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HARNESSING ELECTRICAL FORCES FOR SEPARATION

CAPILLARY ZONE ELECTROPHORESIS, ISOELECTRIC FOCUSING, FIELD-FLOW FRACTIONATION, SPLIT-FLOW THIN-CELL CONTINUOUS-SEPARATION AND OTHER TECHNIQUES

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SUMMARY

A simple analysis, first presented twenty years ago, showed that the effectiveness of a field-driven separation like electrophoresis, as expressed by the maximum number of theoretical plates (N), is given by the dimensionless ratio of two energies

$$N = \frac{-\Delta\mu^{ext}}{2RT}$$

in which $-\Delta\mu^{ext}$ is the electrical potential energy drop of a charged species and RT is the thermal energy (R is the gas constant and T is the absolute temperature). Quantity $-\Delta\mu^{ext}$ is the product of the force F acting on the species and the path length X of separation. The exceptional power of electrophoresis, for which often $N \approx 10^6$, can be traced directly to the enormous magnitude of the electrical force F.

This paper explores the fundamentals underlying several different means for utilizing these powerful electrical forces for separation, including capillary zone electrophoresis, gel electrophoresis, isoelectric focusing, electrical field-flow fractionation and split-flow thin continuous separation cells. Remarkably, the above equation and its relatives are found to describe the approximate performance of all these diverse electrically driven systems. Factors affecting both the resolving power and separation speed of the systems are addressed; from these considerations some broad optimization criteria emerge. The capabilities of the different methods are compared using numerical examples.

INTRODUCTION

Electrical forces are powerful agents for the achievement of separation[1,2]. Therefore, it is not surprising that numerous configurations have arisen for harnessing electrical forces to achieve the separation of electrically charged species. Among the methodologies developed are capillary zone electrophoresis (CZE), gel electro-

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phoresis, two-dimensional gel electrophoresis, isoelectric focusing (IEF), isotachophoresis, electrical field-flow fractionation (FFF), electrical split-flow thin (ESPLITT) cell separation, electrodialysis, dielectrophoresis and other electrically driven techniques[3–5].

The various approaches to the utilization of electrical forces differ substantially in their inherent separative capabilities and, obviously, in their levels of practical development. The object of this paper is to examine the factors underlying the theoretical capabilities of a limited group of these techniques. Such an examination shows that diverse electrically based techniques are surprisingly similar in performance criteria. This simplifies the task of pointing out directions to be taken for optimization and highlighting some promising areas for future advances. A comprehensive review of all electrically based techniques is beyond the scope of this work.

The most straightforward electrically-based separation method is electrophoresis in a gradient-free and support-free conducting liquid. The separation in such a system occurs along the axis of the electrical field gradient. Ideally, transverse gradients and transport processes are absent; the separation (unlike chromatography) can be visualized as a true one-dimensional process[6]. The major strength of CZE is that its thin heat-dissipative structure allows it to closely approach this model separation process. An analysis of the performance of ideal CZE is therefore a useful precursor to the characterization of other electrically driven separation methods.

It is useful to have an index of performance for CZE and other electrophoretic methods that allows some measure of comparison with other separation systems, particularly chromatography. There is no single index that properly serves to compare all separative processes, but the plate height H and the number of theoretical plates N are quite broadly (although not universally) relevant indices of performance for analytical separation methods[2].

Although theoretical plate indices have not traditionally been applied to measure the performance of electrophoresis systems, I showed in 1969[1] that H, defined operationally as $d\sigma^2/dX$, is a valid measure of band broadening in electrophoresis and sedimentation just as it is in chromatography. For uniform systems, H becomes simply σ^2/X, where σ^2 is the variance in the peak distribution and X the distance of migration of a particular component through the surrounding liquid. Likewise, the derivative parameter, the number of theoretical plates, $N = X^2/\sigma^2$, could be utilized to describe performance in electrophoresis.

Specifically, it was shown in the 1969 paper[1] that the upper theoretical limit of N for electrophoresis and related processes was simply one-half of the ratio of two energies

$$N = \frac{-\Delta\mu^{ext}}{2RT} \tag{1}$$

where $-\Delta\mu^{ext}$ is the chemical potential change or, equivalently, the potential energy loss, experienced by the species migrating across the voltage drop prior to measurement. Quantity $-\Delta\mu^{ext}$ is a measure of the energy that structures the separation whereas thermal energy RT (R is the gas constant, T is the absolute temperature) represents the energy driving the diffusive band broadening that obliterates separation[6]. The enormous power of electrophoresis is attributable directly to the fact that

electrical energies are enormous, capable of reaching levels six orders of magnitude larger than ordinary thermal energy. Thus N can reach levels as high as 10^6 or more plates.

