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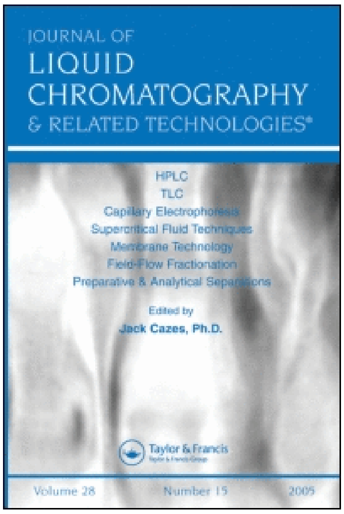

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Isoperichoric Focusing Field-Flow Fractionation for Characterization of Particles and Macromolecules

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ISOPERICHORIC FOCUSING FIELD-FLOW FRACTIONATION FOR CHARACTERIZATION OF PARTICLES AND MACROMOLECULES

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ABSTRACT

The discoveries and innovations in science reflect always a symbiosis of the continuity of thought, knowledge, and experience inherited permanently from the predecessors, and of the outstanding capability of imagination and creativity in breakthrough moments. The invention of the field-flow fractionation concept and its impressive growth belong to such a step forward on the evolution spiral and represent a most important contribution to the science of separation of large molecules and particles in the course of the last 30 years. A generic focusing field-flow fractionation principle has developed both in theory and in analytical methodology. Particular methods and techniques were proposed and implemented independently by several laboratories in the world. This review summarizes the progress achieved during the last few years.

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INTRODUCTION

Known and exploited from about 5000 years B. C., recognized by Archimedes some 2222 years ago, and reviewed for the first time in 1556 by Georgii Agricolae[1] (a german physician living in Jáchymov, Bohemia), the recovery of the fine gold grains or other metals from the sand, soil, and clay by panning or sluicing is based on different buoyancies of the particles in a gravitational field and perpendicular water stream. This antique craft work, lying somewhere between the art and science, is beautifully represented in Fig. 1, reproduced from Ref. 1. An aerostat, shown in Fig. 2, created by Joseph and Etienne Montgolfier,[2] is a phenomenological predecessor of the generic inventions of the isopycnic[3] and isoelectric[4] focusing methods. Regarding the long pathways of history, the ingenious and sophisticated field-flow fractionation (FFF) principle in its polarization variant was proposed by J. Calvin Giddings very recently.[5]

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The idea[6] of the focusing FFF appeared in 1982. This emerging principle was mentioned also in a review on FFF[7] without any reference that could be given at the time of submission of the paper. Giddings[8] preferred the non-generic term hyperlayer FFF attributed to this principle. Later, the focusing FFF[9-11] was clearly identified as a new methodology beside the classical polarization FFF,[5] and the first theoretical and experimental results were cited. In this article, the developments derived from the original idea are described. Several laboratories all over the world contributed independently to this progress. As a result, various focusing mechanisms were subsequently proposed and exploited under conditions of dynamic FFF. The generalized principle of the isoperichoric focusing FFF belongs to the newest achievements in this field. The aim is to report on the new focusing FFF methods and their experimental implementations or potential analytical applications, and to acknowledge their adherence to the family of FFF methodology, a great challenge raised by Giddings in 1966.

Some principles not frequently known are briefly explained to facilitate the understanding of the complementarity, but also of the differences concerning the new focusing and the classical polarization FFF.

FOCUSING AND POLARIZATION FIELD-FLOW FRACTIONATION METHODOLOGIES

FFF is based on a simultaneous action of the effective field forces and of the fluid flow on the fractionated sample inside a separation channel. The mutual orientation of the field forces and of the carrier fluid flow is

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Figure 1. Medieval sluicing technology for gold and other metals separation from the sand, soil, and clay particles.

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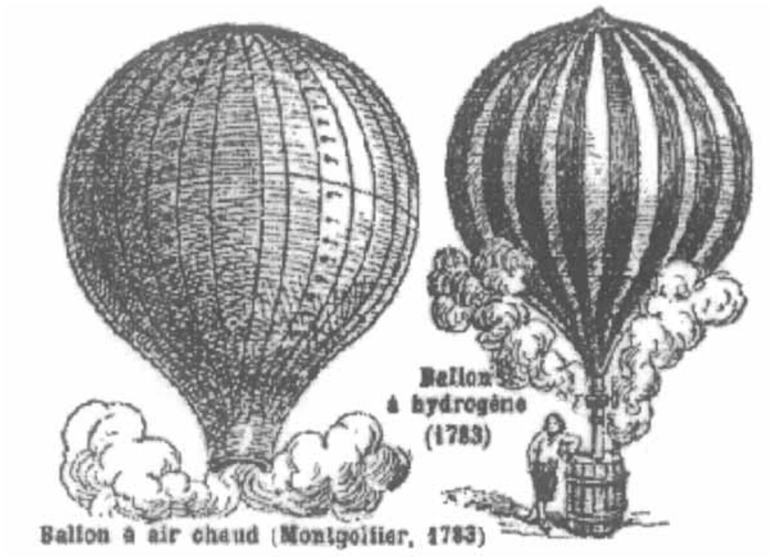

Figure 2. Aerostats of Montgolfier's brothers.

perpendicular, as shown in Fig. 3a. The recent applications of the FFF concern the fractionation of the macromolecules and particles dissolved or suspended in a liquid. The sample to be fractionated is injected into the channel as a short pulse, or as a continuous stream, carried by the liquid flow, as in chromatography. The field interacts with the sample and induces the formation of the concentration gradient of each sample component across the channel. This gradient is balanced by an opposite, dispersive flux. Consequently, a steady-state concentration distribution is established. Simultaneously, a flow velocity profile is formed across the channel due to the viscosity effects that accompany the flow processes. As a result, the sample components are eluted at different velocities, corresponding to their positions within the flow velocity profile and leave the channel at different elution times. This situation is shown schematically in Fig. 3b for the focusing separation mechanism and in Fig. 3c for the polarization mechanism. The resulting zones are either focused at different altitudes or differentially compressed at the accumulation wall of the channel. The substantial difference between the focusing and polarization mechanisms lies in the driving field force whose intensity and direction is dependent on the position across the channel in focusing FFF, while it is position independent in polarization FFF.

