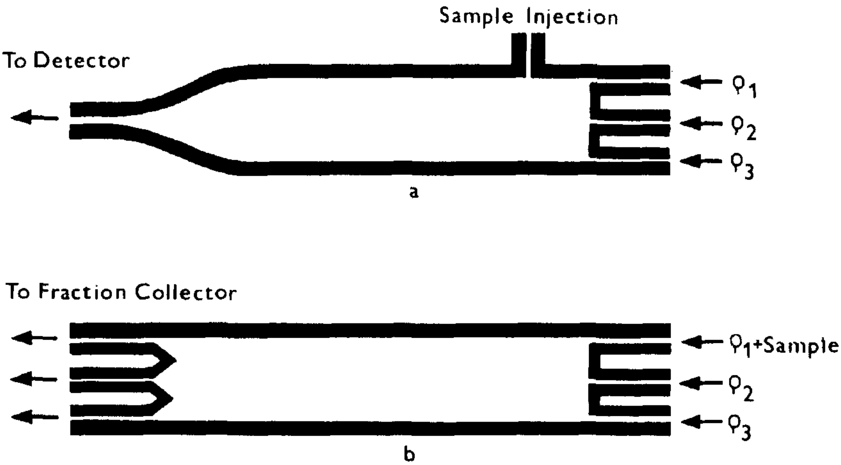


FIGURE 1

Various designs of channels for SFFFFF.
a - Discontinuous analytical fractionation
b - Continuous preparative fractionation
$\rho_1$ $\rho_2$ $\rho_3$ - Various densities of liquid media.



mentally. The channels were composed of one or several layers of Teflon foil, 0.28 mm thick, inserted and clayed between the two thin-walled glass sheets, 200x20x1 mm in dimensions. The channel proper was cut into a Teflon foil and its approximate dimensions were: the length of 170 to 185 mm and the width of 5 to 10 mm. The thickness was constant (0.84 mm) for the channel with the rectangular cross-section or varied in transversal direction in the range from 0.28 to 0.84 mm for the channel with trapezoidal cross-section. The channel was equipped with five capillary tubes. Three of them for the inlet of various density media, one for the sample injection and one for the outlet from the channel leading to the detector. An LD 2 linear displacement feeder (Development Workshops, Czechoslovak Academy of Sciences, Prague, Czechoslovakia) was used to pump simultaneously three solutions of various densities. It was equipped with three injection syringes whose pistons moved at an equal linear velocity. The flow rate of each density medium was determined by the diameter of the piston of the injection syringe used.

Either solutions of sucrose (E. Merck, Darmstadt, W. Germany) of various concentrations or Percoll solutions (Pharmacia Fine Chemicals, Uppsala, Sweden) diluted to required concentrations were used as density media. The solutions of two densities were always prepared. 1.081 and 1.176 $gcm^{-3}$ of sucrose and 1.075 and 1.129 $gcm^{-3}$ of Percoll. All the solutions were stabilized with fungistatically active sodium azide (its resulting concentration in the solutions was 0.05 %). Pure distilled water was used as the topmost layer of the step density gradient in combination with sucrose solutions and 0.15 M solution of NaCl was used with Percoll solutions. Crosslinked dextran density standards (Pharmacia Fine Chemicals, Uppsala, Sweden) were used for the model fractionation. A mixture of red density standard, 1.067 $gcm^{-3}$, and green density standard, 1.107 $gcm^{-3}$, was injected with the use of water and sucrose solutions as density media. When solutions of NaCl and Percoll were used, a mixture of red density standard, 1.062 $gcm^{-3}$, and yellow density standard, 1.087 $gcm^{-3}$, was injected.

Some of the model experiments were photographed to demonstrate both the formation of the step density gradient in the SFFFFF channel and the model separation of density standards.

## RESULTS AND DISCUSSION

In order to observe how the step gradient is formed in the channel, water and one sucrose solution of higher density which were led into the channel via the inlet capillaries were dyed with bromphenol blue, similarly as 0.5 M solution of NaCl and one Percoll solution of higher density in other experiments.

With the use of sucrose solutions and water as density media, the step density gradient was maintained along the whole channel length at sufficiently high total flow rates (200, 400 and 600 $\mu$l/min). Figure 2 illustrates an example of the step gradient formation. An unsharped boundary between the colored zone occurred, i.e., the continuous density gradient was thus formed at lower flow rates in the range of 20 - 100 $\mu$l/min, due to diffusion. Dextran density standards were focused to lower positions than it would correspond to their densities. Sucrose is preferentially solvated by water from the micellar layer of particles of density standards, their effective density beingthen, as a result of this proces, higher[5].