Rather remarkably, eqn. 1, in describing the capabilities of a transport process electrophoresis, has no dependence on transport properties. This is because the basic transport coefficient (the friction coefficient) governing the speed of separation also governs the speed of diffusive band broadening. The underlying transport rates exactly offset one another in formulating N.

CAPILLARY ZONE ELECTROPHORESIS

The derivation of eqn. 1 is rather simple and it helps us understand the origin (and optimization) of the extraordinary resolving power of many electrophoretic methods. It is most closely applicable to ideal capillary zone electrophoresis.

We start by expressing the distance X that a species is displaced through the conducting liquid as the product of the velocity U of the species in the liquid and the time t of migration

$$X = Ut \tag{2}$$

If the species is simultaneously undergoing diffusive band broadening, the increment in variance σ^2 due to the diffusion process is described by

$$\sigma^2 = 2Dt \tag{3}$$

where D is the diffusion coefficient. Thus H, equal to σ^2/X, becomes simply

$$H = \frac{2D}{U} \tag{4}$$

If we express the velocity U by the product of mobility μ and electric field strength E, H becomes

$$H = \frac{2D}{\mu E} \tag{5}$$

Alternately, if we express U in terms of the fundamental transport coefficient f, termed the friction coefficient, we have[2]

$$U = \frac{F}{f} \tag{6}$$

The relationship of D to f is given by

$$D = \frac{RT}{f} \tag{7}$$

Eqn. 4 now takes the form

$$H = \frac{2RT}{F} \tag{8}$$

where F is the force exerted on a mole of the species by the electrical field. Since $N = X/H$, we have

$$N = \frac{FX}{2RT} \tag{9}$$

The product FX is simply the potential energy change experienced by one mole of the charged species in its migration along path length X. This is equal to the chemical potential change $-\Delta\mu^{ext}$ experienced by the species in the course of electrophoretic migration, thus leading to eqn. 1. Eqns. 8 and 9 both lack a dependence on transport rates because f is cancelled out in taking the ratio of D/U to get from eqn. 4 to eqn. 8. (Technically, the f in eqn. 6 may differ slightly from the f in eqn. 7 because the former is perturbed by the counterflow of the ionic cloud surrounding the charged species.)

The above picture is complicated only slightly if the species is displaced in part (typical in CZE) by electroosmotic flow. In this case, the distance of electrophoretic migration X will differ from the distance L between the point of injection and the point of detection (see Fig. 1). If we wish to define the plate height and plate count in reference to the migration over distance L rather than distance X, then $H' = \sigma^2/L$ and the plate count would become $N' = L^2/\sigma^2$, thus modifying the above equations. Eqn. 9, for example, would become

$$N' = \left(\frac{L}{X}\right)^2 \frac{FX}{2RT} = \left(\frac{U + v}{U}\right)^2 \frac{FX}{2RT} \tag{10}$$

where v is the electroosmotic migration velocity, which may be positive or negative relative to the electrophoretic migration velocity U. This treatment is similar to that presented in 1981 by Jorgenson and Lukacs[7], who in addition showed how peak resolution was affected by electroosmotic flow.

Both eqns. 9 and 10 present acceptable indices of separation. Eqn. 9 may be somewhat preferable from the viewpoint of theory and eqn. 10 in connection with

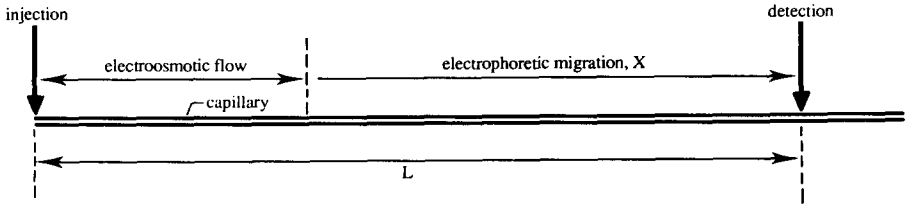

Fig. 1. Diagram showing distances displaced by electroosmotic flow and electrophoretic migration in passage from injection to detection point in a capillary tube.

experimental measurement, but otherwise the choice of index is arbitrary. For simplicity we will work with H and N rather than H' and N'.

