Electrochromatography

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A review of some of the theoretical and practical aspects of chromatography and electrophoresis. Models are generally considered either as stochastic or continuum in nature.

Introduction

Chromatography is a separation process that exploits different chemical and/or physical affinities between the different components of a mixture and a fixed solid sorbent or gel matrix. The solid or gel may be chemically modified to enhance its affinity for a particular component. A source of energy, in the form of a temperature or chemical gradient, is often used to drive or improve the separation. Chemical gradients are usually used to specifically desorb a component, or to focus that component in a small volume of eluent. The scale-up of chromatography of protein mixtures has progressed along the lines of other fixed-bed operations, with special attention being paid to sorbent chemistry, sorbent particle size, and bed dimensions [1].

In comparison, electrophoresis employs an electric field gradient to drive the separation of charged molecules. The technique is frequently employed in the analytical separation of such biological molecules as proteins and nucleic acids. Its scale-up, however, has been elusive. Problems with non-uniform heating, electrically induced flow, and lack of speed have required modifications in laboratory techniques upon scale-up. These modifications include the incorporation of stabilizing gels, a design of special large-scale flow systems [2], and the operation of a large apparatus under microgravity conditions on the space shuttle [3].

Electrochromatography represents the combination of a sorbent-based separation with an electrical gradient. The purpose of the combination is to obtain electrophoretic and chromatographic separation of molecules in a system configuration whose scale-up characteristics are well understood. Along with potential scale-up advantages, more delicate separations may become possible, with two or more energy gradients superimposed on the system. In this article, the basic characteristics of each system are reviewed, and their combination in electrochromatography is discussed.

Liquid Chromatography

The separation of a multicomponent solution is often necessary in biotechnological processes. Chromatography is used for this purpose when a sorbent can be found that has a different equilibrium with each component. The equilibrium of a component with the sorbent is described in terms of a capacity factor, $k_i$,

$$k_i = \frac{n_i}{c_i}$$

where, for the ith component, $n_i$ is the moles of solute in the stationary phase or sorbent, and $c_i$ is the moles in the mobile phase, or fluid. The capacity factor is not necessarily constant, but may be expressed as a function of $c_i$ or $n_i$ [1]. When the capacity factor or distribution coefficient is constant, the equilibrium between the component (solute) and sorbent is said to be linear. In most analytical applications, solute concentrations are low, sample volumes are small, and solute molecules are assumed to interact only with the solvent and the sorbent. When these conditions do not apply, non-linear equilibria and interference effects must be considered [4].
Chromatography Models

Theoretical Phase Theory

The classical view of chromatographic and adsorptive separations, developed by Martin and Synge [5], is to treat the column as a series of stirred tanks in which the contents are well mixed and at equilibrium. The sorbent remains in each tank while solution is passed continuously from one tank to the next. Each stirred tank is called a plate, analogous to a distillation plate. The concentration of solute, obtained from a mass balance written around a single plate is given as:

\[ c_n = c_{n-1} + \frac{\partial t}{HA(K(1-a)+a)} (c_{n-1} - c_{n+1}) \]  

(2)

where \( c \) is the concentration of a single component in plate \( j \) in the mobile phase after \( n \) incremental volumes of eluent have passed, \( K \) is the distribution coefficient, \( a \) is the void fraction, \( A \) is the cross-sectional area of the column, and \( H \) is the height of a theoretical plate.

If a unit pulse of feed in the first plate \( (c_1 = 1) \), is followed by \( n \) incremental volumes of pure eluent, \( \partial t \), the solute concentration in the \( j \)th plate can be represented by the \( j \)th term of the binomial expansion:

\[ 1 = \left( 1 - \frac{\partial t}{V_a} + \frac{\partial t}{V_a} \right)^n \]  

(3)

where \( V_a \) is \( HA(K(1-a)+a) \), the accessible volume per plate.

This expression can be written for each plate as:

\[ c_{ji} = \frac{n!}{j!(n-j)!} \left( \frac{\partial t}{V_a} \right)^j \left( \frac{1}{V_a} \right)^n \]  

(4)

which becomes through Stirling’s approximation for sufficiently large \( n \):

\[ c_{ji} = \frac{(nh)^j}{j!} \left( \frac{1}{V_a} \right)^n \exp \left( -\frac{nh}{V_a} \right) \]  

(5)

When \( j \) is taken to be large, (long column with respect to the height of a plate) and \( nh \) is equal to \( \tau \) (the total volume passed), Stirling’s approximation can again be used to find:

\[ c_{ji} = \left( \frac{\tau}{V_a} \right)^j \left( \frac{1}{\sqrt{2\pi}} \right) \exp \left( -\frac{\tau}{V_a} \right) \]  

(6)

This result was the first of its kind, and explained the zone-spreading phenomena of chromatography from an equilibrium point of view. The solution allowed the calculation of the height of a theoretical plate through the determination of \( \tau \). However, this solution does not describe the exact physical parameters that actually control chromatography. This approach assumes: there is no diffusion from one plate to the text, there are no effects from other solutes, that mass transfer is rapid compared to the average residence time in a tank; and that equilibrium behavior is not a function of concentration. This model is important because it describes observed sample diffusion in an LC column, and so has not been abandoned by practicing chromatographers.