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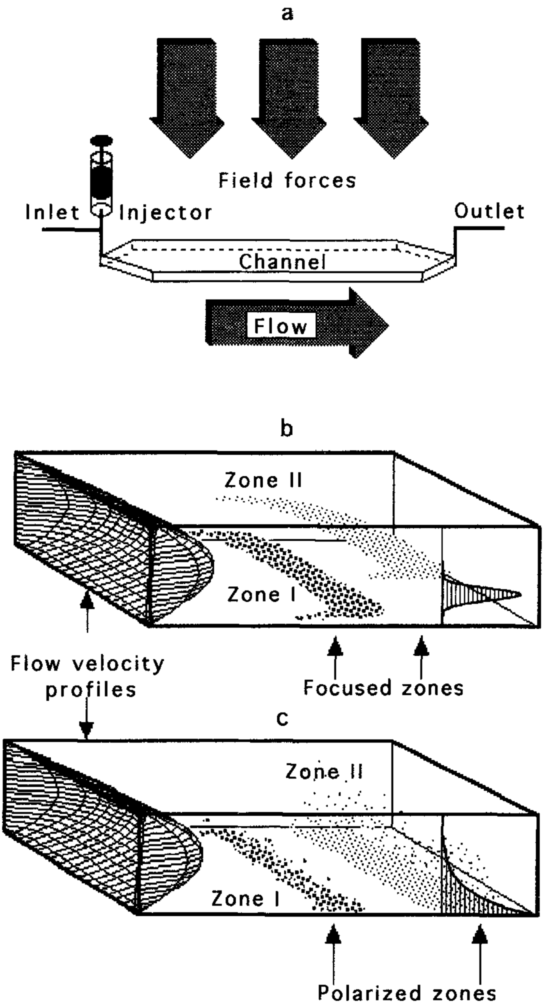

Figure 3. Principle of focusing and polarization field-flow fractionation. a) Mutual orientation of the field forces and of the fluid flow inside the separation channel; b) Section of the channel demonstrating the principle of focusing FFF with two distinct zones focused at different positions, the parabolic flow velocity profile, and the schematic representation of the Gaussian concentration distribution of focused species within a particular zone; c) Section of the channel demonstrating the principle of polarization FFF with two distinct zones concentrated differentially at the accumulation wall, the parabolic flow velocity profile, and the schematic representation of the exponential concentration distribution of one separated species.

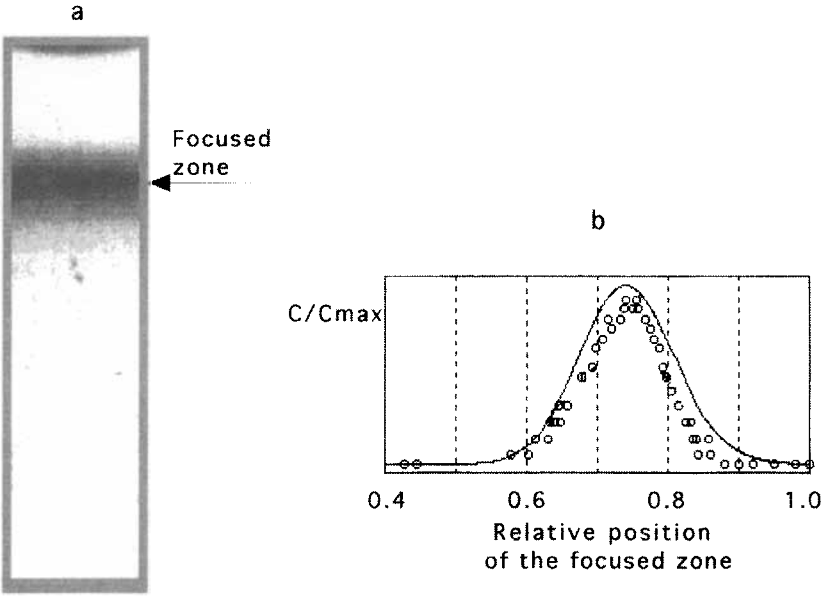

Figure 4. Isoperichoric focusing of the colored nanosize polyaniline particles in a density gradient formed in a suspension of the colloidal silica particles. a) Scanned macrophotograph of the zone of the colored polyaniline particles focused under conditions of static thin-layer isopycnic focusing; b)Theoretical (—) and experimental (o) shapes of the steady-state concentration distribution within a focused zone in a bidisperse suspension of the nanosize polyaniline particles in silica colloidal particles.

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ISOPERICHORIC FOCUSING AND FIELD-FLOW FRACTIONATION

The gradient of the effective property of the carrier liquid, combined with the action of a field, can lead to the focusing of the separated species. For example, the particles of a given density focus in the density gradient combined with the gravitational or centrifugal field at their isopycnic positions, the amphoteric species focus in the pH gradient combined with the electrical field at their isoelectric points, etc. All these particular focusing phenomena are referred to by the general term *isoperichoric focusing*, introduced by Kolin in his seminal paper[12] on the genesis of isoelectric focusing and generalization of the idea. This means that the responding quality of the focused species and the corresponding local effective property of the carrier liquid do not induce the displacement of that species at the isoperichoric point, but induce the converging transport outside this point.