The step density gradient formed by NaCl and Percoll solutions was retained along the whole channel length even at very low flow rates (20 $\mu$l/min). Individual boundaries between the layers of density media were sharp, markedly distinct. Diffusion of large particles of Percoll density medium (silica particles covered with polyvinylpyrrolidone) is substantially lower. After the density standards had been injected, individual particles were focused rapidly into isopycnic points, corresponding to the nominal values of these standards. The particles were virtually focused and carried along the boundaries of the layers of various densities. The particles

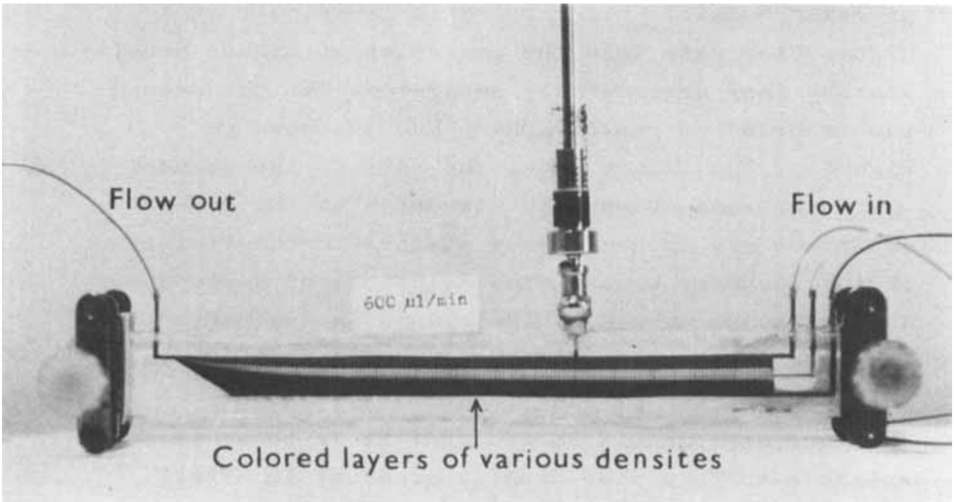


FIGURE 2
Step density gradient forming in channel for SFFFFF.

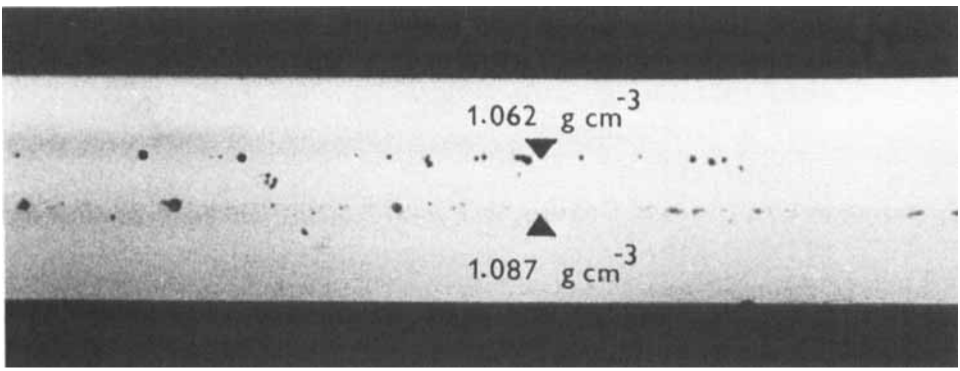


FIGURE 3
Separation of particles of two density standards in step density gradient formed inside the SFFFFF channel.

of lower density were carried in the stream at a higher flow rate than the particles of higher density. Thereby they were totally separated. The phenomenon was recorded by photographing and is shown in Figure 3. The figure shows the part of the channel where two zones of density standards of the given densities are focused. Grey stripes in the vicinity of the boundary between the solutions of various densities are caused by the change in the refractive index in this region and thereby by the change in the intensity of the passing light.

Our experiments showed that it is possible and suitable to form step density gradient in SFFFFF in the channels of adequate design and that the SFFFFF method itself is applicable to the separation of particles even in natural gravitational field. Moreover, the experimental equipment proper can be designed for analytical purposes and for continuous preparative fractionations as well.



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