Stochastic Models

The theoretical plate theory may be contrasted to stochastic models, which give more physical insight into the mechanics of chromatographic separations, and continuum models, such as those proposed by Lapidus and Amundson [6]. While most chromatographic models give similar results, (Gaussian shaped peaks), more advanced models give predictions about particle size effects, or eluent velocity effects, which earlier models were incapable of.

The stochastic model treats chromatography as a random process, in which a solute undergoes a random steps of length \( L \) in its passage through the column. The simplest random walk involves \( n \) adsorbed steps, and \( n+1 \) desorbed steps. Statistical theory then leads to the result that the variance in the column, \( \sigma^2 \), is equal to:

\[ \sigma^2 = L^2(a(n + 1) + L^2) \]  

(7)

where \( n \) is the number of steps taken, \( L \) is the distance traveled while desorbed, and \( L \) is the distance traveled while adsorbed.

Since the length of an adsorbed step is zero, the second term drops out and only desorbed steps are accounted for. The variance in a solute band is related to the plate height by the equation:

\[ H = \frac{\sigma^2}{L} \]  

(8)

where \( L \) is the length of the column. This is useful, because the variance generated by independent processes are directly additive, i.e.,

\[ \sigma^2 = \sigma_1^2 + \sigma_2^2 \]  

(9)

where \( \sigma_1^2 \) and \( \sigma_2^2 \) represent two random walks occurring simultaneously.

This has allowed the development of the stochastic model to include radial, kinetic, hydrodynamic, and tortuosity effects. Using the random walk approach and some intuition, Giddings [7] arrived at an algebraic expression for the height of a theoretical plate of the form:

\[ H = \frac{B}{v} + C \tau + \frac{1}{1 + \frac{1}{A + C \tau}} \]  

(10)

where \( v \) is the eluent superficial velocity. The \( B \) term accounts for longitudinal diffusion in the mobile phase, \( C \) is related to adsorption-desorption processes, and \( A \) and \( C \) are proportional to hydrodynamic column processes due to solute diffusion and mixing in the mobile phase. The result is qualitative, in that each constant is a grouping of physical constants with at least one adjustable parameter such as tortuosity. The determination of these parameters is very involved experimentally, and requires on the order of one hundred experimental observations for statistically significant results [8]. This expression is identical in form to one due to van Deemter, et al. [9].

The Continuum Approach

The continuum approach facilitates calculation of a complete elution profile for a solute based upon a mass balance on the solute, and either an equation of state describing the stationary phase capacity, or a kinetic rela-


tionship describing the rate at which solute approaches equilibrium with the sorbent. One early continuum model is that of Lapidus and Amundson [6]. They proposed a mass balance on a single solute,

\[ D \frac{\partial c}{\partial \tau} = \frac{\partial}{\partial \xi} \left( \frac{\partial c}{\partial \xi} + \frac{1 - \alpha}{\alpha} \frac{\partial n}{\partial \tau} \right) \]

and either an equilibrium relationship

\[ k_C = n \]

or a kinetic relationship

\[ \frac{\partial n}{\partial \tau} = k_C c - k_0 n \]

where \( k_C \) and \( k_0 \) are lumped kinetic coefficients, to describe solute/sorbent interactions.

An analytical solution for each case was derived, with the assumption of a semi-infinite column. Their solution for the equilibrium model reduces quite nicely when an initially clean column and a constant inlet concentration are assumed. These conditions are represented mathematically:

\[ c = 0, \tau = 0, \xi > 0 \]

and

\[ c = c_{o1}, \tau = 0, \xi > 0 \]

This relationship is given as:

\[ c = 0.5 \left[ 1 + \text{erf} \left( \frac{\xi}{c \sqrt{4D\tau}} - x \sqrt{\frac{\gamma}{4D\tau}} \right) \right] + \exp \left( \frac{\xi}{c \sqrt{4D\tau}} \right) \text{erfc} \left( \frac{\xi}{c \sqrt{4D\tau}} + x \sqrt{\frac{\gamma}{4D\tau}} \right) \]

where \( \text{erf} \) is the error function, and \( x = 1 + (1 - \alpha)\gamma/\alpha \). Note that Lapidus and Amundson's solution gives no information on the origin of second order dispersion; it simply assigns a number \( D \) to quantify it. In this sense, their analytical solution is qualitative, because a relationship is not drawn between dispersion and other column parameters as eluent velocity or resin particle diameter.

Subsequent continuum models have addressed this by substituting a kinetic relationship in spherical coordinates to describe diffusion into and out of a stationary particle, given by:

\[ \frac{\partial n}{\partial \tau} = D_s \left( \frac{\partial^2 n}{\partial \xi^2} + \frac{2}{r} \frac{\partial n}{\partial r} \right) \]

[10-12] where \( D_s \) is the apparent diffusivity of the solute in the support particle. This equation must be accompanied by additional boundary conditions for the individual support particles. These conditions are given as

\[ \frac{\partial n}{\partial \tau} = \frac{6k_C}{4D} \left( c - \frac{\gamma}{\gamma} \right), \quad r = \frac{d_p}{2}, \quad \xi > 0 \]

and

\[ n < \omega, \quad r = 0, \quad \xi, \tau > 0 \]

where \( n \) is the particle surface concentration, \( d_p \) is the particle diameter, and \( k_C \) is the mass transfer coefficient from the mobile phase to the particle surface.

A "dispersive" model has also been developed, in which solute both diffuses into and out of the resin, and is also retained by reaction on resin active sites [13, 14]. This model seems to be most appropriate in ion exchange chromatography, and has been applied to the adsorption of bovine serum albumin [14].

