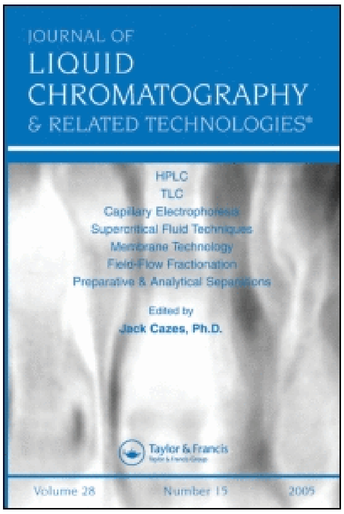

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AN ATTEMPT AT EXPERIMENTAL ELUTRIATION FOCUSING FIELD-FLOW FRACTIONATION

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ABSTRACT

The reliability of the elutriation focusing field-flow fractionation is discussed with respect to the simplified separation mechanism and potential accompanying phenomena. The fractionation of polystyrene latex particles and silica gel particles of various sizes indicated that the proposed technique of fractionation can, in principle, be applicable.

INTRODUCTION

The separation principle of field-flow fractionation (FFF) is based on a simultaneous influence of a physical field on separated macromolecules or particles and of the flow of carrier liquid in the direction perpendicular to the field action. The separation is accomplished inside the thin ribbon like fractionation channel. The cross section of the channel is usually of rectangular shape, the ratio of the width to the thickness of the channel being large. The driving forces of the field act across the channel thickness and the carrier liquid flows in the direction of its longitudinal axis. The field interacts with the separated sample macromolecules or particles and causes the generation of a concentration gradient of the sample components across the channel. The resulting concentration distribution of each component at equilibrium has an exponential shape. The longitudinal velocity of the carrier liquid also changes in the direction across the channel due to the formation of the flow velocity profile under the laminar flow conditions. The different components of the separated sample have different concentration distributions across the channel according to the magnitude of their interaction with the field. As a result, their longitudinal separation occurs in dependence on their position in the flow velocity profile. This principle was invented and elaborated by Giddings (1). It is not possible to present here all of the methods and techniques based on this principle and published in the literature, but the information can be found in a recent monograph on FFF (2).

By combining various driving forces acting on fractionated sample, its components can form narrow focused zones under the special conditions. The focused zones are located at layers where the resulting intensity of the driving forces is zero. The effect of focusing can appear, e.g., in a density gradient where particles of given densities are focused at their isopycnic points under the influence of the natural gravitational or centrifugal forces or in a pH gradient where the ampholytes are focused at their isoelectric points under the influence of electrical field. The idea to apply the focusing processes in FFF was proposed theoretically (3). Several modifications of the original concept were published. They differ in the nature and combinations of the individual driving focusing forces thus giving rise to various methods and techniques of focusing FFF, named alternatively hyperlayer FFF (4). Elutriation focusing FFF (EFFFF) is one of such hypothetical techniques (5). It is in principle similar to the formerly proposed hyperlayer flow FFF (6). The difference lies with the proposed channel geometry and, consequently, with the shape of the flow velocity profiles formed inside the fractionation channels. In EFFFF, the cross section of the channel is not rectangular as in flow hyperlayer FFF but its side walls are declined, thereby constituting the trapezoidal cross section. The external field, e.g., the natural gravitation, acts in one direction across the channel and the cross flow of a carrier liquid passing through the upper and lower semipermeable walls across the channel acts in the opposite direction. The external field causes the migration of different sample components at different but constant linear velocities in the direction of the field action. As the linear velocity of the cross flow decreases in the direction across the channel due to the diverging streamlines caused by declination of the side walls, the sample components can be focused at different positions across the channel. The focused zones are located at the positions where the velocities of the migration of sample components due to the field forces are equal to the velocity of the opposite cross flow. The resulting focused zones are eluted from the channel at different velocities due to the velocity profile formed in the longitudinal flow of the carrier liquid. As mentioned above, this principle was originally proposed by Giddings (6) for rectangular cross section channel in which the cross flow gradient was established due to the difference between the volumetric flow rates of the liquid flowing through the upper and lower semipermeable walls of the fractionation channel.

The complex hydrodynamic conditions inside the trapezoidal cross section channel for EFFFF are established and should be analyzed exactly. However, a simple experimental approach could verify the reliability of the idea of EFFFF technique and specify the problems related to its implementation.

