Counteracting Chromatographic Electrophoresis and Related Imposed-Gradient Separation Processes

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Introduction

Counteracting chromatographic electrophoresis is a multiple-field fractionation technique for which the fields counteract longitudinally so that the entire length of the column can contribute to the simultaneous separation of several constituents. Giddings and Dahlgren (1971) discussed some aspects of such counteracting field separation processes when the fields can be represented as longitudinal gradients. Their analysis, restricted to continuous gradients, was applied to isoelectric focusing and density gradient (isopycnic) sedimentation. In counteracting chromatographic electrophoresis, the electric field opposes velocity changes due to varying matrix porosity.

Giddings (1979) effectively described the common features that unify a range of separation processes in which diffusion and convective forces are counterbalanced. The treatment centralized on the description of the steady state concentration profile, without discussing how the positions of the accumulation zones could be predicted for either discrete or continuous fields, such as in counteracting chromatographic electrophoresis.

O'Farrell (1985) proposed that in addition to gel permeation, other chromatographic matrix properties (surface forces) could be utilized as the basis for separation, e.g., affinity, hydrophobic, or charge interactions with the solutes. The chromatographic properties would vary along the length of the column in a graduated manner, either discretely or continuously. It is also apparent that a variation in column diameter, either discrete or continuous, will cause the fluid velocity to vary along the column distance and lead to stationary zones for accumulation of a solute.

The electrical force of electrophoresis can in general be substituted by another type of external body force, e.g., magnetic or gravitational, as indicated in Table 1. For example, separating sedimenting particles into size classifications by elutriation in a column of varying diameter is possible, although it is made difficult by convective velocity nonuniformities that can be present within the open column (Baver, 1948). The same limitations are expected for counteracting velocity gradient electrophoresis, in which the electrophoretic force would oppose liquid flow into a column of increasing diameter. Another practical problem is to produce a uniform electrical field gradient over the column length.

Sedimentation, or combined electrophoresis and sedimentation of charged particles, in a density gradient is a process in the same class. Density gradient sedimentation or zoned centrifugation, often referred to as isopycnic settling or centrifugation, may be performed with either continuous gradients or step gradients (Hsu, 1981). For combined electrophoresis and sedimentation to be feasible, the solute particles must have sufficient size and mass to be subject to the gravitational or centrifugal force.

Isoelectric focusing is based on an electrophoretic force counteracting the effect of a pH gradient, which causes the solute (usually an amphoteric protein) to have an electrical charge varying with position (Haglund, 1971).

Several types of metals (Cummings et al., 1976), and red blood cells as well (Melville et al., 1979) are paramagnetic and respond to magnetic forces. In principle, a magnetic force acting on such magnetically endowed particles could be counterbalanced by a velocity gradient, due either to graduated column diameter, liquid density, or medium porosity, to effect an accumulation zone of vanishing particle mobility. These processes might be named, respectively, counteracting velocity gradient, density gradient, or chromatographic magnetophoresis.

A quantitative understanding of these processes, in particular of counteracting chromatographic electrophoresis, is desirable for the design and scale-up of industrial and preparative systems. The present note provides a mathematical analysis that describes the concepts and experiments presented by O'Farrell (1985), and suggests a basis for extending the application of the fundamental idea.

Table 1. Types of Column Separation Processes with an External Body Force Counterbalancing a Graduated Property

Separation Process	External Force	Graduated Property	Reference
Counteracting chromatographic electrophoresis	Electrophoresis	Medium porosity	O'Farrell (1985)
Counteracting velocity gradient electrophoresis	Electrophoresis	Column dia. (velocity)	This paper
Isoelectric focusing	Electrophoresis	Ionic charge (pH)	Haglund (1971)
Density gradient sedimentation	Gravity or centrifugal force	Liquid density	Hsu (1981)
Density gradient sedimentation with electrophoresis	Electrophoresis and gravity	Liquid density	This paper
Elutriation	Gravity or centrifugal force	Column dia. (velocity)	Baver (1948)
Counteracting chromatographic magnetophoresis	Magnetic force	Medium porosity	This paper
Counteracting velocity gradient magnetophoresis	Magnetic force	Column dia. (velocity)	This paper
Density gradient sedimentation with magnetophoresis	Magnetic force and gravity	Liquid density	This paper