The theory predicts the isopycnic focusing in bidisperse suspension of the nanosize particles of different densities without *a priori* imposed large size ratio of the focused to the density gradient forming particles.[13] This prediction was evidenced experimentally by the focusing of the polyaniline particles in a suspension with silica particles of close average diameters, and partly superposed particle size distributions.[14] The image of the focused zone in the isoperichoric focusing experiment, carried out under static conditions, and the comparison of the experimental result with the theoretical prediction is shown in Fig. 4.

Usually, the same primary field forces which produce the density or pH gradient, such as centrifugal forces or electric field forces, etc., are used to generate the isoperichoric focusing effect. An original proposal[15] consists of the use of the secondary field forces of different nature to generate the focusing phenomenon within the corresponding gradient established by the primary field. This new isoperichoric focusing principle, exploiting the combined action of two fields of different nature, applied under static or dynamic FFF conditions, is very promising for high performance analytical and micropreparative separations. The experimental test was performed by using the static thin-layer and the dynamic isoperichoric focusing FFF of model polystyrene latex particles.[16] While separation was not observed under static conditions, focusing FFF resulted in very good resolution as shown in Fig. 5a. An important advantage is that both the static thin-layer focusing cell and the channel for dynamic isoperichoric focusing FFF, applying the combination of the electric and gravitational fields, are very simple, as can be seen in Fig. 5b and c, and are much less expensive compared to the centrifuge. As the distance between the electrodes is short (roughly from 100 μm to a few mm), a low voltage (of the order of 100 mV to a few V) is enough to create the high electric field strength (Vcm^{-1}). Theoretical calculations[13] verified the above mentioned increase of resolution of separation of the dynamic isoperichoric focusing FFF compared with the experiments under static conditions.

The simultaneous use of two fields of different nature represents a challenging alternative of the isoperichoric focusing FFF, but not the only one. Natural gravitation alone was used to separate large particles (100 to 200 μm) of density marker beads in a density gradient under conditions of isopycnic focusing FFF.[17] The scanned macrophotographs of this former experiment in Fig. 6a shows, clearly, the focusing inside the channel under different operational conditions. More powerful centrifugal forces were applied to demonstrate the focusing of the polystyrene and poly(glycidylmethacrylate) latex particles in a density gradient formed by sedimentation of colloidal silica particles in a centrifuge rotor.[18] Unfortunately, the experimental work was

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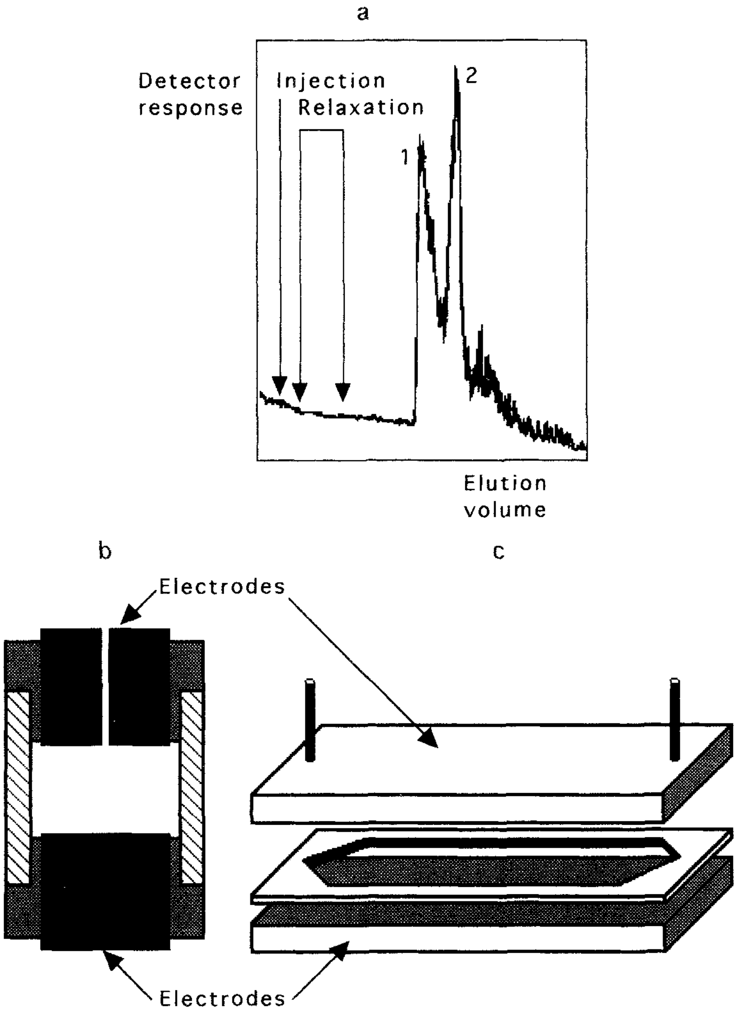

Figure 5. Isoperichoric focusing field-flow fractionation in coupled electric and gravitational fields. a) Fractogram of two samples of polystyrene latex particles. Particle diameters were 9.87 μm (1) and 40.1 μm (2). While this dynamic FFF separation shows a good resolution of the separated samples, no detectable resolution was achieved under static conditions in thin-layer isoperichoric focusing cell; b) Design of the cell for static thin-layer isoperichoric focusing and of the channel for dynamic focusing field-flow fractionation.