The aim of this work was to try to fractionate the model samples of polymer latex particles and silica gel particles of micron sizes by using the trapezoidal cross section channel and applying the natural gravitational field and the cross flow thus simulating the mechanism of EFFFF.

The experiments were accomplished on the analytical scale by detecting the particles eluting from the channel and evaluating the recorded fractograms and on the micropreparative scale by collecting the fractions eluting from the channel and measuring the size of the particles in the fractions by optical microscopy.

THEORETICAL

The mean volumetric flowrate per unit area in the plane perpendicular to the direction of the liquid flow across the trapezoidal cross section channel shown schematically in Fig.1 changes in the direction of the

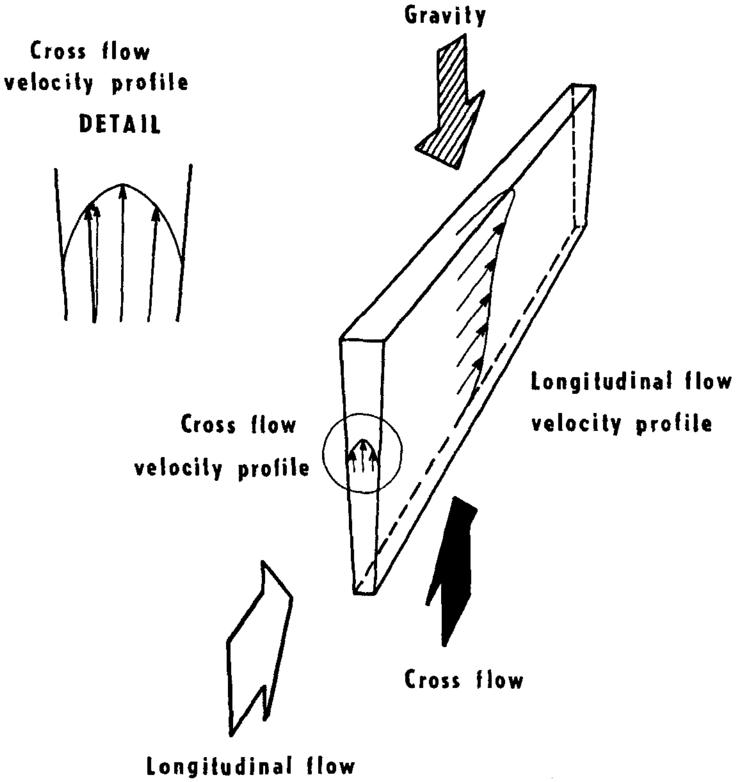

Fig.1. Schematic representation of the simplified principle of EFFFF.

flow from the lower semipermeable wall to the upper one in dependence on the angle between the declined side walls and on the ratio of the total inflowing to outflowing liquid flowrates through the semipermeable walls of the open channel. The open channel means that casual difference between the inflowing and outflowing liquid volumes is compensated by the carrier liquid flowing longitudinally along the channel. The mean volumetric flowrate is an average value per unit area in the plane from one declined side wall to the other. The geometry of the fractionation channel together with the ratio of the inflowing to the outflowing liquid flowrates are the main experimental variables allowing to adjust the required linear velocity gradient in the direction opposite to gravity action across the channel.

The local linear velocity of the cross flow generating the effective force opposite to the action of gravity is position dependent not only as a consequence of the mentioned experimental variables but also due to the flow velocity profile formed in the plane of the channel cross section (see Fig.1). This flow velocity profile, as unavoidable, appears to be an undesired phenomenon accompanying the above described image of the simplified mechanism of EFFFF.

The direct consequence of the flow velocity profile formed in the plane of the channel cross section is that the sedimenting particles should experience more complicated pathways than to be simply focused in a layer shaped according to the form of the flow velocity profile. The vector of the diverging cross flow can be decomposed into vertical and horizontal components (see Fig.1). The horizontal component should cause the lateral flux of the sedimenting particles toward the side walls. Near the side walls where the linear velocity of the cross flow approaches zero value, the particles should not focuse at all but sediment under the influence of the gravity. However, the finite size of the particles does not allow the approach of their centers to the side wall closer than to the distance equal to their radii. Consequently, the particles can never reach the completely stagnant layer of the cross flowing liquid and will permanently be influenced by lifting forces due to the cross flow. On the other hand, the friction of the particles in contact with the side walls will retard their sedimentation due to gravity.