A Comparison of Chromatography Models

It has been useful to combine information that can be obtained from both the stochastic and continuum models. This can be done by relating the dispersion coefficient to the plate number through the Peclet number:

\[ 2N = Pe - \frac{vL}{D} \]

where \( Pe \) is the Peclet number, and \( L \) is the length of the column. Substituting the van Deeneter [9] equation for \( D \),

\[ D = \frac{vL}{2e} - \frac{1}{2} \left( \frac{1}{2} + \frac{B}{v} + Cc + A \right) \]

where \( A, B, \) and \( C \) are on the order of \( 10^{-1}, 10^{-7}, \) and \( 10^{-14} \), respectively. If a rough estimate of the van Deeneter coefficients can be obtained, the dispersion coefficient can be estimated for different flow rates. The Lapidus and Amundson solution can then be used to generate an elution profile. An example of \( \beta \)-lactoglobulin B elution from a Sephadex column is shown in Figure 1.

A number of issues need to be resolved in the modeling and prediction of chromatographic separations. Most

![Figure 1. Elution profiles of the whey protein \( \beta \)-lactoglobulin B given by the Lapidus and Amundson equation (Equation (16)) [6], for different eluent velocities, with the dispersion coefficient estimated from the van Deeneter Equation (9). Solid lines are model predictions, points are from column experiments on Sephadex G-75.](image)

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models still retain at least one adjustable parameter, requiring extensive experimentation before the model can be employed to predict elution profiles with confidence. Few models employ surface chemistry to elucidate the interaction of solutes with the stationary phase [15]. Most analytical solutions thus far have used a semi-infinite approximation to avoid describing the effects of the solutes leaving the bed, or a no-flux condition ($\delta \sigma = 0$), which is obviously not the exact physical situation in pulsed operations. This problem has recently been addressed [16]. Suitable outlet boundary conditions will help improve our understanding of chromatography; allowing us to predict phenomena in shallow beds with overloaded samples. This in turn will give a means of understanding and controlling the selected chromatographic processes of current industrial interest [1].

Electrophoresis

Electrophoresis is a standard method of high-resolution protein and nucleic acid separation in modern biology laboratories. It achieves high resolution on an analytical scale, and consequently, is the target of scale-up attempts [2, 17]. The various complexities inherent in a system through which current flows, particularly Joule heat dissipation and electromosotic flow, have made the scale-up of electrophoresis a challenging problem. Some special steps to deal with these problems, such as the operation of electrophoresis in space [3], have shown promise, but these approaches also have obvious limitations. The characteristics inherent to electrophoresis and their impact on separation scale-up is described in more detail below.

Electrophoretic Mobility

The motive force for solutes in electrophoretic systems is the electric field. The rate of electrolyte displacement per unit electric field strength is known as the electrolyte’s electrophoretic mobility, $\mu$. Thus, the net distance a solute migrates in an electric field in a given time, $t$, is given as:

$$d_s = \mu E t$$

where $d_s$ is the distance migrated, $t$ is the time allowed for migration, and $E$ is the strength of the applied electric field. This mobility is a characteristic of the charge on the solute. However, the charge is effected by the pH, ionic character (strength and composition), and temperature of the media.

A general mobility has been proposed [17] that is applicable to solute electrophoretic mobility (motion driven by electric field force) and to solute diffusivity (motion driven by thermal agitation). This general mobility would essentially be a shape factor for a Stokes Einstein mobility analysis and would not take such electric double-layer effects as relaxation or Stern layer polarization into account. To be general, the effect of the surrounding media on the charge and hydrodynamics at the particle surface must be understood. The effect of the surrounding media on protein charge appears to be a property of the protein itself, because most proteins bind one ion specifically. The hydrodynamic effects have been studied theoretically for some cases of defined geometry.

The simplest case is that of a rigid sphere, which is uniformly charged and large compared to the surrounding molecules. von Smoluchowski and Helmholtz [18] showed that electrophoretic mobility of such a sphere could be represented by the relation

$$\mu = \frac{6 \epsilon A}{\eta}$$

where $\xi$ is the electric potential of the electrolyte at its surface of shear, and $\epsilon$ and $\eta$ are the relative permittivity and viscosity of the surrounding media, respectively [18], and $\epsilon$, is the permittivity of free space. This equation represents the movement of a hard sphere by the action of an electric field on its fixed charge, through a viscous, conducting fluid, when the electric field is parallel to the surface of the particle in the double layer. A theoretical prediction of electrophoretic mobility for proteins under various conditions has not yet been derived [19]. The rigid sphere model is therefore used to describe the effects of an electric field on proteins in solution [20].

When a polyelectrolyte, i.e., a protein, is placed in a solution of smaller molecules such as salt water, the charges at the surface of such a particle attract ions with opposite charges. These ions form a layer of charge, which is then free to diffuse in the local media. The fixed and free layers of charge together are referred to as the electric double layer. When an electric field is applied to such a system, there are at least four phenomena that occur. The large particle, or protein, will be attracted to the electric pole of opposite charge. Then, as it moves, the molecules around it will form a viscous flow field and will shear along the particle, and any other associated molecules, such as bound water. These two effects are reflected by the parameters $\xi$, $\epsilon$, and $\eta$, respectively, in Equation 23.