If now we express the force in eqns. 8 and 9 as[1,2]

$$F = z\mathscr{F}E \tag{11}$$

the plate height becomes

$$H = \frac{2RT}{z\mathscr{F}E} \tag{12}$$

and N becomes

$$N = \frac{z\mathscr{F}EX}{2RT} = \frac{z\mathscr{F}V}{2RT} \tag{13}$$

where z is the effective charge of the migrating species in proton units, $\mathscr{F}$ is the Faraday, and V, the voltage exerted over path X, is given by the product EX. (With finite electroosmotic flow, $V = V'X/L$, where V' is the system voltage applied between the inlet and detector.)

While effective charge z is less than the true ionic charge because of the opposing drag of the cloud of counterions[8], this parameter describes an essential feature of electrophoresis: the need for a net electrical charge. Furthermore, N increases in proportion to z, which is subject to variation through changes in pH, ionic strength, etc. Because z is so easily visualized, equations incorporating z are particularly easy to interpret and to manipulate for electrophoretic optimization.

Eqn. 13 shows that N is proportional to the voltage applied to the electrophoretic system, thus demonstrating why high voltages are needed for optimal performance[1]. (Note that N is proportional to V independent of the length of path over which the voltage is applied.) The early calculations showed that if $V = 10\,000$ V, it is possible to obtain $N = 10^5$ even for a low effective charge of unity ($z = 1$)[1]. Higher N values can be (and now have been) realized for higher values of V and z. The enormous resolving power implied by such large values of N is directly related to the large energies and forces arising when electrical fields are applied to charged molecules[9].

It is useful to calculate a range of H values expected in CZE. If E ranges from 10^4 to 10^5 V/m (0.01 to 0.1 V/μm) and z from 1 to 5, then H from eqn. 12 will fall between 0.1 and 5 μm at room temperature. This is consistent with the observations of Jorgenson and Lukacs[7], who observed $H' \approx 2$ μm.

In many cases the speed of separation is also an important consideration. We can use the rate of generation of theoretical plates, N/t, as an index of speed[1,2]. By combining eqns. 2, 6, 11, and 13 we get[1]

$$\frac{N}{t} = \frac{(z\mathscr{F}E)^2}{2RTf} \tag{14}$$

which achieves its highest value at maximum E (in proportion to E^2) rather than

maximum V. Since $E = V/X$, it is as important to decrease migration distance X as it is to increase V for speed optimization.

If a specific number of plates N is needed for a given separation, eqn. 14 can be rearranged to express the time t of separation as

$$t = \frac{2RTf}{(z\mathcal{F}E)^2} N \tag{15}$$

The time t is proportional to N, which can sometimes be reduced by factors enhancing selectivity.

Because t is proportional to the product Tf, and since f decreases rapidly with increasing temperature, the experimental temperature corresponding to minimum t should be at the highest possible level consistent with sample stability[2]. This important aspect of optimization is further clarified by using Stokes' law, $f = 6\pi\eta a\mathcal{N}$, which shows that f is proportional to viscosity η, Stokes radius a, and Avogadro's number $\mathcal{N}$. The substitution of this f into eqn. 15 yields

$$t = \frac{12\pi\mathcal{N}RT\eta a}{(z\mathcal{F}E)^2} N \tag{16}$$

While a, z, and plate number N can exhibit a significant temperature dependence in special cases, the product $T\eta$ is universally dependent on temperature, decreasing substantially as temperature increases. This is illustrated in Fig. 2 for water where $T\eta$ (relative to its value at the normal boiling point) is plotted against T. We see that $T\eta$ decreases four-fold upon increasing T from 4 to 100°C, and two-fold as T is raised from 20 to 60°C. Species tolerant of higher temperatures can thus be separated several times faster than normal at temperatures elevated above 50 or 60°C. This gain could be enhanced further by applying a few atmospheres of external pressure to the system to further elevate the boiling point[10]. (Some incremental pressure may be needed for operation close to 100°C to counteract local joule heating.)