Theory for Discrete Zones

Consider a column with discrete regions of chromatographic media composed of porous particles; each region of uniform matrix properties is indicated by the subscript i. The mass balance equation that describes the movement of a solute with time t and distance z in each region i is

$$\alpha_i\left(\frac{\partial c}{\partial t}+v_i\frac{\partial c}{\partial z}\right)=D_i\frac{\partial^2 c}{\partial z^2}-\beta_i(1-\alpha_i)\left(\frac{\partial c_{ads}}{\partial t}+\frac{\partial c_p}{\partial t}\right) \tag{1}$$

in terms of the concentration of solute in the interparticle fluid, $c(t,z)$, the concentration of solute in the intraparticle liquid $c_p(t,z)$, and the adsorbed concentration, $c_{ads}(t,z)$. The void fraction is α_i, the porosity of particles is β_i, the effective dispersion coefficient is D_i (assumed constant over z), and the migration velocity of ions is v_i, which is the net effect of electrophoretic and convective flows. (In a more general context the external field would be an unspecified body force).

If v_o is the volumetric flowrate to the column divided by the column cross-sectional area, then v_i is given by

$$v_i=v_o/\alpha_i-v_e \tag{2}$$

where v_e is the electrophoretic velocity, which is assumed constant with z. In Eq. 2 we have assumed that longitudinal flow cannot occur within the particle pores. If particle porosity is such that liquid can flow through the pores to the particles then we have

$$v_i=v_o/[\alpha_i+\beta_i(1-\alpha_i)]-v_e \tag{3}$$

when $c=c_p$. Obviously, v_i may be positive or negative, depending on the relative magnitudes of the two counteracting velocities in Eqs. 2 and 3. This possibility of velocity reversal is the essential feature for separation processes of the counteracting field type. It is at the zone of zero species migration velocity that the solute accumulates. In Figure 1 we have let $v_c=v_i-(-v_e)$, which we refer to as the chromatographic velocity. We assume that a linear equilibrium relation describes the adsorption or affinity process, which is independent of the external field,

$$c_{ads}=K_ic. \tag{4}$$

For the present article we ignore intraparticle diffusion and liquid-to-particle mass transfer, and thus

$$c_p=c. \tag{5}$$

The equation to be solved is then

$$\alpha_i[(1+k_i)\,\partial c/\partial t+v_i\partial c/\partial z]=D_i\partial c^2/\partial z^2 \tag{6}$$

where we have let

$$k_i=\beta_i(1-\alpha_i)(1+K_i)/\alpha_i. \tag{7}$$

When dispersion is ignored the moment method and the method of characteristics give identical results for the case of discrete gradations in separation properties. For the present separation problem the dispersive process during solute migration is not a primary issue, for the objective is to cause solute to accumulate continuously at a stationary zone, where band-spreading by diffusion occurs. However, for analytical applications band-spreading by dispersion during pulse migration may be of interest, and we present the second moment expressions for completeness.

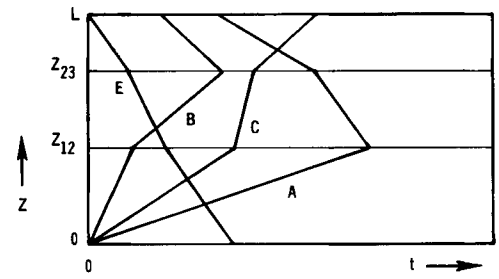

Figure 1. A column with three different zones can separate four species, *A*, *B*, *C*, and *E*.

Species A accumulates at $z=z_{12}$ because its migration velocity changes signs there, as shown by the change of slope of its characteristic path. Species B accumulates at $z=z_{23}$ in a like manner. Species C proceeds through the column without accumulating, because the electrophoretic force on it is not sufficiently large to change the sign of its velocity. Species E, if introduced at the column exit $z=L$ as shown, will migrate to the column entrance, since its electrophoretic force exceeds the chromatographic effect.