The first effect is a property of the particle itself, and is only affected by the surrounding media insofar as the surrounding media regulates and participates in direct reactions with the charge on the particle surface. For example, the solution pH will affect the degree of dissociation of several amino acid residues that may contribute to the total surface charge of the protein. These reactions give rise to the isoelectric point, the pH at which a protein has no net mobility in an electric field. The temperature of the solution also affects the association-dissociation equilibrium of charged amino acid residues, as well as the amount of bound water on the molecule. This, in turn, will also be reflected by the molecule’s hydrodynamic characteristics.

The third and fourth effects arise from the diffuse layer in the surrounding media and its own tendency to migrate in the electric field [21-23]. Ionic charges in the diffuse layer will also be acted upon by the electric field, and, being oppositely charged from the particle they surround, will be moved in the opposite direction. This motion in the diffuse layer exerts an additional drag on the particle, known as electrophoretic retardation. This is shown in Figure 2. Similar interactions also result in the macroscopic phenomena known as electromososis, which is discussed later.

The fourth retarding effect arises from the distortion of the diffuse layer in the electric field. With the particle moving in one direction and the diffuse layer in the other, a uniform diffuse layer does not remain around the particle. The diffuse layer is continuously restored by the
Coulombic forces of the particle [24]. These forces do take a finite time to set, known as the relaxation time. Since the particle is in motion, this time is translated into a short, steady-state distance by which the particle is offset from its electroneutral position in the ionic environment. In other words, electroneutrality is an equilibrium condition that requires a finite time to occur. The build-up of opposite charge in the direction opposite of the motion of the particle then exerts an electrical force that tends to restore the particle to the center of the ionic atmosphere. This is known as the relaxation effect.

Electroretardation and electrolelaxation are the result of the existence of a diffuse layer and are highly dependent upon such bulk phase properties as the ionic strength, pH, and temperature. These effects apply to all electrolyte systems where one or more electrolytes attract a diffuse layer. A schematic of all four effects is given in Figure 2.

Determination of Electrophoretic Mobility

In the determination of the electrophoretic mobility of a molecule, all four forces must be accounted for. It is important to understand the calculation of an electrophoretic mobility, and the dependence of such a calculation on the nature of the solution. A short description of the equations for the electrophoretic mobility of a rigid sphere, and the limitations of these equations follows.

In order to account for electrophoretic retardation, the properties of the double layer need to be investigated. The double layer potential extends from the particle surface (where the potential is denoted $\psi$) and considered to be homogeneously distributed on the surface) according to the Poisson-Boltzmann equation:

$$-\nabla \psi = \frac{e}{\kappa} \sum_{i} n_{i} z_{i} \exp \left( -\frac{z_{i} e \psi}{k_{b} T} \right)$$

(24)

where $\psi$ is the electric potential, $e$ is the elementary charge, $n_{i}$ is the ion concentration, $z_{i}$ is the charge of the ion, $k_{b}$ is the Boltzmann constant, and $T$ is the absolute temperature.

A Taylor’s series expansion of the exponential term, truncated after the second term, yields:

$$\nabla \psi = \kappa \psi$$

(25)

where

$$\kappa = \frac{e^{2} \sum_{i} n_{i} z_{i}^{2}}{k_{b} T}$$

(26)

when $z_{i} e \psi \gg k_{b} T$. $\kappa$ has the dimension of length, and is known as the Debye length. The Debye length is considered a measure of the thickness of the double layer.

Huckel [25] and von Smoluchowski [26] used this linearized form of the Poisson-Boltzmann equation to calculate the drag on moving charged particles. Von Smoluchowski considered a flat surface, which is approximately true when the diffuse layer is thin compared to the radius of the molecule. Huckel considered a spherical surface, and found the result

$$\mu = \frac{2 \kappa \psi}{3 \eta}$$

(27)

Henry [26] resolved these two models by expanding von Smoluchowski’s calculation (Equation 23)) for cases where the double layer was not thin compared to the particle. The extra terms in Henry’s calculation where introduced as a scaling function, dependent on the ratio of the particle radius, $a$, to the double layer thickness (Debye length).

$$\mu = \frac{\kappa \psi}{\eta} f(\kappa a)$$

(28)

FIGURE 3. The function $f(\kappa a)$ as given by Henry [26].
When the ratio \( k_a \) approaches infinity, i.e., the double layer is thin, \( \phi_{ea} \) approaches 1. When \( k_a \) becomes very small, i.e., the double layer is thick, \( \phi_{ea} \) becomes 2/3. When \( k_a \) is on the order of one, Henry's solution does not converge, and the linearization of the Poisson-Boltzmann equation may not be valid [22]. The function \( \phi_{ea} \) is given graphically in Figure 3. The region in which \( k_a \) is near unity in the region in which relaxation effects have been found to be significant [21, 22].

Booth and Overbeek [21, 22] solved the non-linearized form of the Poisson-Boltzmann equation to find the effects of relaxation. Their solutions, while quite elegant, require a priori knowledge of certain surface parameters. The application of these equations to experimental data is necessarily iterative, and thus, difficult to prove. Wiersma, et al. [23], have obtained computer solutions for the higher order Poisson-Boltzmann equation where relaxation effects have been accounted for, for rigid spheres. Their solution shows that under certain conditions, relaxation can account for up to a 50% difference in electrophoretic mobility, versus the mobility predicted by Henry's equation. Wiersma, et al. developed a chart showing the regions in which different solutions to the electrophoresis problem may be considered approximately correct. O'Brien and White [27] have considered the force to move a particle at its electrophoretic velocity in the absence of a field and to hold a particle in place in an applied field, to calculate the electrophoretic mobility. Zinowski and Saville [28] have incorporated transport in the Stern layer (adsorbed ion layer) to calculate electrophoretic mobility.

