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# Different elution modes and field programming in gravitational field-flow fractionation

## I. A theoretical approach

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### Abstract

Gravitational field-flow fractionation utilizes the Earth's gravitational field as an external force which causes settlement of particles towards channel accumulation wall. It is sometimes considered that gravitational field-flow fractionation is not a versatile technique because the force field cannot be changed as in the other field-flow fractionation techniques. However, there are several possibilities to change the force acting on particles (e.g., by changing the angle between the Earth's gravitational field and the longitudinal axis of the channel, or the use of carrier liquids having different densities. Hydrodynamic lift forces are also known to act on particles in the carrier liquid flow and give some other means of modulating the force field in this technique by varying the flow-rate. This change can be reached by using programmed pumps or channels with non-constant cross-sections. Some of these possibilities can be exploited for field programming if a unified approach to control the retention variables in gravitational field-flow fractionation can be worked out. Several different elution modes can be indeed observed in gravitational field-flow fractionation, even mixed in some experiments. The equations describing the concentration profiles of analytes and their dependence on the elution mode mechanisms are used to gain insights into the different ways of field programming for each particular elution mode. © 1999 Elsevier Science B.V. All rights reserved.

*Keywords:* Field-flow fractionation

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### 1. Introduction

Separation effect in field-flow fractionation (FFF) is reached by a combined action of the non-uniform flow velocity profile of a carrier liquid and a transverse physical field applied [1]. The force field forms a specific concentration profile of sample components which determines the elution mode. Based on the magnitude and the course of the acting field, on the properties of the analytes and, in some cases, on the flow-rate of the carrier liquid, different elution modes can be observed in FFF.

Gravitational field-flow fractionation (GFFF) is the experimentally simplest FFF technique [2]. It

was successfully applied to separation, characterization and micropreparation of various particles, e.g., silica gel particles [3–7], polymer latexes [8,9] and cells [10–12]. GFFF utilizes the Earth's gravitational field as an external force which causes settlement of particles towards channel accumulation wall. However, theory for general description of retention in GFFF still needs significant improvement with respect to other FFF techniques. For instance, there are some other forces acting on the micron-sized sample particles as they elute in the carrier liquid flow; the most important of which are the hydrodynamic lift forces [13,14]. Opposed to gravitation, they tend to drive the particles away from the accumulation wall

and to focus them into narrow zones. These zones are located in the flow velocity profile according to the particle size and density because of the simultaneous action of the gravitational force field and of the hydrodynamic lift forces. The reached equilibrium position corresponds to the point where the effective Archimedes weight of the particle equals the lift force: the lift forces cause the focusing (hyperlayer) elution mode [15,16]. As a consequence, it was shown that this technique is suitable for the measurement and evaluation of the hydrodynamic lift forces [14,17].

For complex sample mixtures, elution times of the most retained components in GFFF are very long and their peaks are too broad. Field programming is an efficient tool for optimizing separation in FFF in order to obtain better separation in terms of time, resolution and detection limit [18,19]. It is sometimes considered that the force field cannot be changed in GFFF and therefore field programming cannot be applied to this technique. However, there are several possibilities to change the gravitational force acting on particles, i.e., change of the angle between the Earth's gravitational field and the longitudinal axis of the channel, the use of carrier liquids having different density. The hydrodynamic lift gives some other possibilities for changing the force field in this technique by changing the flow-rate. This change can be reached by using programmed pumps or using channels with non-constant cross-section. Some of these possibilities can thus be utilized for field programming.

In this work, a unified approach describing the concentration profiles of analytes and the mechanisms of the different elution modes in GFFF was used to focus on the different ways by which programming in the particular elution modes of GFFF can be achieved.

## 2. Theory

As stated above, FFF is in principle based on the coupling of a non-uniform flow velocity profile of a carrier liquid with a non-uniform transverse concentration profile of the analyte caused by a field applied perpendicularly to the direction of the flow (Fig. 1). Three different elution modes can be

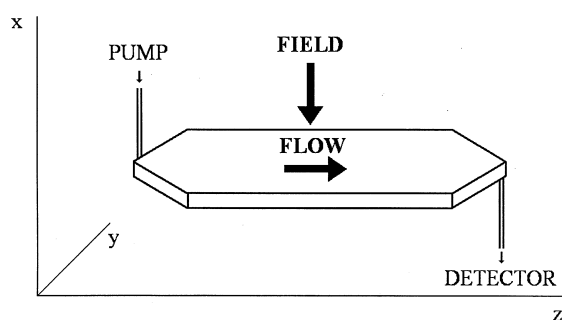


Fig. 1. Schematic representation of the GFFF channel and orientation of the gravitational field and the carrier liquid flow used in this work.

derived from the general transport equation following the same procedure. The differences among them arise from the course and magnitude (in comparison to the effect of diffusion of the analyte) of the resulting force acting on the analyte (Fig. 2).