The first normalized temporal moment gives the centroid of the pulse for the point z,

$$\mu_1' = \int_o^\infty t\, c(t,z)\, dt \Big/ \int_o^\infty c(t,z)\, dt = (1 + k_i) z/v_i. \quad (8)$$

The second central moment gives the variance of the pulse at z,

$$\mu_2 = \int_o^\infty (t - \mu_1')^2 c(t, z)\, dt \Big/ \int_o^\infty c(t, z)\, dt = 2D_i(1 + k_i)^2 z/\alpha_i v_i^3 \quad (9)$$

demonstrating that spreading increases with dispersion and distance. The inverse behavior with velocity obtains because dispersion acts for a shorter time at higher velocities. Unencumbered by nonlinearities, the moment theory yields the same results as the method of characteristics, and includes band-spreading by dispersion in the second moment.

To apply the method of characteristics, set D_i equal to zero. Equation 6 then shows that concentration does not change along the characteristic lines, whose slope on a plot of z vs. t is simply given by

$$\text{slope} = v_i/(1 + k_i). \quad (10)$$

Equation 10 has an obvious relationship to Eq. 8 when z/μ_1' is identified as the slope of characteristic lines, i.e., the migration velocity. The slope will have the sign of v_i, which can change sign at an interface between two media, if α_i or β_i change sufficiently. Figure 1 depicts such interfaces where characteristic paths meet. Solute introduced at the column entrance, $z = 0$, will migrate at the velocity $v_i/(1 + k_i)$ to an interface, where it will stop. If solute A is introduced at the column outlet, where $z = L$, the electrophoretic force will cause the solute to migrate upstream against the flow. Thus the solute tends to accumulate at the interface. The behavior would be the same if v_i changed sign due to a variation in v_o, for example when the cross-sectional area varies with distance. Figure 1 depicts the column with three different discrete medium properties. Characteristic paths for four hypothetical solute species are drawn. Thus four components can potentially be separated in the column with three discrete media properties.

Stationary Zone Band-Spreading

According to our analysis, solute cannot pass an interface at which the velocity v_i changes sign. As the concentration of the accumulating species increases, the concentration gradient between the interface and the upstream and downstream regions increases, causing diffusion of the species away from the interface. On either side of the interface, diffusion is opposed by the convective velocity v_i. A pseudosteady state treatment serves to describe this band-spreading of slowly accumulating solute.

Consider the diffusion equation in the absence of adsorptive effects,

$$v_i dc/dy = D dc^2/dy^2 \quad (11)$$

in which D is the diffusion coefficient and y is the distance away from the interface in either the upstream or downstream direction. Two boundary conditions are

$$c(y = 0) = c_o \quad (12)$$

and

$$c(y \to \infty) = 0 \quad (13)$$

where c_o is the concentration at the interface. Integration of Eq. 11 yields an exponential decline of concentration away from the interface,

$$c(y) = c_o \exp(-v_i y/D) \quad (14)$$

The exponential shape of the concentration profile agrees with Gidding's (1979) conclusion that exponential concentration profiles are formed at barriers. The width of the band, defined for given values of c and c_o, is found from Eq. 14 by solving for y. The total accumulated solute is found by integrating over the upstream and downstream regions:

$$M = A_{\text{col}} \left[\int_0^\infty c_1(y)\, dy + \int_0^\infty c_2(y)\, dy \right] \quad (15)$$

Solving for the concentration at the interface one obtains

$$c_o = M/(D/v_1 + D/v_2) A_{\text{col}} \quad (16)$$

Continuous Gradation of Separation Property

O'Farrell (1985) observed that a continuous gradient of separation property along the column should generate many distinct stationary accumulation zones for differently charged species. For example, two media mixed in continuously varying proportions could constitute the column packing. The void fraction α_i and the property k_i would vary considerably with z, causing the migration velocity, $v_i/(1 + k_i)$, to vary continuously. The position for accumulation of the solute is where $v_i = 0$, i.e., where the solute migration velocity changes sign. Vanishing migration velocity is approached from above by upstream solute and from below by downstream solute. This behavior is depicted schematically in Figure 2, in which species A and B accumulate at different positions in the column. The characteristic paths are curved since α_i and k_i vary with z. Species C migrates through the column to the outlet because its migration velocity does not vanish. Species E will migrate to the inlet of the column because its electrophoretic velocity exceeds v_o/α_i. Figure 2 illustrates how the four different species could be separated in the continuous gradient. Overlapping of the accumulation zones owing to diffusional band-spreading will limit the number of species that can be separated.