**Double Layer Thickness**

The thickness of the double layer is dependent on the ionic strength of the solution or, on the concentration and valence of all the electrolytes. Electrolyte valence is selected more than \( z \), and concentrations are only rarely below 1 mM or above 10 M. Thus, the Debye length varies between \( 3.25 \times 10^{-4} \) and \( 3.25 \times 10^{-6} \) for this condition. Applying Henry's solution, particles with radii less than approximately \( 10^{-6} \) obey the Debye-Hückel relation (Equation 27), while particles above \( 10^{-6} \) obey von Smoluchowski's solution (Equation 23). All other charged particles or colloids are affected by relaxation, Stern layer transport, etc., and more complicated relations must be used in the interpretation of their electrophoretic mobilities because their radii and double layer thicknesses are on the same order of magnitude.

Since proteins are amphoteric electrolytes, solution ionic strength, ionic composition, and pH become important for their contribution to protein surface potential. Due to the amino acid composition and structure of proteins, and the fact that different residues have different pKa's, proteins have different degrees of dissociation at different pH's. At low pH's, proteins will have a net positive charge, while at high pH's, proteins will have a net negative charge. The point at which a protein shows no net motion in an electric field is called the isoelectric point of the protein, and is a property of the protein and the surrounding solution. The isoelectric behavior of \( \beta \)-lactoglobulin was determined by Pedersen [29] and is compared to its titration behavior [20] in Figure 4. This point varies slightly with ionic strength [24], and with ion type, due to specific ion adsorption. Therefore, a protein's environment can have a profound effect, not only on the thickness of its double layer, but also on its surface potential \( \psi \). This property can be exploited to assist in the separation of protein mixtures.

**Stochastic Models in Electrophoresis**

The plate height concept, introduced earlier, has been extended to electrophoresis by Giddings [30]. Einstein [31] showed that the variance generated from an ideal pulse in time \( t \) could be represented as:

\[
\sigma^2 = 2Dt
\]

Giddings [30] noted that the plate height was a measure of the variance generated \( \sigma^2 \) per length, and could be represented as:

\[
H = \frac{2Dt}{L}
\]

In electrophoresis, a component will traverse a length \( L \) in a time \( t \),

\[
t = \frac{L}{\mu E}
\]

where \( E \) is the electric field strength, \( V/L \), and \( V \) is the total applied voltage. This gives an expression for the plate height

\[
H = \frac{2L}{\mu E}
\]

and for the plate number
Since the variance generated is assumed to be directly proportional to time and independent of such other variables as the electric field strength, system width, or ionic strength (according to this model), this result shows that the plate height is dependent on the system length, and the number of plates is independent of system length. This predicts that the most efficient electrophoresis system is one in which the length between electrodes is a thin layer, and the potential applied is infinitely high. In a crude way, this is similar to assuming that mobile phase diffusion is the only contributor to dispersion in chromatographic systems, and shallow beds with high flow rates give the most efficient separations. In either analysis, kinetic effects are ignored.

Continuum Models of Electrophoresis

While electrophoresis has become one of the most widely used analytical techniques for the study of molecular biology, the mathematical modeling of the transport processes involved has lagged [32]. One reason for this is the complex, coupled and non-linear equations needed to describe electrophoresis. Another reason may be lack of easily quantifiable experimental techniques for testing theoretical models.

A unified model for electrophoretic processes has recently been proposed [17, 32, 33]. The model considers the electrophoretic, diffusive and convective (or electroosmotic) flux of electrolytes in electric fields. It takes into account the electrochemical reactions at the electrodes, and the equilibrium between varying degrees of ionization displayed by electrolytes. These equations are written

\[
\frac{\partial c_i}{\partial t} = - \nabla ( - D_i \nabla \Phi ) - \nabla ( \Phi \nabla c_i ) + \frac{c_i \mu_i}{RT} \frac{\partial \Phi}{\partial x}\]

(34)

with

\[
K_{i}^{-1} = c_0 c_h c_i c_1; \quad K_{i}^{+1} = c_0 c_h c_i c_1 \]

(35)

and

\[
K_{e} = c_0 c_h c_i
\]

(37)

where \(c_0\) is water, \(c_1\) is hydrogen ion, \(c_2\) is hydroxyl ion, and \(c_0\) and \(c_1\) have one less, and one more hydrogen ion, respectively, than \(c_0\). The detailed accounting of all the ionic species in this model allows calculation of electric field and pH gradients in the system. However, the equations are coupled, non-linear, very stiff, and require a very high speed computer for their efficient numerical integration. The details of the numerical solution are given in [32].

Electrophoresis Considerations Summarized

The stochastic and continuum approaches to electrophoresis modeling elucidate some of the problems inher-

ent in the analysis of these systems. Simple approaches, such as the stochastic model presented, are probably only applicable over a small range of conditions. Their predictions are not consistent when extrapolated to extreme, or even normal experimental conditions. On the other hand, a general continuum model is difficult to utilize, due to the non-linearities and stiffness inherent in the equations. The results are also difficult to confirm experimentally due to lack of hardware and techniques. The results obtained by Bier, et al. [32] do appear to be qualitatively consistent with experimental observation. Asymptotic analytic solutions are also important in this field [34, 35].