Simultaneously, the longitudinal flow of the carrier liquid could influence the lateral migration of the particles owing to the so called pinch effect that causes the concentration of the moving particles certain distance apart from the side channel walls.

The above considerations indicate that the hydrodynamic conditions inside the channel will be rather complex. A detailed quantitative analysis can provide the definite and exact picture of the mechanisms and processes coming into play. Only on the basis of such an analysis, the geometry and dimensions of the fractionation channel as well as the appropriate experimental conditions can be optimized and the objective comparison of relative advantages and drawbacks of the EFFFF configuration in trapezoidal cross section channel with the flow hyperlayer FFF in conventional rectangular cross section channel can be effected.

EXPERIMENTAL

An experimental arrangement for EFFFF is shown in Fig.2. It was composed of two linear displacement pumps, one providing the longitudinal flow of the carrier liquid (Doser M-122, Mikrotechna, Czechoslovakia), the other equipped with two syringes providing the cross flow (LD-2, Development Workshops, Czechoslovak Academy of Sciences). The use of two identical syringes functioning simultaneously, one for pumping and the other for suction, is necessary in order to keep the constant flow rate across the channel, undisturbed by changes in the longitudinal flow of the carrier liquid. A six port valve, (Inst.Anal.Chem., Czechoslovakia), was used for the injection of separated samples. The injection valve was connected to the input of the fractionation channel with a short capillary tubing. The output capillary of the channel was connected with a UV spectrophotometric detector for analytical fractionations (UVM-4, Development Workshops, Czechoslovak Academy of Sciences). The detector was working at a wavelength of 254 nm. The separation channel (see Fig.3) was composed

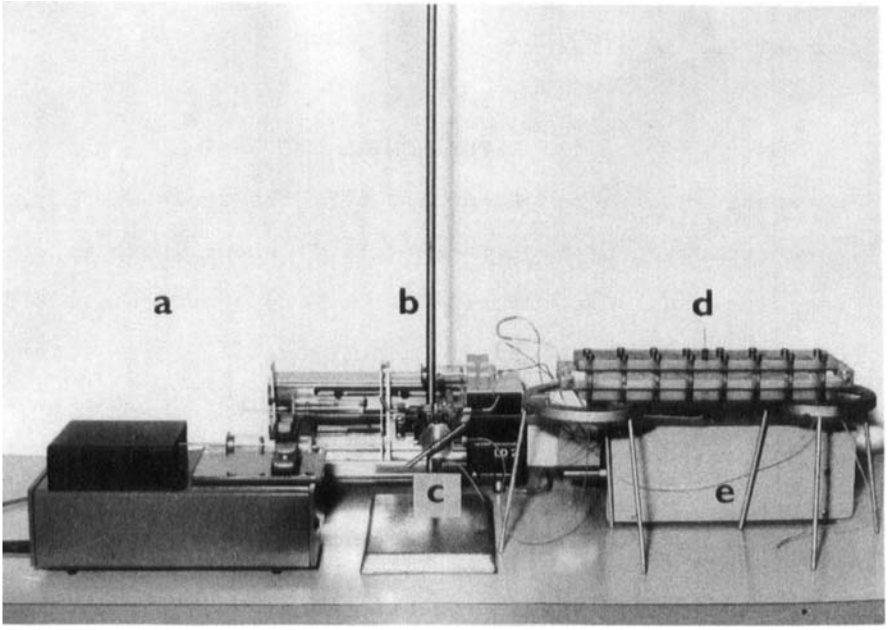

Fig.2. Experimental arrangement for EFFFF.

