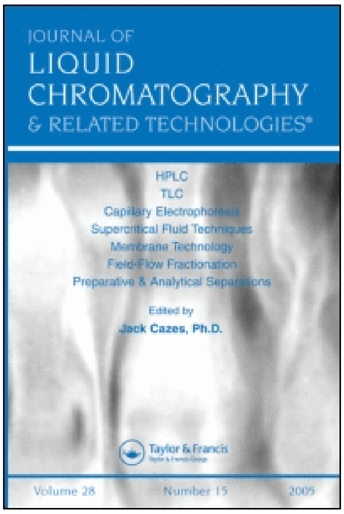

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SEDIMENTATION-FLOTATION FOCUSING FIELD-FLOW FRACTIONATION IN CHANNELS WITH MODULATED CROSS-SECTIONAL PERMEABILITY. II. EXPERIMENTAL IMPLEMENTATION

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ABSTRACT

The first experimental separation of the model particles according to their differences in densities by a new Sedimentation-Flotation Focusing Field-Flow Fractionation (SFFFFF) method is described. The SFFFFF was accomplished in a simple fractionation channel by using a density gradient medium and applying the natural gravitational field of 1 G. Although the experimental results are of the preliminary character, they proved decisively the real potential of the SFFFFF.

INTRODUCTION

In this communication we describe the first successful experimental implementation of a new

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separation method Sedimentation-Flotation Focusing Field-Flow Fractionation (SFFFFF), proposed recently (1). It is based on sedimentation-flotation processes in the density gradient of a liquid phase created across a fractionation channel. These processes cause the formation of the focused concentration zones of the components of the sample to be separated. When the resulting narrow zones of Gaussian or nearly Gaussian shapes are carried by flow of the liquid inside a channel along its longitudinal axis in the direction perpendicular to the density gradient they are separated axially due to the flow velocity profile formed in the liquid.

This focusing principle was firstly described in 1982 (1) for the special case of SFFFFF. Later on, we generalized this original idea and developed an advanced theory of separation and dispersion processes occuring in the course of fractionation by Focusing Field-Flow Fractionation method (2).

In the first paper of this series (3) we proposed the use of a fractionation channel with modulated cross-sectional permeability for Focusing Field-Flow Fractionation. The improvement of such a channel stands on the advantageous shape of the flow velocity profile formed inside the channel. A detailed theoretical analysis of the shapes of velocity profiles was developed for special cases of the channels of trapezoidal and parabolic cross-sections (3). This analysis was further extended in order to describe quantitatively the retention and efficiency in Focusing Field-Flow Fractionation (2).

This paper then represents the first step of our approach in developing the highly promissing SFFFFF method also from the experimental viewpoint.

EXPERIMENTAL

The fractionation channel of a simple design with a trapezoidal cross-section was described in detail in the first paper of this series (3). The only modification consisted in situating a separate injection capillary few centimeters after the inlet capillary in the centerline of the channel. This modification allowed to inject the samples in a more reproducible manner. The thickness and consequently the permeability of this channel changed in the direction of the density gradient. The injection was effectuated by using a conventional injection syringe. A linear displacement feeder LD 2 (Development Workshops of the Czechoslovak Academy of Sciences, Prague, Czechoslovakia) was used to pump the density gradient liquid through the separation system. The flow rates of the density gradient liquid were kept constant between 10 to 100 μl/min. A variable wavelength spectrophotometer Varian 6340 with a 8 μl flow-through cell (Varian Techtron Pty. Ltd., Springvale, Australia) was used as the detector at the end of the separation system. The detector response was recorded by a TZ 4200 line recorder (Laboratory Instruments, Prague, Czechoslovakia). A tabletop laboratory high speed centrifuge type 310 b (Mechanika Precyzyjna, Warszawa, Poland) equipped with a fixed angle rotor was used for some preliminary experiments. A density gradient medium Percoll (Pharmacia Fine Chemicals, Uppsala, Sweden) used as the carrier fluid in this study is a sterile colloidal suspension of silica particles coated with polyvinylpyrrolidone. Density Marker Beads kit (Pharmacia Fine Chemicals) was used as density standards to model the separation by SFFFFF. Further we used a monodisperse polystyrene latex standard (Duke Scientific, Palo Alto, California) having the mean particle diameter of 624 nm and the density of 1.05 g/cm^3, and a polyglycidylmethacrylate latex having the mean particle diameter of 675 nm and the density of about 1.20 g/cm^3.