Generally, the concentration profiles of analytes in FFF can be obtained from the solution of the general transport equation [20]

$$J = Wc - D\nabla c \quad (1)$$

where  $J$  represents the flux density of the analyte,  $W$  is the transport velocity of the analyte which equals the sum of the transport velocity of the analyte induced by the external field applied  $U$  and the transport velocity of the analyte induced by the carrier liquid flow  $v$  ( $W=U+v$ ),  $c$  is the analyte concentration,  $D$  is the total effective diffusion coefficient and  $\nabla$  is the gradient operator. The term  $Wc$  corresponds to the convective flux of the analyte and  $D\nabla c$  corresponds to the diffusive flux of the analyte.

In the case of the non-constant concentration profile of the analyte along the axis of the applied field, the expression in Eq. (1) can be given [20] in differential form as

$$J_x = W_x c(x) - D \cdot \frac{\delta c}{\delta x} \quad (2)$$

where  $J_x$ ,  $W_x = U_x + v_x$  are the components and  $c(x)$  is the concentration profile along the direction of the applied field. Because of the direction of the carrier liquid flow inside the GFFF channel, the component

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$$U_x = \text{const.}, U_x t \approx \sqrt{2Dt}$$

$$U_x = \text{const.}, U_x t \gg \sqrt{2Dt}$$

$$U_x = -a(x - s)$$

$$c(x) = c_0 e^{-\frac{|U_x|}{D}x}$$

$$c(r_p) = c_0, c(x \neq r_p) = 0$$

$$c(x) = c_0 e^{-\frac{a}{2D}(x-s)^2}$$

$$R = 6kT / Fw$$

$$R = 6 r_p / w$$

$$R = 6s / w$$

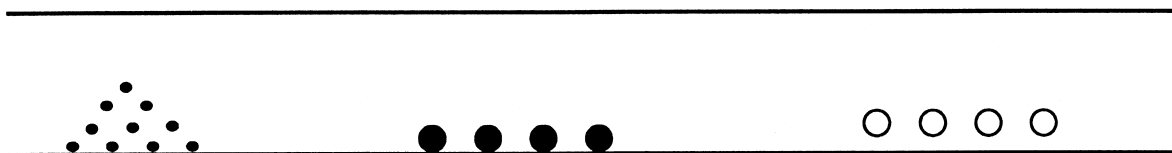


Fig. 2. Comparison of the expressions for the transport velocity of the analyte induced by the external field applied  $U_x$ , the resulting concentration of the analyte  $c$ , the retention ratio  $R$ , and graphic representation of the concentration profiles of the analytes in three elution modes. For explanation of the symbols see Theory.

$v_x$  equals zero (the axis  $x$  is perpendicular to the direction of the flow), and thus  $W_x$  equals  $U_x$ .

Following the treatment given by Giddings [20], imposing for the condition of the steady-state zone of the analyte, which is characterized by the null flux density, and applying the equation of continuity, the general solution of the analyte concentration profile can be expressed in the form

$$c(x) = c_0 e^{\int_0^x \left(\frac{U_x}{D}\right) dx} \quad (3)$$

where the integration limit  $x=0$  corresponds to the accumulation wall boundary. Therefore particular solutions for the concentration profile are dependent on the course of the force field, which induces the transport of the analyte, and the ratio of  $U_x$  and  $D$ .

The equation of the field induced transport velocity was derived by Giddings [21]

$$U_x = -ax^n \quad (4)$$

where  $a$  is constant and  $n$  equals 0 or 1. If  $n=0$  then  $U_x$  is constant, if  $n=1$  then  $U_x$  is dependent on the position inside the channel. These conditions identify the different elution modes in GFFF.