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suspended before the reproducible results could be obtained. The last version of the apparatus, built up in 1982[19,20] for these applications, is shown in Fig. 6b. In isopycnic focusing FFF experiments, using the natural gravitational field forces alone,[17] some doubts existed as to the possibility to establish the density gradients sufficiently strong to generate the focusing phenomena during the elution time allowed. Another plausible explanation of the observed focusing phenomenon is that the density gradient formed inside the channel is, in fact, stepwise due to the injection of the focused particles in a liquid whose density is different from the density of the bulk carrier liquid at the injection point. A careful experimental investigation of the analytical and preparative focusing FFF in preformed stepwise density gradient[21,22] confirmed that the focusing under such conditions is very rapid. The gradient of the effective property can be preformed, for example, by pumping carrier liquids of various densities through different inlets of the channel. If the layers of different densities flow under conditions hindering their mixing, a step density gradient is formed which can be stabilized by the primary field.[21,22] The preforming of the pseudo-continuous gradient is typical for, but not limited to, the density gradient. The unpublished results obtained by Nováková and Janča within the frame of the work published in Refs. 21, 22 are reproduced in Fig. 7. They show a very rapid focusing of the density marker particles on an in situ preformed stepwise density gradient, as well as an excellent stability of the different density layers, along the separation channel. On the other hand, a recent study of the transient state in the settling of the same colloidal silica[23] as used previously[17] indicates that the kinetics of this transport phenomenon is quite a slow process, even at high centrifugal field forces.

The electrical field alone was applied to establish a pH gradient and to separate the acidic and basic components of horse heart myoglobin by isoelectric focusing FFF.[24] This separation is demonstrated in Fig. 8a. The design of the separation channel, published originally in Ref. 25, is shown in Fig. 8b, which was reproduced in Ref. 26 without the citation of the original source. Another attempt to separate the amphoteric proteins was less successful.[27] A former colleague continued the investigation of the operational variables influencing the formation of the pH gradient under conditions of isoelectric focusing FFF.[26,28] His results generally agree with the known theoretical and experimental findings. The original idea to use the channels with modulated cross-sectional permeability in focusing FFF, elaborated in 1983,[29] was applied[28] without the accurate citation. These channels allow to overcome the problem of the identical longitudinal velocities of two distinct zones focused at the same, but opposite, distances from the central axis of the channel of rectangular cross-section in which the axially symmetrical flow velocity profile is formed (see Fig. 9).

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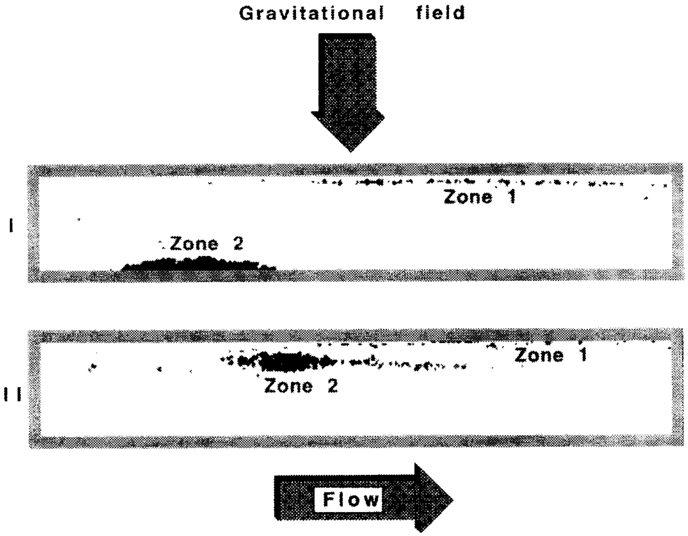

b

A detailed experimental study[30-32] of the isoelectric focusing FFF in a channel with modulated cross-sectional permeability (trapezoidal cross-section) resulted in the distinct focusing of cytochrome c and in the efficient separation of horse spleen ferritin, equine myoglobin and horse heart cytochrome c. Although the resolution was clearly superior to the former results,[27] it was, nevertheless, largely inferior compared with dynamic capillary isoelectric focusing with electroosmotic flow.

The focusing process is intrinsically related to the gradient of the effective forces. Only if the magnitude of the converging forces is position dependent and only if they vanish at the isoperichoric point, the focusing effect can appear. Various combinations of the fields and gradients determining other focusing FFF methods are described in the following paragraphs.

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CROSS-FLOW VELOCITY GRADIENT COMBINED WITH FIELD ACTION

The focusing can be achieved by the action of a velocity gradient of the cross-flow of the carrier liquid opposite to the action of an external field. The longitudinal flow of the carrier liquid is imposed simultaneously.[33,34] This elutriation focusing FFF was used to separate model mixtures of polystyrene latex particles and silica particles in a trapezoidal cross-section channel, shown schematically in Fig. 10 together with the fractogram of a mixture of polystyrene latex particles. Giddings[35] proposed similar focusing FFF principle, but operating in a rectangular cross-section channel with two opposite semipermeable walls. The flow rates through the walls should be different to form the velocity gradient.

Figure 6 (left). Isoperichoric focusing field-flow fractionation using gravitational or centrifugal field forces. a) Scanned macrophotographs of the channel demonstrating the isopycnic focusing FFF of two different densities particles in gravitational field (diameter ca. 200 μm) in two different average densities carrier liquids. Two distinct focused zones are formed and the flow of the carrier liquid causes their longitudinal separation. In low density carrier liquid (I), higher density particles are in contact with the lower wall and the lower density particles are focused in the upper part of the channel. In high density carrier liquid (II), higher density particles are focused in the upper part of the channel and the lower density particles are focused near the upper wall; b) Apparatus for sedimentation FFF which was applied for isopycnic focusing FFF using centrifugal field forces.