To determine how long it takes for the migration velocity to vanish (at the accumulation zone) requires a differential treatment. Duarte and McCoy (1982) studied chromatographic separations in a column with a temperature gradient imposed along the column. The mathematical model has similarities to programming techniques in chromatography, for which temperature (McCoy, 1979), velocity (McCoy, 1984), or some other parameter changes continuously (or discretely) with time. The mathematical treatment can be applied when other properties,

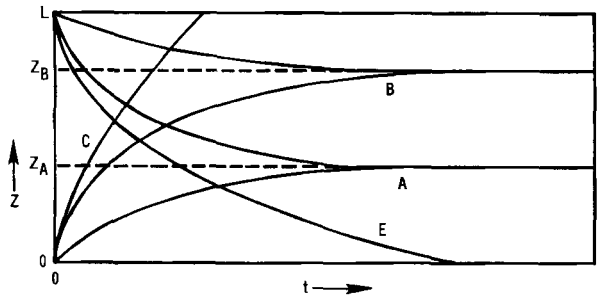

Figure 2. A continuous gradient of separation property along the column can allow species *A*, *B*, *C*, and *E* to be separated.

At points z_A and z_B the solutes mobilities for *A* and *B*, respectively, change sign so that they accumulate asymptotically. For species *C* the migration velocity is always positive. For species *E* the migration velocity is always negative.

e.g., void fraction α_i or porosity β_i, vary along the column. The essential idea is that the change of the first moment is proportional to the distance, thus a differential change in μ_1' is proportional to dz as follows:

$$d\mu_1' = f_i\, dz \tag{17}$$

where

$$f_i = (1 + k_i)/v_i \tag{18}$$

When μ_1' is identified as the time for the solute to reach to position z, Eq. 18 is seen to be the differential form of the slope of the characteristic path.

To illustrate how the solute migrates to its stationary position, consider the simplest nontrivial example, a linear dependence of α_i on z, and $\beta_i = 0$,

$$\alpha_i = \gamma z + \alpha_0 \tag{19}$$

Then the expression of Eq. 18, with $v_i = v_o/\alpha_i - v_e$, integrates to

$$\mu_1'(z) = -\frac{1}{v_e}\left[z + \frac{v_o}{\gamma v_e}\ln\left(\frac{v_o - \alpha_i v_e}{v_o - \alpha_o v_e}\right)\right] \tag{20}$$

Notice that when v_i approaches zero, μ_1' increases without limit; that is, the solute slows as $v_i \to 0$, and the solute requires increasingly more time to reach the stationary point. In actuality, back-diffusion smears the point of zero velocity into a finite zone for accumulation. Equation 20 reduces to Eq. 8 in the limit as the void fraction gradient γ vanishes.

This treatment of continuous gradients of the separation property can be extended to other counteracting fields, such as those in Table 1. The pertinent design quantity will be the position in the column where the solute migration velocity reverses direction. The differential expression for the first moment is available to describe the motion of the solute. The differential expression of the second moment (not presented here) can be utilized to understand the dispersive spreading of a band of solute as it approaches its stationary zone.

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Notation

A_{col} = column cross-sectional area
c = concentration of a species
c_{ads} = concentration of adsorbed species
c_p = concentration of a species in pores of a particle, i.e., intraparticle concentration
D_i = effective axial dispersion coefficient for region i
D = effective diffusion coefficient for stationary band-spreading
c_o = concentration of a species at an interface, Eq. 12
K_i = equilibrium adsorption coefficient
$k_i = \beta_i(1 - \alpha_i)(1 + K_i)/\alpha_i$, Eq. 7
M = total mass of a species introduced into column
t = time
v_c = chromatographic velocity
v_e = electrophoretic velocity, i.e., product of electrophoretic mobility of charged species and electric field gradient
v_i = species migration velocity (net) in region i
v_o = superficial velocity, i.e., volumn flow rate/column cross-sectional area
y = distance from interface between two column regions
z = distance coordinate along the column
Z_{12}, Z_{23} = interface between regions 1 and 2, and 2 and 3, respectively

Greek letters

α_i = interparticle void fraction of region i
β_i = intraparticle porosity of region i
γ = linear gradient of void fraction with z, Eq. 20
μ_1' = first normalized temporal moment, Eq. 8
μ_2 = second central moment, Eq. 9

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