RESULTS

The intention of this study was to verify by experiment that the SFFFFF method proposed theoretically works in reality, and to prove the viability of the modulated cross-sectional permeability channel idea. The latter initiated in the first paper of this series (3), where the formation of the flow velocity profile, anticipated theoretically, was proved experimentally. The following step consisted in verification of the possibility to separate the particles of various densities in a density gradient medium into narrow zones close each other. The density gradient in Percoll was formed in microvials by centrifugation at moderate rotations of 5000 rpm. Figure 1 shows the separation of the density standards in microvials of 4 mm I.D. The sharp zones of all density standards

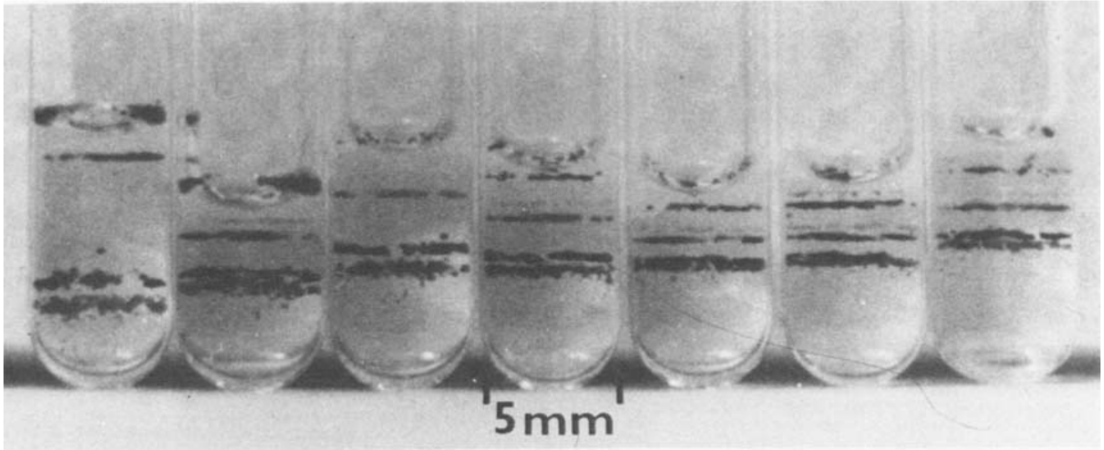
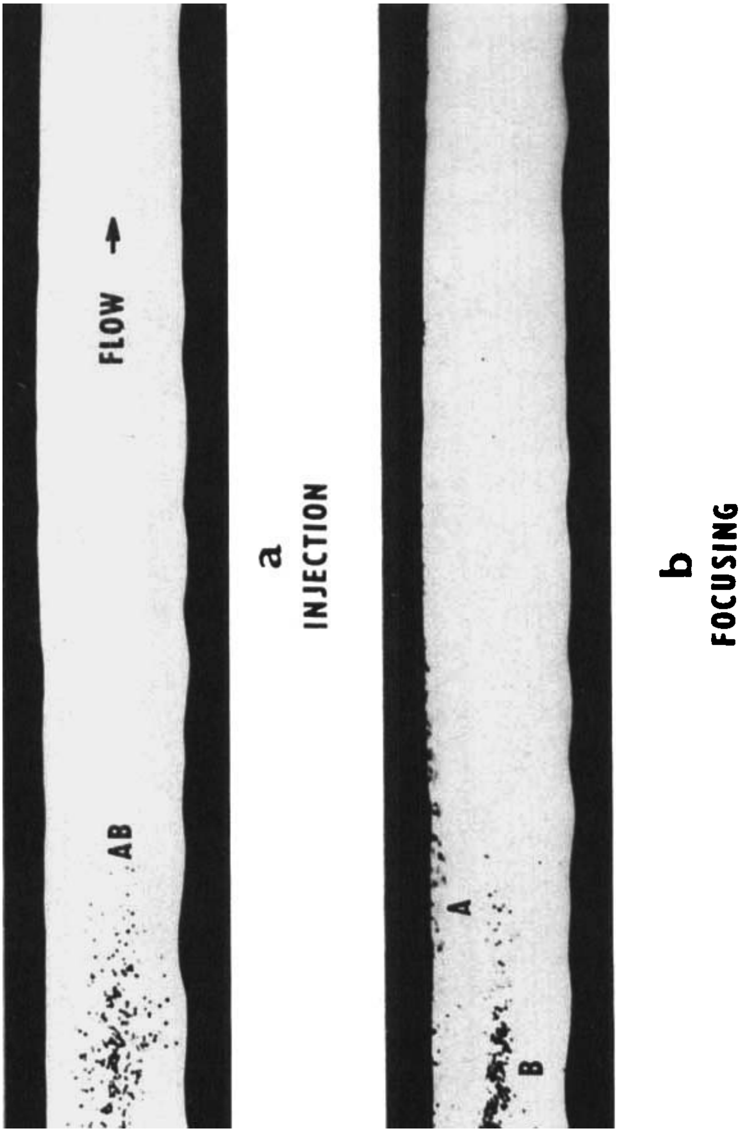
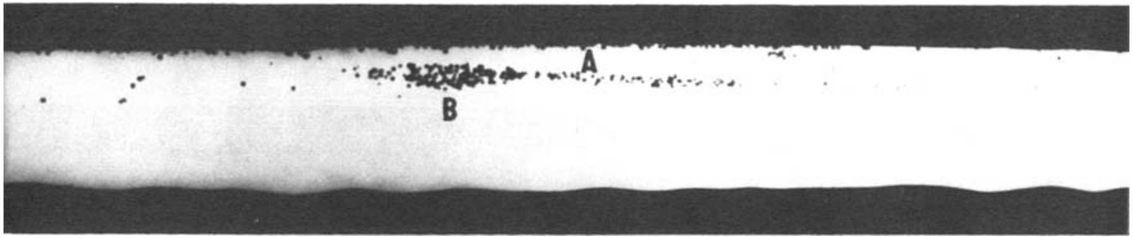