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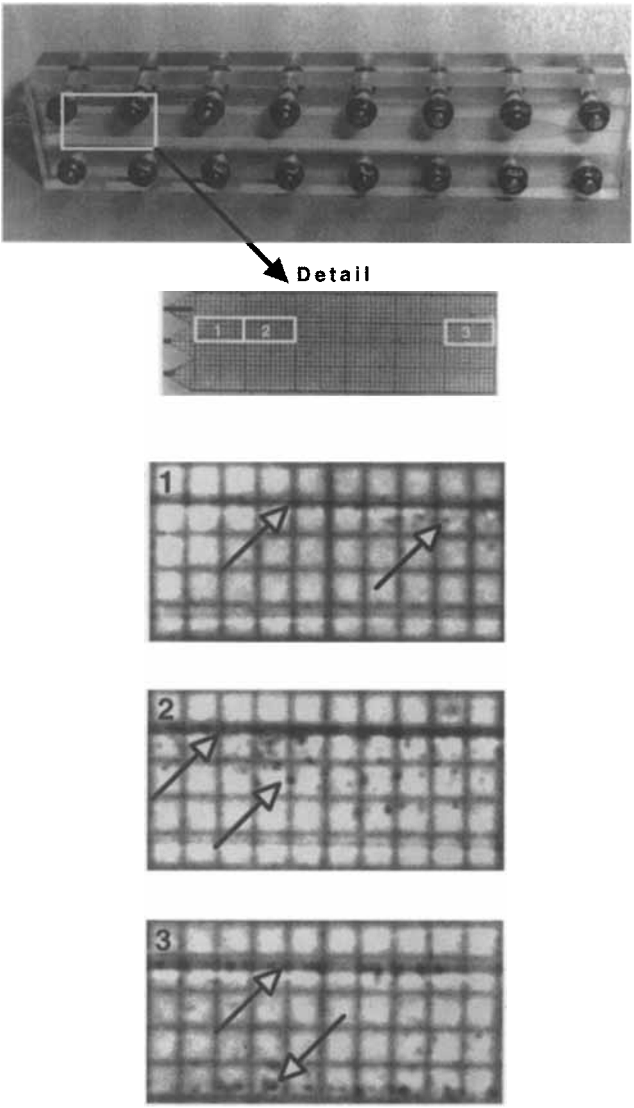

Figure 7. Isopycnic focusing FFF in preformed stepwise density gradient. Separation channel and the scanned photographs showing the evolution of two focused layers of the particles at three different longitudinal positions inside the channel. The detail 1 shows one layer of the particle mixture positioned at the interface between two different densities liquids and the beginning of the separation of the more dense particles, detail 2 shows the separation in progress of different densities particles, detail 3 shows two different densities particles focused at the interfaces of different densities liquids.

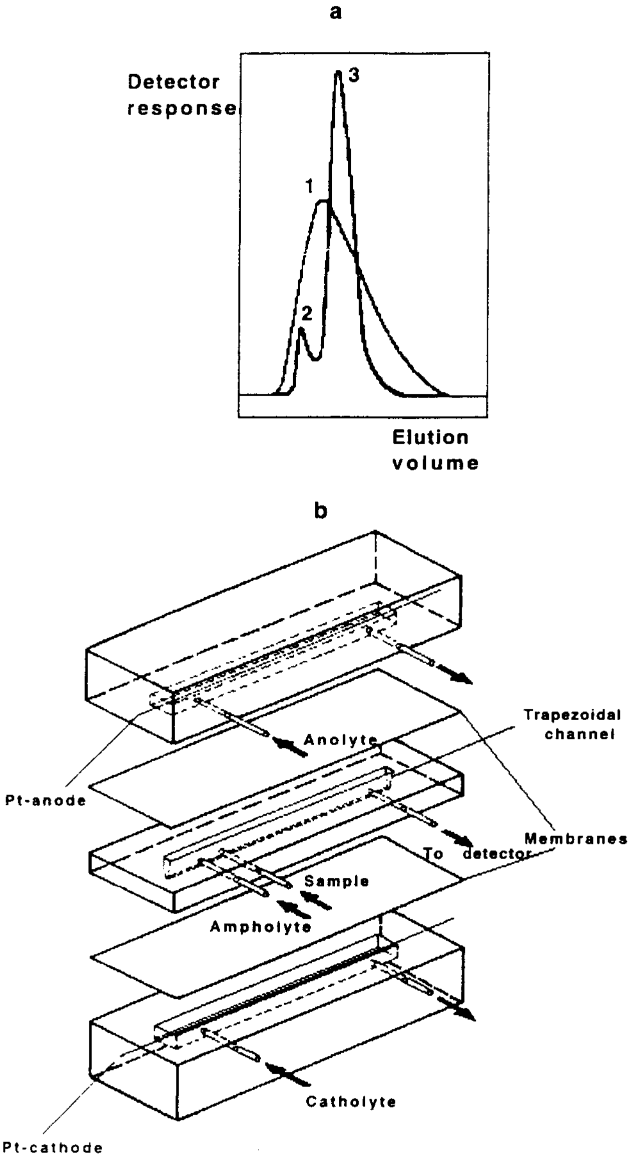

Figure 8. Isoperichoric focusing field-flow fractionation using the electric field. a) Fractograms without the electric field applied (1) and with the active field showing the separation of acidic (2) and basic (3) components of horse heart myoglobin by isoelectric focusing field-flow fractionation; b) Schematic representation of the channel for isoelectric focusing FFF.