*2.1. Brownian elution mode*

The field induced velocity of analyte in the separation channel is constant and comparable with its diffusive motion ( $U_x = \text{constant}$ ,  $U_x t \approx \sqrt{2Dt}$ , where  $t$  is time). The resulting concentration profile of the analyte is given by the well-known exponential relationship [22]

$$c(x) = c_0 e^{-\frac{|U_x|}{D}x} \quad (5)$$

where  $c_0$  is the maximum concentration at the accumulation channel wall. The elution mode with the exponential concentration profile is in FFF called Brownian [23] (this mode is also called normal in the literature).

It is known that there are two main factors influencing behavior of analytes in this elution mode: the properties of the analytes (characterized by so-called analyte–field interaction parameter [24] and diffusion coefficient) and the strength of the field applied. In the case of GFFF, the analyte–field interaction parameter is the effective mass of the particle  $m_{\text{ef}} = V_p(\rho_p - \rho_{\text{cl}})$ , where  $V_p$  is the volume of the particle,  $\rho_p$  and  $\rho_{\text{cl}}$  are densities of the particle and of the carrier liquid, respectively, and the gravitational field is characterized by acceleration  $G = 9.81 \text{ m/s}^2$ .

In Brownian elution mode for GFFF, of course, the general expression for retention in which the retention ratio  $R$  is indirectly dependent on the force applied and independent on the flow-rate [25] still holds true. It can be expressed in an approximate form

$$R = 6kT/Fw \quad (6)$$

where  $k$  is Boltzmann constant,  $T$  is the absolute temperature,  $F = Gm_{\text{ef}}$  is the gravitational force acting on the analyte inside the channel, and  $w$  is the thickness of the channel. The resulting force acting on a particle in the case of Brownian elution mode can, thus, be changed by the changing the angle  $\alpha$  between the direction of Earth's gravitational field and the longitudinal axis of the channel, the effective force being  $F_{\text{ef}} = G(\sin \alpha)m_{\text{ef}}$  (Fig. 3a), or by changing the density of the carrier liquid (Fig. 3b). Moreover, retention can be influenced by using the channels with non-constant thickness because a reduction of the channel thickness (Fig. 3c) turns out a reduction of retention, i.e., faster elution of analytes. However, because of the absence of the focusing action of the hydrodynamic lift forces, in this case a reduction of efficiency occurs, since the peaks of the eluted analytes become more broader as a result of the increasing linear velocity of the carrier liquid flow.

## 2.2. Steric elution mode

The velocity of separative transport induced by the

force field in the separation channel is constant and much higher than the velocity caused by diffusive motion of the analyte ( $U_x = \text{constant}$ ,  $U_x t \gg \sqrt{2Dt}$ ). In this case the analyte forms a layer on the accumulation channel wall and its concentration in any other position inside the channel equals zero. Thus, the particle radius  $r_p$  describes the distance of the particle center from the accumulation wall

$$c(r_p) = c_0 \text{ and } c(x \neq r_p) = 0 \quad (7)$$

The elution mode is called steric [2]. The retention ratio can be expressed in the form

$$R = 6r_p/w \quad (8)$$

It shows that  $R$  is independent both of the field applied and of the flow-rate, and it is dependent only on the particle radius and the channel thickness. There are no possibilities for field changing and programming induced by any changes of the carrier liquid density or the flow-rate. Decreasing thickness of the channel can cause faster elution of analytes. However, no substantial separation improvement can be expected in comparison to the channel with constant thickness. Moreover, the increasing linear velocity of the carrier liquid flow induce a mixed elution mode caused by the onset of the hydrodynamic lift forces in the narrower part of the channel, which complicates the evaluation of the observed retention data. In fact, the retention ratio values corresponding to the pure steric elution mode have been seldom observed experimentally [5]. The observed values often correspond to the focusing elution mode as a result of the action of some additional forces influencing retention behavior of analytes.