a: linear displacement pump for the longitudinal flow of the carrier liquid

b: linear displacement pump for the cross flow

c: injection valve

d: separation channel

e: detector

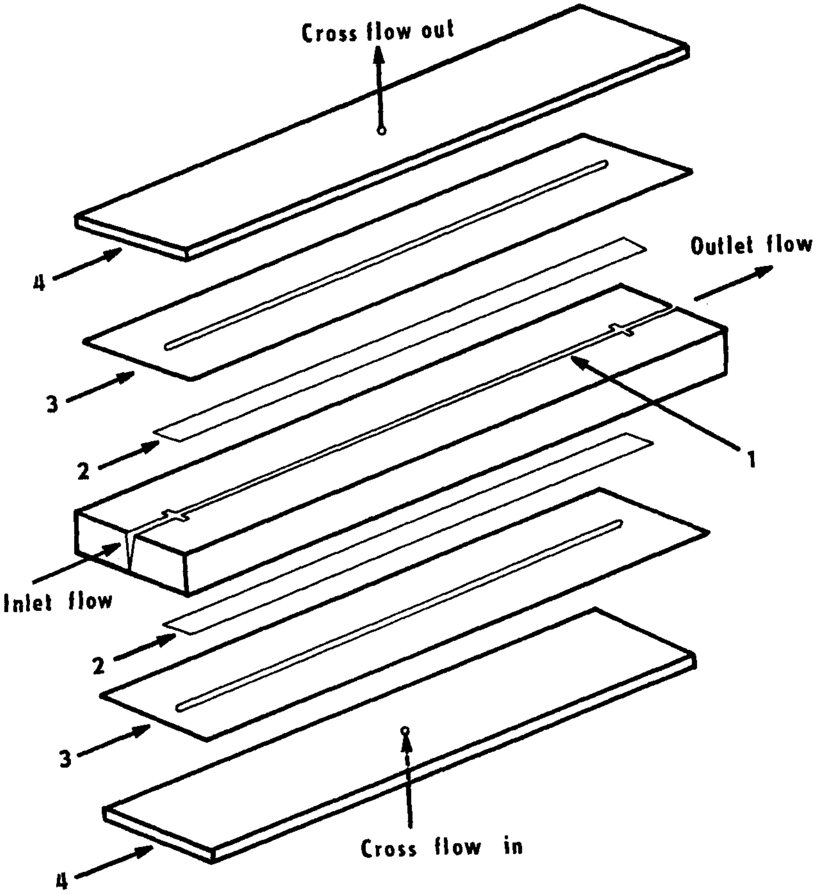

Fig.3. Schematic representation of the fractionation channel.

1: trapezoidal channel

2: frits

3: silicone rubber spacer

4: perspex blocks

of two perspex blocks bevelled so that they included the required angle thus forming the trapezoidal cross section channel. The lower and the upper walls of the channel were made of stainless steel frits sealed with the 5mm thick silicone rubber spacers in which the longitudinal slots were cut to conduct the cross flowing liquid homogeneously across the whole area of the semipermeable frits. The carrier liquid and the samples to be fractionated were introduced into the channel by an inlet capillary and taken out by an outlet capillary situated at the end of the channel. The channel for micropreparative use was equipped at the end with two outlet capillaries each situated near the lower and the upper semipermeable walls, resp., for collection of two different fractions. The whole system was kept between the two perspex blocks. The separation channel itself was 20 mm high, 250 mm long, and 0.2 and 0.55 mm thick in the lower and the upper parts, resp. The volume of the channel was 1.875 ml.

The solution of 0.1 % of Tween 60 detergent (Fluka AG, Switzerland) in distilled degassed water was used as a carrier liquid in the case of the fractionation of polystyrene latex samples and distilled degassed water in the case of silica gel particles fractionation. Two samples of polystyrene latex particles of the diameters of 1.6 and 5 μm and of the density of 1.05 g/cm^3 were provided by Dr.K.Bouchal from the Institute of Macromolecular Chemistry, Prague. Three samples of silica gel particles used in this study were provided by Dr.K.Slais from this Institute and were of the diameters of 3 and 10 μm, and one was a mixture of particles of 3 to 5.9 μm in diameter. The densities of all silica gel particles were 2.3 g/cm^3.

RESULTS

Analytical EFFFF was used to separate the samples of polystyrene latex particles. The experiments were accomplished under various longitudinal and cross flow rates of the carrier liquid. Based on the practical experience from these experiments, the following operational procedures were adopted.

If only the particles of 5 μm in diameter were injected at a longitudinal flow rate of 200 μl/min and with the cross flow stopped, a rapid sedimentation was observed, and the particles were retained at the lower semipermeable wall. After the period of 30 min a cross flow of 200 μl/min was started, the sedimented particles were lifted from the lower wall and eluted from the channel. The record of this procedure is a fractogram shown in Fig.4 A.