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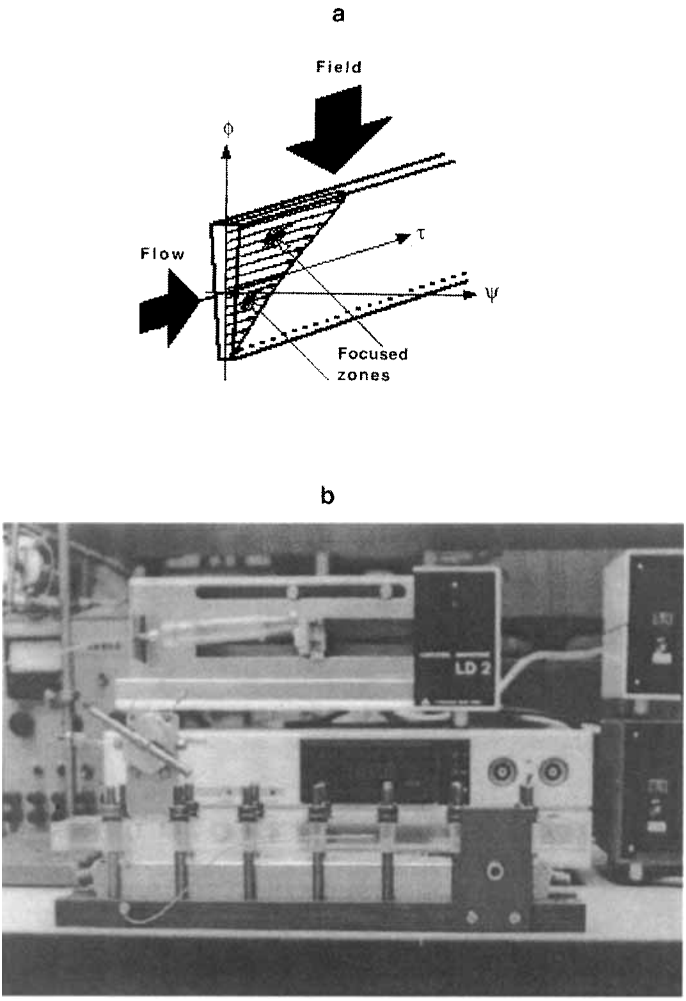

Figure 9. Modulated cross-sectional permeability channel for focusing FFF. a) Schematic representation of the trapezoidal cross-section channel and the corresponding flow velocity profile with two focused zones; b) Apparatus for isoelectric focusing FFF with the trapezoidal cross-section channel which was used for the separation demonstrated in Fig. 8.

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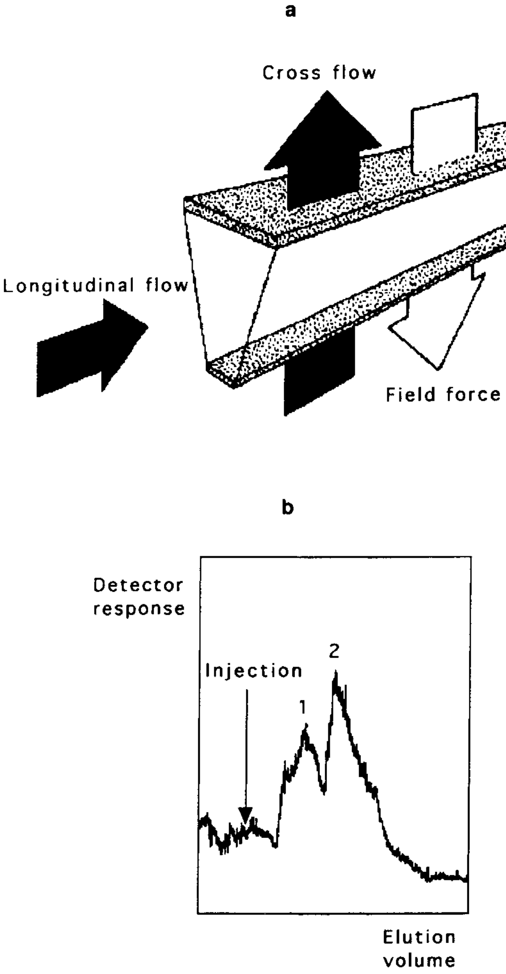

Figure 10. Principle of elutriation focusing field-flow fractionation in trapezoidal cross-section channel and the separation of two different diameter polystyrene latex particles. a) Focused zones are formed due to the field forces and the opposite velocity gradient of the carrier liquid flow across the semipermeable lower and upper walls of the channel. Separation of the focused zones in the direction of the longitudinal flow in trapezoidal cross-section channel is facilitated by the formation of axially asymmetrical flow velocity profile; b) Fractogram showing the separation of two polystyrene latexes of 1.6 μm in diameter (1) and of 5 μm in diameter (2) particles.

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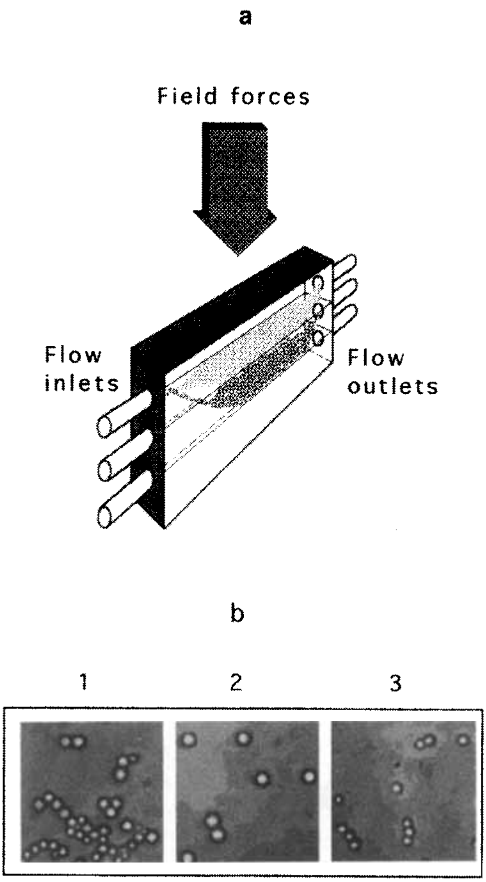

Figure 11. Continuous preparative focusing field-flow fractionation. a) Separation channel for continuous preparative focusing FFF operating in natural gravitational field with three inlet capillaries allowing to preform the step density gradient by pumping three liquids of different densities and with three outlet capillaries to collect the separated fractions; b) Optical microscopy photographs showing the preparative fractionation of the polydisperse silica particles (3 to 5.9 μm in diameter). Original unfractionated sample (1) and the fractions taken from the lower (2) and the upper (3) outlets demonstrate clearly the narrowing of the particle size distributions of the fractions.