## 2.3. Focusing elution mode

In this elution mode, the velocity of analyte transport induced by a force field in the separation channel is dependent on the position across the channel ( $U_x \neq \text{constant}$ ). Based on Eq. (4), the non-constant transport velocity can be, in the simplest case, described as

$$U_x = -a(x - s) \quad (9)$$

where  $s$  is the distance of the center of the focused

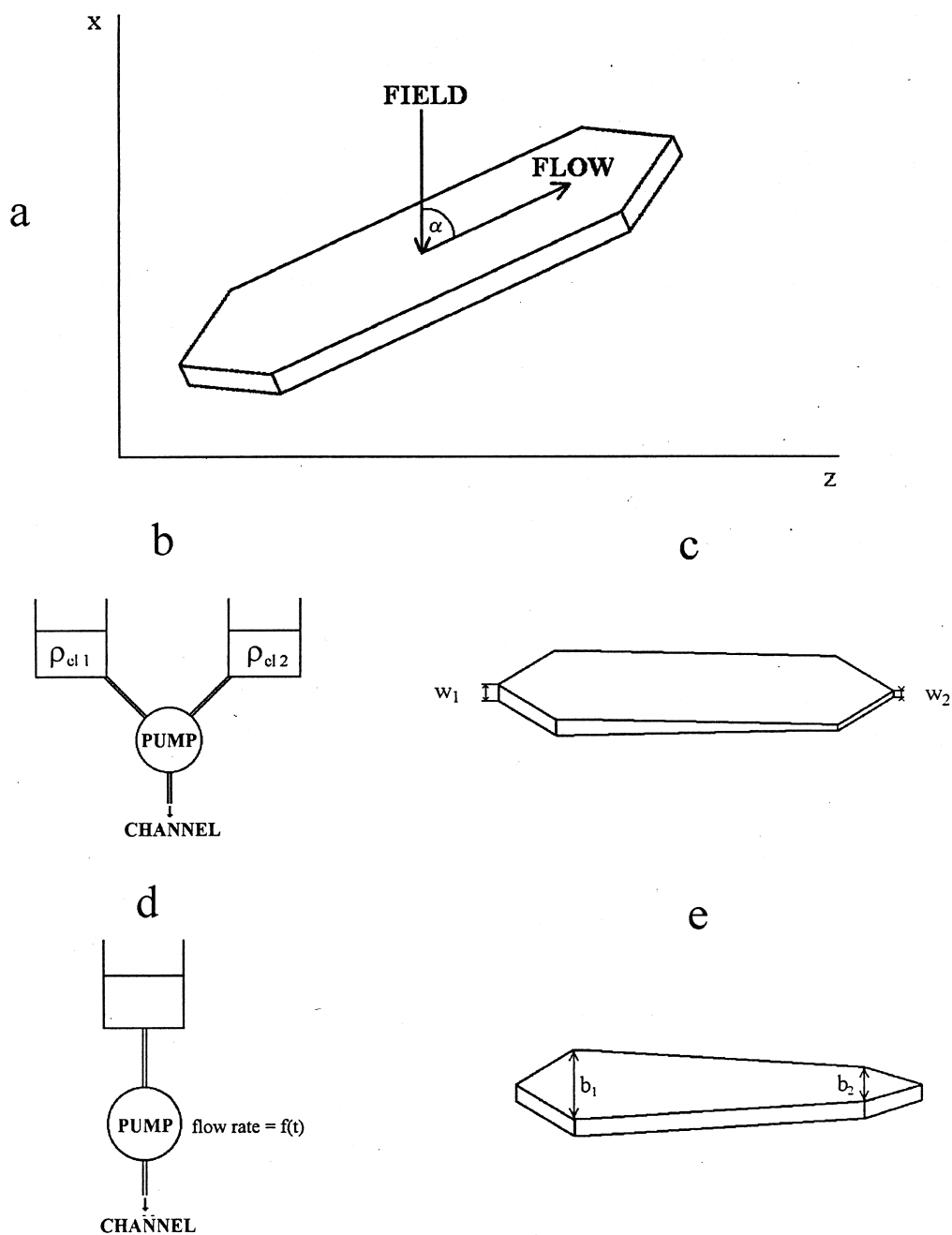


Fig. 3. Schematic representation of different means of field programming in GFFF. (a) The change of the angle  $\alpha$  between the direction of the Earth's gravitational field and the longitudinal axis of the channel, (b) the density gradient of the carrier liquid formed from two liquids having densities  $\rho_{cl1}$  and  $\rho_{cl2}$ , (c) the channel with non-constant thickness (from  $w_1$  to  $w_2$ ), (d) the flow-rate gradient of the carrier liquid and (e) the channel with non-constant breadth (from  $b_1$  to  $b_2$ ).