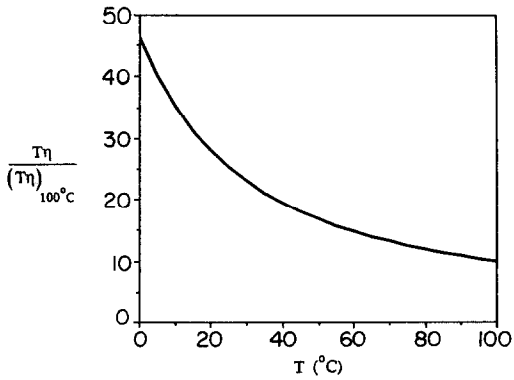

Fig. 2. Plot of product of absolute temperature T and viscosity η (relative to its value at the normal boiling point) *versus* temperature.

We must caution that the above equations and calculations apply only when the sole source of band broadening is molecular diffusion, as expressed by eqn. 3. Otherwise, larger H and smaller N values will be observed. Increments in H can be introduced by the temperature non-uniformities generated by joule heating, by non-uniformities in gels or other support media, by non-uniform electroosmotic flow, by finite injection and detection volumes, and by the non-equilibrium effects arising from wall adsorption and non-uniform flow velocity profiles. In most cases these extraneous sources are responsible for an additional variance term, $\sigma^2(\text{ext})$, leading to an overall variance of

$$\sigma^2 = \sigma^2(\text{ext}) + 2Dt \tag{17}$$

replacing the σ^2 of eqn. 3.

GEL ELECTROPHORESIS

Electrophoresis is frequently carried out on slabs of gel, particularly polyacrylamide gel. Cohen and Karger[11] have recently developed effective methods for introducing a gel media suitable for electrophoresis into a thin capillary. Highly efficient electrophoretic separations were achieved by this approach.

The use of a gel not only inhibits electroosmotic flow, it alters the transport properties (specifically the mobilities) of charged species. Because of the sieving action of the gel network, the mobility of high-molecular-weight components is inhibited more than that of low-molecular-weight species. Accordingly, when macromolecules are charged evenly, such as proteins when subjected to treatment by sodium dodecyl sulfate (SDS), gel electrophoresis becomes an effective means for achieving separation according to differences in molecular weight.

While the transport properties and the basis of selectivity are both altered by using a gel medium, it is useful to examine the effects of gels on overall separation efficiency. Insight on this matter is gained by noting that the reduced mobility caused by the gel network is equivalent to an increase in the friction coefficient f. The increase in f is also associated with the reduction in the diffusion coefficient (see eqn. 7). As noted earlier, however, the number of theoretical plates is independent of the friction coefficient because its role in influencing the velocity of a charged species is exactly offset by its role in inhibiting diffusion. Consequently, one would not expect a significant increase or decrease in the number of theoretical plates that could be realized in ideal electrophoresis because of the use of gel materials. However, a number of non-idealities might be substantially influenced by the presence of a gel. For example, it is possible that thinner initial zones could be introduced into a capillary filled with a gel as opposed to an empty capillary. Also non-uniformities in electroosmotic flow will distort and broaden bands in open capillaries, whereas such non-uniformities will be largely eliminated when using gels. These differences require further study.

ELECTRICAL FIELD-FLOW FRACTIONATION

Electrical FFF is the electrically driven member of the FFF family of techniques[12]. The mechanisms of electrical FFF and electrophoresis differ markedly,

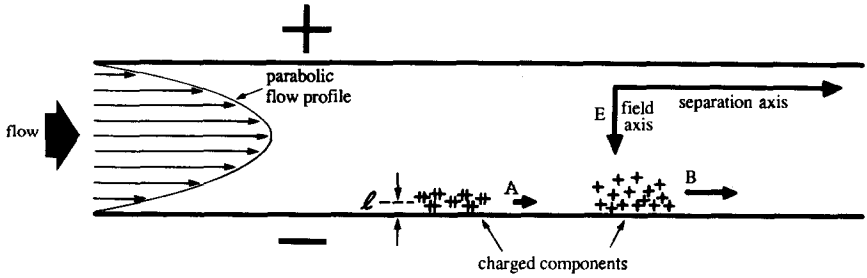

Fig. 3. Separation of charged species (A and B) by electrical FFF.

first because the direction of the electrical forces and the axis of separation are perpendicular in electrical FFF and parallel in electrophoresis, second because the electrically induced displacement in FFF is limited to the few hundred micrometer thickness of the FFF channel, and third because charged species in FFF rapidly reach a steady state distribution along the electrical field axis.