Practical Aspects of Electrophoresis

For an electrophoresis system to be efficient, the dispersion of solute zones must be minimized. There are several dispersions that may occur in electrophoresis, such as electrode gassing, Joule heating, and electroosmosis. These effects can be responsible for non-uniformities in bulk flow patterns, or electric fields. The mechanism of their action is briefly described below.

Electrolysis Reactions

Electrolysis reactions, which are responsible for generating current, will occur at the electrodes in an electrophoresis system. These reactions are accounted for in the general electrophoresis model, in terms of the generation and consumption of aqueous electrolyte species. However, these reactions are also responsible for gas generation at the electrodes. The amount of gas generated at an electrode may be calculated by:

\[
G = \frac{I}{2F}
\]

(38)

where \(G\) is the number of moles of gas produced, \(I\) is the current in amperes, \(s\) is the stoichiometric coefficient for production or consumption of electrons for the favored reaction (per mole of gas), \(t\) is the amount of time in seconds over which the current is applied, and \(F\) is the Faraday constant. For example, a 20 mA current will result in the formation of \(20 \times 10^{-3} \times 10^{-3} \) moles of oxygen per second at the cathode, or 0.001 to 0.002 ml of gas (at STP) per second. This roughly translates into 4 to 8 ml of gas per hour. Gassing can disrupt electric fields and lead to dispersion of electrophoresed zones. Pressurized gas may also lead to bulk flow in an airright system. Pressurized hydrogen gas is also not considered desirable in a large-scale operation.

Joule Heating

The passage of current through a medium also results in the generation of heat in that medium, due to the dissipation of energy. If the medium is homogeneously resistant, then this heat generation will also be uniform with respect to position. Heat is generated according to the current and the voltage required to drive the current. It is given by the work done on the system, \(W\):

\[
W = IV = \frac{1}{2}Fr
\]

(39)
FIGURE 5. Schematic diagram of Counteracting Chromatographic Electrophoresis described by O’Farrell [38]. (A) Shows different solutes in the column without an applied potential. (B) Shows the accumulation of one solute near the gel/gel interface after the application of potential.

Legend
- Convective velocity
- Electrophoretic velocity
- Charged species 1
- Other species 2, 3

where \( I \) is the applied current and \( R \) is the resistance of the system.

Heat is removed from the system either conductively or convectively through the system’s boundaries, but the efficiency of this removal is dependent on the location of heat generation. This results in a temperature gradient (and therefore a density gradient) in the medium, which often leads to unwanted natural convection or viscosity differences that can disrupt the migration, or electrophoretic mobility, of identical molecules along the temperature gradient.

Electroosmosis

Electroosmosis is another phenomenon that occurs in electrophoretic systems and can distort free band separations. Electroosmosis, like electrophoretic retardation, takes place when the small ions in a diffuse layer migrate in the electric field. In this case, the double layer forms on a fixed surface that interfaces with the electrolyte, such as the glass wall of a gel or the surface of a chromatographic support. These surfaces are frequently charged, due to ionizable groups on the surface or adsorption of charged molecules to the interface, leading to the formation of a double layer. When an electric field is applied, surface molecules remain on the surface, but hydrophilic molecules in the diffuse layer migrate. Electroosmosis may induce a bulk flow in the apparatus that mixes or distorts the zones that are formed during the separation. The mechanisms and problems associated with electroosmosis have been reviewed by Vanderhoff and Micale [36].

Solutions

There are two generally applicable solutions to dispersing phenomena in electrophoresis. The current in the cell can be minimized, or the media can be stabilized (or both). Reducing the current in the cell decreases the rate of gas formation linearly, and decreases the rate of heat generation as its square. The current can be reduced at constant potential by increasing the resistivity of the medium. This is accomplished by using dilute buffers. The trade-off is a poorer pH control, and a greater likelihood that the solute of interest will not be stable or soluble, or may adsorb to a surface. This is particularly true with proteins.

The media can be stabilized against non-uniform flow fields with porous packing materials or gels. These create mixing cells with smaller mixing lengths than an empty tube and can effectively dampen out non-uniform flow effects due to electroosmosis or heating. Polyacrylamide and agarose have been popular gel materials, although Sephadex has also been used as a packing [37]. The trade-off here is solute recovery. If a gel is used in an electrophoresis apparatus, it typically must be destroyed in order to recover purified solutes. If a packing such as Sephadex is used in a chromatography column, solutes can be eluted out with flow after a given period of time. However, this also increases the dispersion of the zones and may change the separation itself.

Another alternative has been to conduct electrophoresis in outer space. The absence of gravity removes the driving force for natural convection by heating. This technique has been tested and proven effective, although it does not address gassing and electroosmotic effects, both of which have been observed [3, 38, 39]. Plans were recently cancelled to make outer space separations commercial, in order to take advantage of the higher loadings a thick free flow electrophoresis system can handle. Products must now be extremely valuable and extremely difficult to process while maintaining biological activity, to economically justify processing in space.

Electrochromatography

This study is concerned with the parallel combination of chromatographic and electrophoretic effects for an enhanced separation. There are many forms that this enhanced separation may take, such as applications in which the convective and electric fields are not parallel and are not covered. Initial concern is devoted to the fundamental properties of the system. A review of the previous work in this evolving science is given first.