zone from the channel wall, i.e., the position where the resulting force acting on the analyte equals zero. Combining this equation with Eq. (3), we obtain a relation for the resulting concentration profile of the analyte

$$c(x) = c_0 e^{-\frac{\alpha}{2D}(x-s)^2} \quad (10)$$

where  $c_0$  is the maximum concentration at the center of the focused zone (i.e., at the position where the resulting force acting on the analyte equals zero). The concentration profile of the analyte across the channel thickness, in this simplest case, is Gaussian. In other cases, where other secondary effects act on retention in the focusing elution mode, the observed concentration profile is more complex. However, even in non-ideal cases, the main feature remains the same, i.e., the maximum concentration of the analyte is at the equilibrium position where the resulting force acting on the analyte is zero, and not on the channel wall as in the case of the steric elution mode. The elution mode is, thus, called focusing (or hyperlayer) [15,16].

In the focusing elution mode, the retention ratio can be expressed in a form formally similar to the expression given for the steric elution mode (see Eq. (8))

$$R = 6s/w \quad (11)$$

At least two counteracting forces are thus necessary for the formation of the focused zone of the analyte at the position where the resulting force is zero, i.e., the center of the zone is located at the position  $s$ . In the case of GFFF, it means that both gravitational and hydrodynamic forces can be changed, and thus the resulting position of the particle zone can be controlled [13,14]. Similarly to Brownian elution, the gravitational force acting on a particle can be changed by varying the angle  $\alpha$  between the direction of the Earth's gravitational field and the longitudinal axis of the channel (Fig. 3a) or by modifying the density of the carrier liquid (Fig. 3b). In contrast to Brownian elution mode, the hydrodynamic forces influence the position of the analyte zone, which is caused by the action of the carrier liquid flow on the sample particles [26]. The change can be reached by using programmed pumps

(Fig. 3d) or using channels with non-constant cross-sections (Fig. 3c and e).

### 3. Discussion

The above described theoretical analysis confirmed that the force field in GFFF can be varied as in the other FFF techniques, which can be utilized for field programming. However, the experimental determination of the type of the elution mode responsible for displacement of the analytes is very important because there might be different elution modes acting on different analytes in the same experiment [5]. Determination of the elution mode is a first requirement for both interpretation of the changed elution conditions, e.g., field programming, and evaluation of the measured retention data.

In Brownian elution mode, the resulting force acting on particles can be tuned by varying the angle  $\alpha$  between the direction of Earth's gravitational field and the longitudinal axis of the channel (Fig. 3a) or by the density of the carrier liquid (Fig. 3b). The former possibility requires one to locate the channel or the whole GFFF instrumentation on a moveable desk. The same holds true also in the case of the focusing elution mode. However, because of the active role of the hydrodynamic forces in this mode, other possible changes of the force field can be reached by flow programming (Fig. 3d) or by using channels with non-constant cross-sections, i.e., channels with changing either their thickness or breadth (Fig. 3c and e). The retention ratios of analytes are influenced by the changing thickness of the channel in all three elution modes. On the other hand, the changing breadth of the channel influences the retention ratio only in the focusing mode in the presence of the hydrodynamic lift forces.

Since one of the main reasons for field programming in FFF is to shorten the elution time, the dimensions of the channel should be higher at the channel input (Fig. 3c and e). The channel of non-constant breadth was already suggested Litzén and Wahlund [27] in asymmetrical flow FFF, although for different purposes.

There is an important difference between channels of non-constant breadth or non-constant thickness. In the case of channels with non-constant breadth, the

hydrodynamic lift forces are increased by higher linear flow velocity values in the narrower parts of the channel. On the other hand, in channel with non-constant thickness, the influence on the hydrodynamic lift forces is enhanced not only by higher linear flow velocity values in the narrower parts of the channel but also amplified by the decreasing thickness of the channels.

The influence of the flow-rate and the density difference between the sample particles and the carrier liquid has been experimentally proven several times [5,10,11,13,14,17,28] as well as the role of the channel thickness has been already shown [4,7,14]. By using a programmable pump, both density and flow-rate gradients can be easily formed. Recently, separation of silica gel particles in various carrier liquids at different flow-rates has been presented [29], and some preliminary experiments with model particles under the influence of the flow-rate gradient have been shown [30]. Our continuing effort is devoted to implementation of field programming in GFFF.

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