FIGURE 1. Separation of Density Marker Beads in Percoll. Densities from above: 1.06 g/cm^3; 1.09 g/cm^3; 1.10 g/cm^3; 1.12 g/cm^3; 1.14 g/cm^3. Time of centrifugation at 5000 rpm from left to right: 1, 2, 3, 4, 5, 10, 15 min.



a
INJECTION

b
FOCUSING

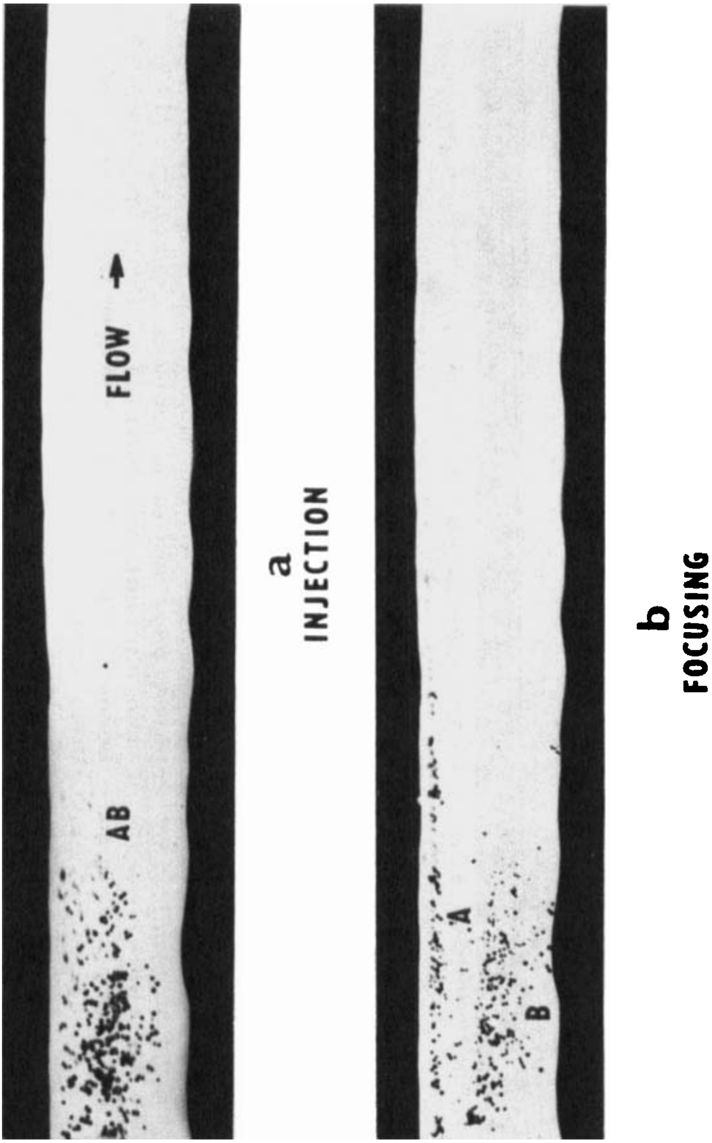


C

ELUTION

FIGURE 2. SFFFFF of two Density Marker Beads in Percoll using the trapezoidals cross-section channel. Sample: A = 1.08 g/cm^3; B = 1.14 g/cm^3. The fastest streamlines of the carrier fluid inside the channel are at the top side of the channel and the slowest ones at the bottom side.

a) Mixture of A and B samples at the moment of injection. b) Beginning of separation. Sample A becomes concentrated at the top side of the channel and moves faster along the channel. Sample B becomes focused in a streamline of slower velocity. c) Developed separation. Sample A is at the top wall of the channel. It is somewhat decelerated by friction against the top wall. Sample B is totally focused at the given streamline and leaves the channel at this position.


FLOW →
AB
a
INJECTION
A
B
b
FOCUSING

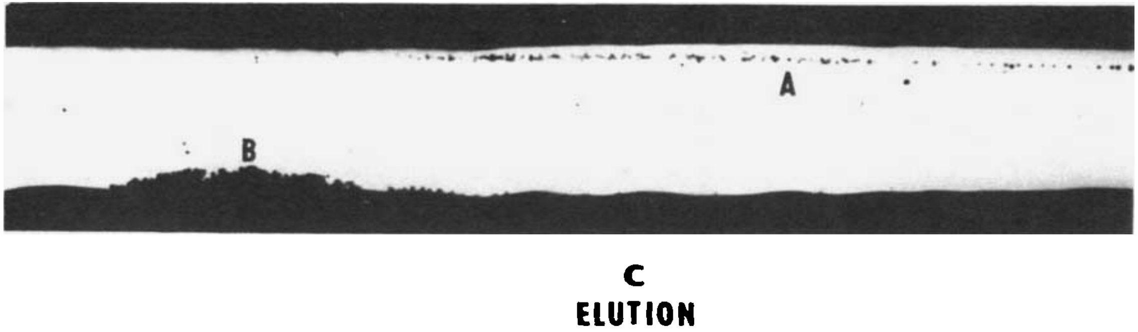


FIGURE 3. SFFFFF of the same samples as in Figure 2, but using Percoll diluted with water.
a) Mixture of A and B samples at the moment of injection.
b) Beginning of separation. Sample A becomes focused in the upper part of the channel and sample B sediments into the lower part. c) Developed separation. Sample A is focused and totally separated in the axial direction. Sample B moves very slowly in lower part of the channel due to the combined action of the slow streamline velocity and of the friction against the bottom wall of the channel.