Counteracting Chromatographic Electrophoresis

O’Farrell [40] recently brought this technology to light with a system he described as Counteracting Chromato-
graph: Electrophoresis (CACE). In this system, two size exclusion gels of different exclusion properties were layered one on top of the other. The lower gel, which included solutes of the larger extent was packed below the less porous gel. Therefore, the partially included solute’s net velocity through the packing was higher in the first (less porous) gel than in the second. An electric field was then applied so that a selected component moved against the flow field electrophoretically. This concept is illustrated in Figure 5.

O’Farrell found that a field strength could be selected that would cause a single solute to be held stationary at the interface between the two gels. He speculated that, at the interface, the electroosmotic velocity of the selected solute was in between the net velocities due to the flow in the different gel layers. This method is limited in capacity by the volume of the second, more porous, gel, packed in the lower part of the column. This limitation indicates in that its observation can be used to make some fundamental deductions about a solute’s behavior in such a fixed bed under an electric field that may be applicable to more general systems. Since the net velocity of the solute is toward the interface in both the bottom and top gel layers (see Figure 5), the solute tends to focus at this interface [40]. Since the volume of the interface is negligible, i.e., the interface is essentially two-dimensional, solute must actually concentrate in either the more or less porous phase. Changes of solute concentration have little effect on the eluent mobile phase velocity, but may influence the local electric field. It was observed that solute concentrates in the lower (more porous) phase. Furthermore, this equilibrium concentration is reproducible, and is independent of feed concentration.

O’Farrell speculated that this concentration must decrease the local electric field so that the protein’s electrophoretic velocity is equal and opposite to the protein’s net velocity due to the mobile phase. This can be visualized as an idealized random walk, where two types of steps occur differently in the three different regions of the column. In the topmost region, a solute has an included step, during which the solute experiences only electromotive forces, and a mobile phase step, during which it experiences both convective and electromotive forces. O’Farrell deduced that the system so that electromotive and convective forces were opposite in orientation. Because the steps must alternate, we conclude that in the upper gel layer, the excluded step carries the solute a greater distance than the included step. Conversely, in the lower layer, the included step is longer than the excluded step, resulting in a negative (against the flow) net mobility. If the external void fraction is the same in both gel phases, the lengths of these steps depend upon the amount of time spent in the resin, if the convective versus electromotive force in the excluded volume is always the same. In the intermediate section, however, where solute may be concentrated, the two steps must be of equal length. Since the convective force does not change in the excluded region, the effect of the electric field must be either increased (if solute is to collect in the upper phase) or decreased (for solute to collect in the lower gel). It was observed that the solute concentrates in the lower gel, implying decreased electric potential in this region. We know that reduced potentials for constant currents occur when the resistivity of a medium decreases, or the conductivity increases. Increases in solute concentration have just this effect.

The process is inherently batch, although continuous modes have been suggested. This result shows that electric potentials can be applied to chromatographic columns, and that a field strength can be safely applied to induce an electrometric velocity comparable to the velocity of a protein in a flow field. It also shows that the electrophoretic flow of solute occurs in two phases, and that the fluid in the resin phase is a significant contributor to net electrophoretic mobility.

This process and other focusing processes, have been described mathematically by McCoy [41] for the mobile phase step. The motion of solute in the included step of the process still needs to be added. Further understanding of this step is also required for the proper design of such systems.

Electrochromatography

The concept of a parallel superimposition of convective and electric fields, electrochromatography, was suggested as early as 1969, by Nerenberg and Poppeoff [42], and by Salak and Roch [43] in 1972. These investigators applied voltage to Sephadex packed size exclusion columns through external column modifications, and operated these columns in the common, linear fashion. While improved separations were reported, substantiating data were not included in their articles.

A more recent application has been reported by Tsuda [44]. Here, an electric field between ±16 kV over a column was used to separate uracil and cis-N-methyl-4-dihydroxyphenylpyridinium iodide (CSI). The resin used was Devon sil; the column was either a 10 cm long glass tube, or 7.4 cm of tetrafluoroethylene tubing. The resulting chromatograms were compared to an elution model, which stated

\[ u = K_c v + K_c E \] (40)

where \( u \) is the solute velocity, \( v \) is the eluent velocity, \( E \) is the electric field strength, \( K_c \) is a convective constant, and \( K_c \) is an electrophoretic constant.

Solute velocity is usually measured as the length of the column divided by the retention time of the solute that is measured. Although the effect of convection and electric field strength are proportional to solute velocity, they are inversely proportional to retention time,

\[ u = \frac{L}{t_r} \]

where \( L \) is the length of the column and \( t_r \) is the retention time.

so that, when \( E \) and \( v \) both have the same sign, the effect on \( t_r \) is less than when they have opposite signs. For example, if \( K_c \) and \( K_e \) are constant for a solute that travels through a column convectively at 1 cm/min, and has a constant \( K_c \) of \( 1 \times 10^{-8} \text{cm}^2/\text{V-s} \), an electric field of 10 V/cm will decrease the retention time by 9%, while one of \( -10 \text{ V/cm} \) will increase the retention time by 11%. Obviously, extrapolation to higher voltages show larger and larger differences in retention times (-100 V/cm will cause the solute not to elute, while 100 V/cm only causes a 50% reduction in retention time).