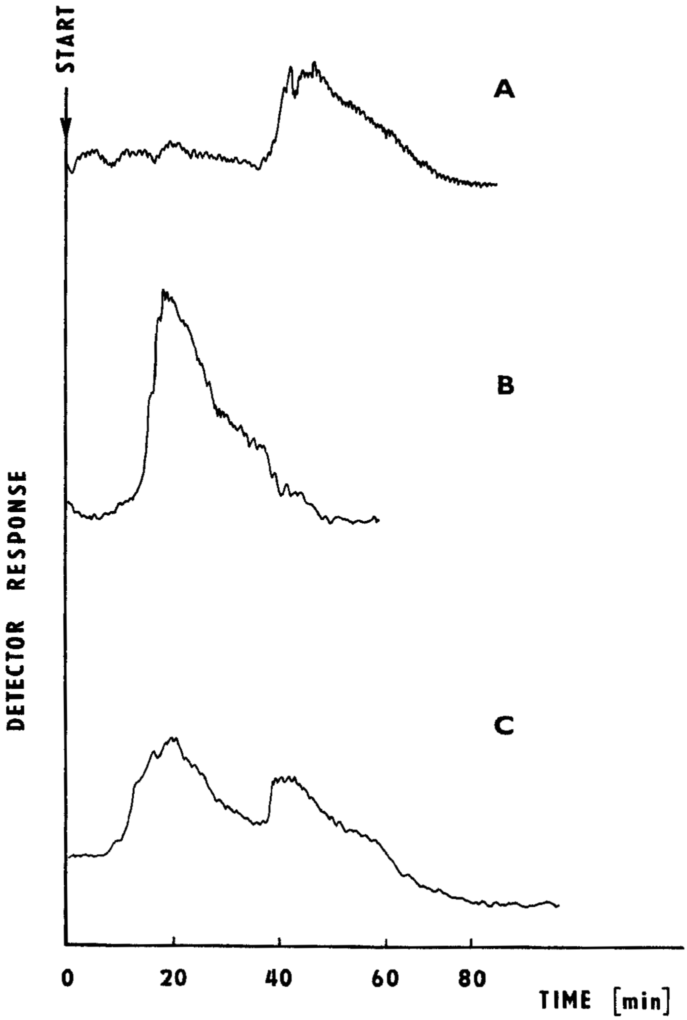

Fig.4. Fractograms of two polystyrene latex particles.

A: 5 μm particles

B: 1.6 μm particles

C: mixture of 1.6 and 5 μm particles

If the sample with particles of 1.6 μm in diameter was injected at a longitudinal flow rate of 200 μl/min and with the cross flow stopped, the sedimentation was not observed in a short time. The particles formed a relatively narrow layer, probably due to the channel hydrodynamics, and eluted from the channel without a remarkable retention. This procedure was recorded in a fractogram shown in Fig.4 B.

If the mixture of both 1.6 and 5 μm particles was injected at a longitudinal flow rate of 200 μl/min and with the cross flow stopped for the initial period of 30 min and later on restarted at 200 μl/min, the 1.6 μm particles eluted first followed by 5 μm ones. The fractogram of this separation is shown in Fig.4 C.

Micropreparative *EFFFF* was used to separate a mixture of two narrow distribution samples of silica gel particles, 3 μm and 10 μm in diameter and a polydisperse sample with particle diameter range from 3 to 5.9 μm. To separate the mixture of 3 μm and 10 μm silica gels, a longitudinal flow rate of 640 μl/min was used simultaneously with a cross flow of 270 μl/min. After the injection of the sample mixture under separation, the longitudinal flow was stopped for the period of 60 min. After this period, the longitudinal flow was