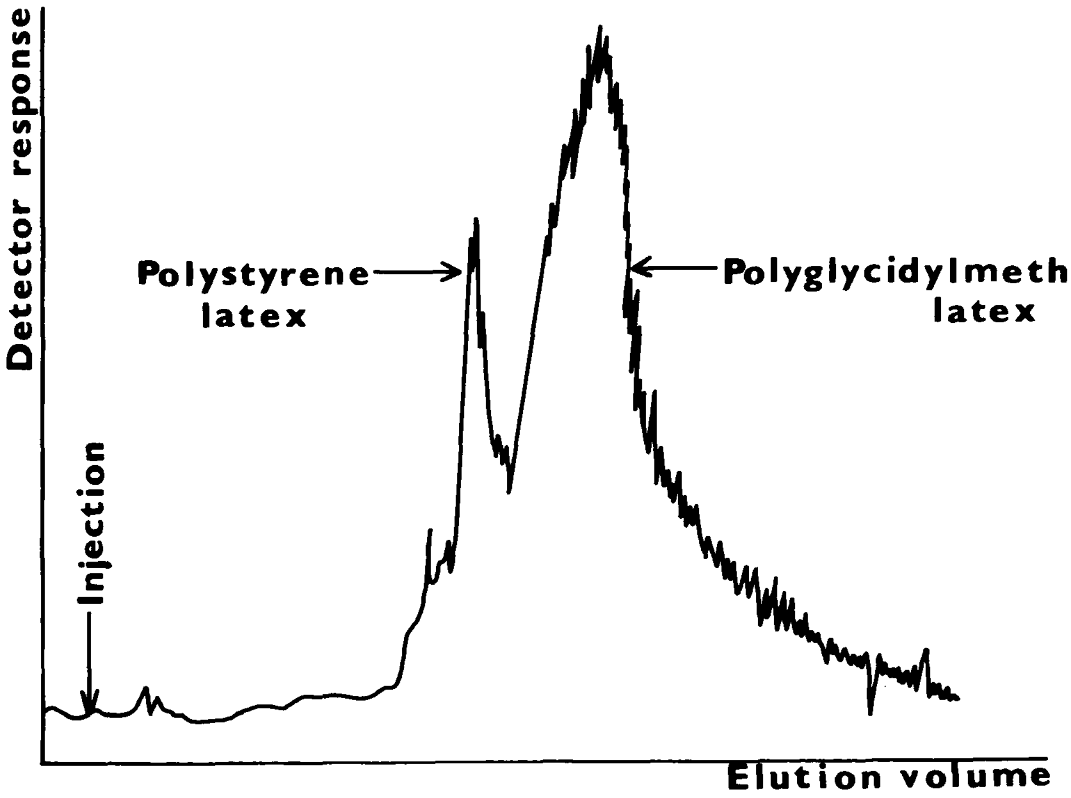


FIGURE 4. Reproduced fractogram of two latex samples separated in Percoll by SFFFFF in modulated cross-sectional permeability channel.

used can clearly be seen. These zones were formed during few minutes centrifugation. Moreover, we observed, that simillar separation was achieved even in natural gravitational field of 1 G. This observation was favorable for the following experiments.

The next step was the real SFFFFF experiment. The focusing of the zones of density standards in Percoll and their axial elution and separation was performed inside the channel of modulated cross-sectional permeability. The whole times of the fractionations were about 5 to 20 minutes. Some of the experiments were photographed and are shown on Figures 2 and 3.

Finally, the mixture of polystyrene latex with polyglycidylmethacrylate latex was injected into the SFFFFF channel and eluted with Percoll as a carrier fluid. The result of the SFFFFF separation (a fractogram recorded by using the spectrophotometric detector) is shown in Figure 4. Here, the clear separation of both latex particles can be seen. The formation and separation of the zones of both latex particles inside the channel in the course of SFFFFF experiment was observed visually, too.

CONCLUSION

Although our results are of preliminary and qualitative character, it is evident, that the proposed SFFFFF method, and generally Focusing Field-Flow Fractionation principle, are viable. The finding that SFFFFF can be performed applying moderate centrifugal forces in convenient density gradient medium and even in natural gravitational field of 1 G is encouraging.

A more detailed investigation is in progress.

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