LIFT FORCES COMBINED WITH A FIELD ACTION

The hydrodynamic lift forces that appear at high flow rates of the carrier liquid, combined with the field forces, are able to concentrate the suspended particles into the focused layers. The original observation of the lift effect was described by Segré and Silberberg.[36] The particles flowing in capillaries tend to concentrate spontaneously into the layers due to the lift forces, even without other effective field, as demonstrated by Small.[37] Whenever the other field forces are superposed, the focused zones can appear. The retention behavior of the particles under the simultaneous effect of the primary field, and lift forces generated by the high longitudinal flow rate, can vary with the nature of various superposed primary field forces. The strong effect of the lift forces in FFF was described by Caldwell et al.[38] for the separation of human and animal cells and of model latex particles. Wahlund and Litzen[39] observed the interference of the lift forces in classical polarization flow FFF, performed in an asymmetrical channel with one semipermeable wall. The combined effect of the gravitation or the cross-flow with the lift forces resulted in a good separation of polystyrene latex or silica particles.[40,41] The field forces should concentrate the retained particles at the accumulation wall, thus generating the conditions of polarization FFF. However, at high flow rates, the lift forces become operational and pull the particles away from the wall. As a result, the transition from polarization to focusing FFF appears first, followed by the proper focusing effect. Another application of this technique to fractionate the coal and limestone particles was published by Barman et al.[42]

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SHEAR STRESS COMBINED WITH A FIELD ACTION

A high shear gradient can lead to the deformation of the macromolecular coils, which results in a decrease of the chain entropy. The entropy gradient generates the driving forces that displace the macromolecules into a low shear zone. The observation by Giddings et al.[43] of the reversed elution order of high molecular weight polystyrenes in thermal FFF at high flow rates can be attributed to this phenomenon. However, a detailed study of the effect of the operational variables indicated another possibility to explain the reversed elution order.[44] More experimental work is needed to draw any conclusions concerning the origin of the observed phenomena.

GRADIENT OF A NONHOMOGENEOUS FIELD ACTION

The use of a high-gradient magnetic field was proposed to separate paramagnetic and diamagnetic species by a mechanism of focusing FFF. In a series of theoretical papers summarized in Ref. 45, Semyonov et al. treated various aspects of focusing FFF but no experimental results appeared until now. However, the idea is highly interesting and merits experimental verification.

PREPARATIVE FRACTIONATION

No substantial difference distinguishes the analytical and preparative use of FFF. While the main objective of the analytical separation is to determine the required parameters of the examined sample, or to prove the existence of the investigated components, preparative fractionation is performed to obtain a sufficient amount of specific fractions to use for subsequent analysis, or for other experimental work.

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Focusing FFF can be used for continuous preparative fractionation.[9,46] If the fractionation channel is equipped with several outlet capillaries at various positions (and occasionally with several inlets to preform a stepwise gradient in the direction of the focusing) and the sample to be fractionated is continuously pumped into the channel, the focused layers eluting through the individual outlets can be collected. This general principle is schematically demonstrated in Fig. 11a. The experimental demonstration of its feasibility was given by the fractionations of various samples of silica particles[34] of which one example is shown in Fig. 11b. In this particular case, the natural gravitation and the counteracting cross-flow gradient produced the focusing effect and the silica particles were separated according to their sizes, similarly as for analytical scale separation shown in Fig. 10b. The particle size of the studied sample was within the range that is interesting, e.g., for the fractionation of blood cells. Although another focusing FFF was not yet implemented under preparative conditions, it is evident that, for example, the isopycnic or isoelectric focusing FFF already performed in analytical scale (see Figs. 5a, 6a, 7, and 8a) can easily be transformed into large scale separations.

OUTLINES AND PERSPECTIVES

All progress passes through the discoveries, inventions, and innovations. The advances in science and technology are due to the understanding and mastering of the laws of nature, as well as due to the prediction and exploration of the phenomena do not appearing spontaneously in the universe. Analytical chemistry follows this process as a particular scientific discipline, but also in context with the whole area of scientific research.

Focusing FFF represents, most recently, a modest contribution to the science and technology of separation and analysis of macromolecules of synthetic or natural origin within a wide range of molar masses, of particles in the submicron and micron ranges, and of organized structures, such as cells and microorganisms. This does not mean that low molar mass species cannot be separated. The molecules not influenced by the field and, thus, not exhibiting the focusing effect can still be separated, providing that an equilibrium between them and the focused species is established. However, this hypothesis has yet to be realized.

The research and technology related to the life sciences and to the macromolecular chemistry and technology, the analytical problems appearing in context with the protection of the environment, and many other scientific and technological activities, have stimulated the development of new analytical separation methods.

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The present review of the achievements in focusing FFF clearly indicates that most of the experimental implementations have been obtained with model systems. Some of them represent a clear proof that *"it works"* rather than a practical application elaborated to minutest details.

The advantages of these methods already mentioned above are evident. Some of them are inherently related to the separation principle of focusing FFF, such as the absence of a large surface area within the separation channel that can be of crucial importance for sensitive biological materials, the operational variables, including the strength of the field, which can be continuously manipulated within a very wide range, etc.

The lack of the commercially available instrumentation represents a limiting factor with regard to the widespreading of the focusing FFF methodology into current laboratory practice. However, this lack is only virtual, because all the particular components of the whole apparatus are absolutely identical as those for liquid chromatography, with the only exception being the separation channel.

A liquid chromatograph can be modular, which means comprised of individual parts commercially available, such as the pump, injector, detector, etc., with a column that can be changed for each specific application. The FFF apparatus can be assembled similarly with various channels which, in most cases, are easy to construct. This approach, rather than to be a limiting factor, can represent a challenge for creativity and invention.