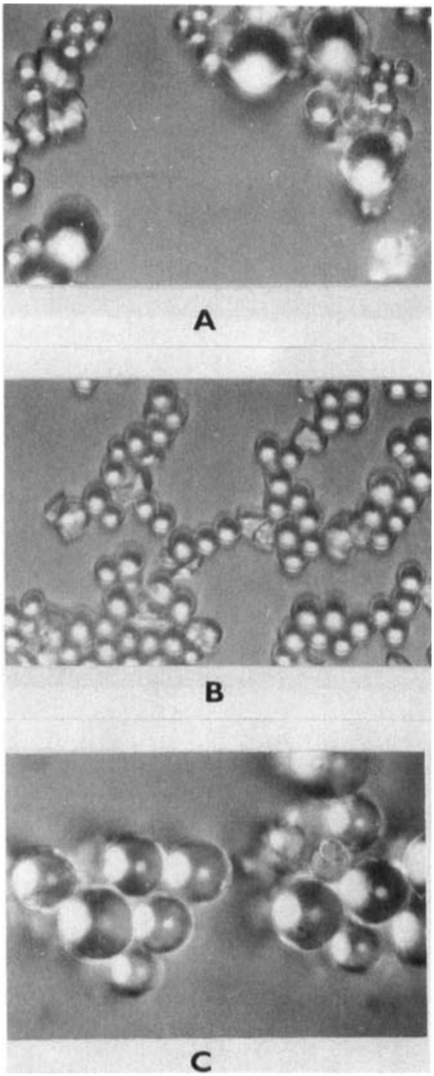

Fig.5. Photographs from optical microscopy showing the separation of the model mixture of two different samples of narrow distribution silica gel particles.

A: mixture of 3 and 10 μm particles

B: fraction taken from the upper outlet

C: fraction taken from the lower outlet

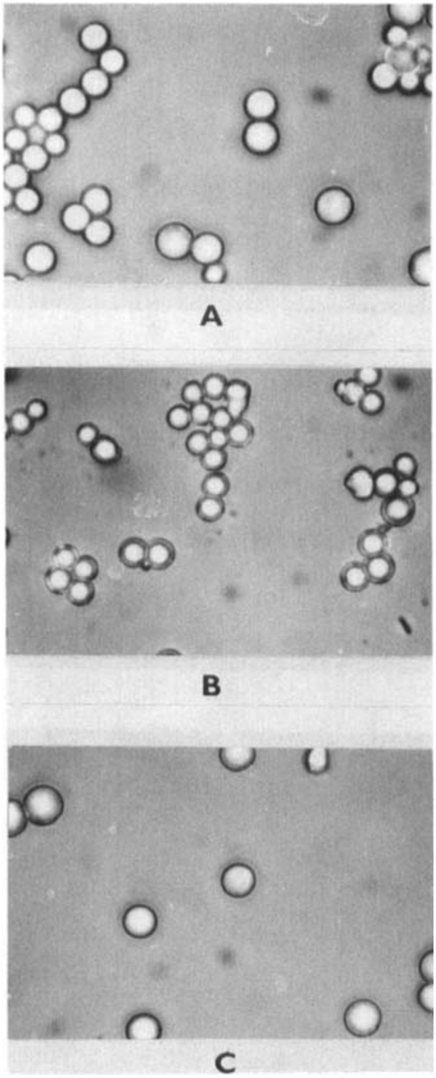

Fig.6. Photographs from optical microscopy showing the fractionation of the polydisperse sample of silica gel particles (3 to 5.9 μm in diameter).

A: unfractionated sample

B: fraction taken from the upper outlet

C: fraction taken from the lower outlet

restarted and smaller particles were eluted via the upper output capillary and collected into a vial. After the elution of smaller 3 μm particles, the cross flow was increased up to the 3200 μl/min and larger 10 μm particles were eluted via the lower output capillary and collected into a vial. The collected fractions were evaluated microscopically. The photographs of the original mixture and of the both fractions are in Fig.5. The photographs demonstrate a fair separation of the 3 and 10 μm silica gel particles.

Finally, a polydisperse silica gel sample was injected at a longitudinal flow rate of 200 μl/min and a cross flow rate of 280 μl/min. After the injection, the longitudinal flow was stopped for the period of 60 min and then restarted. The fractions collected simultaneously from both lower and upper output capillaries were collected and evaluated microscopically. The result of the fractionation, shown in Fig.6, demonstrates the unfractionated sample and both fractions. It can clearly be seen that the upper fraction contains larger proportion of smaller particles and, on the other hand, the lower fraction contains larger proportion of larger particles.

CONCLUSION

The theoretical considerations as well as the preliminary experimental results indicated that the proposed EFFFF can be realized. On the other hand, it is evident that more extensive investigation, both theoretical and experimental, is necessary to determine the actual separation mechanism and to elucidate the full potential of this technique.

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