If Tsuda’s simple model for electrochromatography is to be accepted, one must investigate the effect of the strength of the fields (convective and electric) on the...

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stants $K_e$ and $K_n$. There is some evidence [45, 46] that the electric field has an effect on the capacity factor for resins, which is related to $K_e$. This effect has not yet been formally elucidated, but may relate to interfacial characteristics of solute-turbine interactions [46].

Random walk and continuum models for electrochromatography have not yet been published. Saville et al. [17], incorporate a convective term into their electrophoretic model, but indicate that additional scaling would have to be worked out to assume it is non-zero. A random walk model has been derived and demonstrated by Jorgenson and Lukacs [47] for capillary electrophoresis, but no adsorption affects, pH, or electric field non-uniformities were considered. The effect of the electric field on the resin seems to significantly affect retention times calculated using either a random walk or continuum model.

Conclusions

Chromatography and electrophoresis are two well developed analytical lab techniques. Chromatography seems particularly well understood. It can be represented by several random walk and continuum approaches, some of which are reviewed here. Both continuum and random walk approaches are easily applied to polymers, although exact solutions to more complicated proposed mechanisms of film mass transfer and interparticle diffusion still require a large set of diverse rate data and a computer to use.

Electrophoresis models are also developing at a rapid pace, but there is still a large gap between stochastic and continuum approaches. While continuum models are helping to increase our understanding of electrophoresis in general, they remain difficult to confirm due to limitations in most available electrophoresis equipment. Existing stochastic models are not very useful unless the most ideal conditions prevail. Much work remains to be done to develop design equations for electrophoresis apparatus.

Reports of electrochromatographic separations are beginning to increase. Many of the simultaneous applications of electric and flow fields are two-dimensional and clever in conception [48-55]. Models that account for electrophoresis in two phases and the effect of the electric field on the properties of the resin phase and transport processes between the two phases need to be developed further.

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Notation

$A = \text{plate height parameter (l), column cross section (P)}$
$a = \text{radius of electrophoresing particle (l)}$
$B = \text{plate height parameter (Pn)}$
$c_i = \text{moles of solute i in mobile phase (mols), inlet concentration in continuum models (mols/ml)}$
$c_{in} = \text{concentration in the nth plate after the passage of the nth incremental volume } \Delta$
$C = \text{plate height parameter (l)}$
$C_0 = \text{plate height parameter (l)}$
$d_p = \text{distance migrated electrophoretically (l)}$
$d_e = \text{particle diameter (l)}$
$D_e = \text{column dispersivity (Pn)}$
$D_s = \text{solute dispersivity in the stationary phase (Pn)}$
$E = \text{charge of an electron (1.6 \times 10^{-19} \text{ vs. coul. equiv.)}}$
$F = \text{electric field strength (V/l)}$
$G = \text{Faraday's constant (96,485 \text{ abs. coul/g equiv.)}}$
$H = \text{number of mols of gas evolved (mols)}$
$I = \text{height of a theoretical plate (l)}$
$j = \text{current (amps)}$
$k = \text{plate number}$
$k_0 = \text{capacity factor}$
$k_B = \text{Boltzmann constant (8.61 \times 10^{-5} \text{ abs. ev/Celeq.)}}$
$k_{t, k_a} = \text{mass transfer coefficient from the mobile phase to a support particle surface (l/\text{mol})}$
$k_b = \text{mass transfer or kinetic coefficients (l)}$
$k_c = \text{convection constant}$
$k_{d, c} = \text{electrophoresis constant}$
$k_{d, s} = \text{distribution coefficient for solute i}$
$k_{eq} = \text{distribution equilibria constants}$
$l = \text{length of an adsorption step (l)}$
$l_s = \text{length of a desorption step (l)}$
$L = \text{length of column (l)}$
$n = \text{number of steps, or number of differential eluent passages } x$
$n_i = \text{mole of solute i in stationary phase (mols)}$
$N = \text{number of plates}$
$P_e = \text{Pecllet number}$
$r = \text{radial distance in a particle (l)}$
$R = \text{resistance (ohms)}$
$R(t) = \text{rate of electrolytic reaction (mol/m! x t)}$
$s = \text{stoichiometric coefficient}$
$t = \text{time}$
$t_e = \text{residence time}$
$T = \text{temperature}$
$u = \text{superficial velocity based on an empty column (l/t)}$
$V = \text{applied voltage (volts)}$
$V_a = \text{the column volume accessible to a solute in a plate (ml)}$
$W = \text{power (watts)}$
$x = \text{distance along the column (l)}$
$x_i = \text{valence of solute i}$

Greek

$\alpha = \text{column void fraction, based on blue dextran}$
$\varepsilon = \text{relative permittivity of media}$
$\zeta = \text{permittivity of free space}$
$\gamma = 1 + 2(1 - \alpha)$
$\eta = \text{viscosity of media (P x t/l)}$
$\kappa = \text{inverse Debye length (l)}$
$\mu = \text{electrophoretic mobility (P x V / l)}$
$\Omega = \text{electrophoretic mobility of a single charge (P x V / t)}$
$\rho_i = \text{electric potential gradient (E, u/l)}$
$\sigma = \text{variance from the mean}$
$\tau = \text{total amount of eluent passed (ml)}$
$\theta = \text{or an arbitrarily small volume of eluent (ml)}$
$\psi = \text{potential of solute (volts)}$
$\xi = \text{potential of solute at surface of shear (volts)}$
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