The main activity of Cal Giddings in the domain of FFF was oriented toward polarization FFF methodology, in which the list of his and his collaborators' papers accounts for hundreds contributions published during exactly 30 years. But, he was remarkably present also in the field of focusing, hyperlayer, FFF and not only as a source of inspiration. I worked in his laboratory, under his expert leadership, during 1978-79 and, as a result, the dominating part of my subsequent research was specifically on FFF, which provided a great challenge on one hand, but a satisfaction in moments of a successful outcome.

ACKNOWLEDGMENT

This paper is dedicated to the memory of J. Calvin Giddings.

Precious contribution of my former and recent collaborators to the development of the focusing FFF methodology in the course of the past 15 years is gratefully acknowledged. Some of them are listed below as co-authors of the cited publications.

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REFERENCES

1. G. Agricolae, **De Re Metallica**, Libri XII, QVI, Basileae, 1556.
2. J. Montgolfier, E. Montgolfier, **Description des Expériences Aérostatiques de MM. de Montgolfier, et de Celles Auxquelles Cette Découverte a Donné Lieu**, Cuchet, Paris, 1784.
3. M. Meselson, F. W. Stahl, J. Vinograd, Proc. Natl. Acad. Sci., U. S. A., **43**, 581 (1957).
4. H. Svensson, Acta Chem. Scand., **15**, 325 (1961).
5. J. C. Giddings, Sepn. Sci., **1**, 123 (1966).
6. J. Janča, Makromol. Chem., Rapid Commun., **3**, 887 (1982).
7. J. Janča, Trends Anal. Chem., **2**, 278 (1983).
8. J. C. Giddings, Sepn. Sci. Technol., **18**, 765 (1983).

9. J. Janča, J. Chmelik, Anal. Chem., **56**, 2481 (1984).

10. J. Janča, Trends Anal. Chem., **6**, 147 (1987).

11. J. Janča, **Field-Flow Fractionation: Analysis of Macromolecules and Particles**, Marcel Dekker, Inc., New York, 1988.

12. A. Kolin, in **Electrofocusing and Isotachophoresis**, B. J. Radola, D. Graesslin, eds., de Gruyter, Berlin, 1977.

13. J. Janča, Mikrochim. Acta, **112**, 197 (1994).

14. J. Janča, M. Špírková, Collect. Czech. Chem. Commun., **61**, 819 (1996).

15. J. Janča, R. Audebert, J. Appl. Polym. Sci., Appl. Polym. Symp., **52**, 63 (1993).

16. J. Janča, R. Audebert, Mikrochim. Acta, **111**, 163 (1993).

17. J. Chmelik, J. Janča, J. Liquid Chromatogr., **9**, 55 (1986).

18. J. Janča, D. Přibylová, unpublished results.

19. J. Janča, M. Janíček, V. Slavik, F. Stehlik, Czechoslovak Patent N° 230086, 2 June 1982 (PV 5061-82).

20. J. Janča, M. Janíček, D. Přibylová, M. Klesnil, Anal. Instrumentation, **15**, 149 (1986).

21. J. Janča, N. Nováková, J. Liquid Chromatogr., 10, 2869 (1987).

22. J. Janča, N. Nováková, J. Chromatogr., **452**, 549 (1988).

23. J. Janča, M. Špírková, J. Coll. Interface Sci., **184**, 000 (1996).

24. J. Chmelik, M. Deml, J. Janča, Anal. Chem., **61**, 912 (1989).

25. J. Janča, J. Chmelik, V. Jáhnová, N. Nováková, E. Urbánková, J. Appl. Polym. Sci., Appl. Polym. Symp., **45**, 39 (1990).

26. J. Chmelik, J. Chromatogr., **545**, 349 (1991).

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27. W. Thormann, M. A. Firestone, M. L. Dietz, T. Cecconie, R. A. Mosher, J. Chromatogr., **461**, 95 (1989).

28. J. Chmelik, J. Chromatogr., **539**, 111 (1991).

29. J. Janča, V. Jáhnová, J. Liquid Chromatogr., **6**, 1559 (1983).

30. J. Chmelik, W. Thormann, J. Chromatogr., **600**, 297 (1992).

31. J. Chmelik, W. Thormann, J. Chromatogr., **600**, 305 (1992).

32. J. Chmelik, W. Thormann, J. Chromatogr., **632**, 229 (1993).

33. J. Janča, Makromol. Chem., Rapid Commun., **8**, 233 (1987).

34. E. Urbánková, J. Janča, J. Liquid Chromatogr., **13**, 1877 (1990).

35. J. C. Giddings, Sepn. Sci. Technol., **21**, 831 (1986).

36. G. Segré, A. Silberberg, Nature (London), **189**, 209 (1961).

37. E. H. Small. J. Colloid Interface Sci., **48**, 147 (1974).

38. K. D. Caldwell, Z. Q. Cheng, P. Hradecky, J. C. Giddings, Cell Biophys., **6**, 233 (1984).

39. K. G. Wahlund, A. Litzen, J. Chromatogr., **461**, 73 (1989).

40. K. Chen, K. G. Wahlund, J. C. Giddings, Anal. Chem., **60**, 362 (1988).

41. S. K. Ratanathanawongs, J. C. Giddings, J. Chromatogr., **467**, 341 (1989).

42. B. N. Barman, M. N. Myers, J. C. Giddings, Powder Technol., **59**, 53 (1989).

43. J. C. Giddings, S. Li, P. S. Williams, M. E. Schimpf, Makromol. Chem., Rapid Commun., **9**, 817 (1988).

44. J. Janča, M. Martin, Chromatographia, **34**, 125 (1992).

45. S. N. Semyonov, A. A. Kuznetsov, P. P. Zolotaryov, J. Chromatogr., **364**, 389 (1986).

Downloaded At: 11:44 24 January 2011

46. J. C. Giddings, Sepn. Sci. Technol., **21**, 831 (1986).

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