The mechanism of separation in electrical FFF is illustrated in Fig. 3. Charged species are compressed against one wall (the accumulation wall) of a thin channel by an electrical field applied between channel walls. Specifically, the electrical field drives charged components into thin exponential distributions near the accumulation wall. The most highly charged species (species A in the example of Fig. 3) is driven closest to the wall as measured by the lower mean elevation l of component A than component B molecules above the wall. When flow is initiated in the channel, the two components are displaced downstream by the ensuing fluid motion. However, the velocity of the parabolic flow profile in the channel approaches zero at the channel walls. Therefore the closer a molecule is to the accumulation wall, the slower is its migration velocity down the channel. A component such as A, driven closer to the wall than its counterpart B, will accordingly move downstream more slowly than B, leading to the separation of A and B.

Since the downstream migration velocity is controlled by mean elevation l, it is important to examine the factors controlling l. It has been shown generally for FFF that l is given by[13]

$$l = \frac{RT}{F} \tag{18}$$

In the case of an electrical field, force F can be expressed by eqn. 11, giving

$$l = \frac{RT}{z\mathcal{F}E} \tag{19}$$

The only parameter in this expression differing from one component to another is effective charge z. Thus separation will occur according to differences in the magnitude of z. In electrophoresis, by contrast, separation occurs according to differences in the

magnitude of μ or equivalently z/f (see eqns. 6 and 11). Thus electrical FFF and electrophoresis are complementary to one another by virtue of the different factors controlling separation.

There are, of course, other substantial differences between electrical FFF and electrophoresis. Electrical FFF is by nature a flexible elution technique whereas electrophoretic elution, if utilized, is rather inflexibly governed by electroosmosis. However, electrical FFF has not yielded high efficiency in the few cases it has been attempted; resolution levels are below those found for other FFF methods and particularly below the levels achieved in CZE and related electrophoretic techniques. Theory, in contrast to these observations, suggests that electrical FFF is capable of performance approaching that of CZE. We will briefly examine the relevant theoretical equations both to illustrate the comparison with electrophoresis and to gain insight on how the discrepancy between theory and practice in electrical FFF might be removed.

According to theory developed in an earlier paper[14], the minimum plate height achievable in an FFF system is equal to 5.66 l or, in view of eqn. 19

$$H_{\min}(\text{FFF}) = \frac{5.66\ RT}{z\mathcal{F}E} \tag{20}$$

Remarkably, despite the different mechanisms, this expression is identical in form to that for ideal CZE (eqn. 12). Specifically, the minimum H for electrical FFF is only 2.8 times larger than the theoretical minimum H achievable by electrophoresis at the same values of E, z and T as expressed by eqn. 12. Thus with E, z and T fixed

$$H_{\min}(\text{FFF}) = 2.8\ H_{\min}(\text{CZE}) \tag{21}$$

We observe that if the electrical field strengh in electrical FFF, instead of being held equal to that in electrophoresis, is raised to a level 2.8 times higher than that utilized in electrophoresis, the two limiting H values become equal. It is possible, with further technological developments in electrical FFF, that the increased E value may prove feasible because of the shorter distance across which the potential is applied, equal only to the channel thickness w in FFF. Thus for electrophoresis, an extreme of 100 000 V applied over a 1-m length of capillary corresponds to a field strength of only 0.1 V/μm. This field strength applied over the thickness w of an FFF channel, typically 254 μm, corresponds to a total potential of only 25 V. For more efficient 50- or 100-μm channels, V would be 5 or 10 V, respectively. (In the past, the voltage has been applied between electrodes outside of the FFF channel, in which case the voltage applied to the system has well exceeded the channel voltage. The 5–25 V discussed above correspond only to the voltage applied across the channel.) It is likely that designs will ultimately evolve in which it is possible to apply potentials much higher than 5–25 V across FFF channels.

Let us consider a highly efficient ideal electrophoretic system that generates 10^6 plates/m for a given component, equivalent to 1 plate/μm. Since the plate height is 1 μm, the value of l found for that component at an identical electric field strength E in an electrical FFF system will be 0.5 μm, as shown by comparing eqn. 12 and eqn. 19. If w = 50 μm, the retention ratio would be 0.06, a small but reasonable value. According

to eqn. 21, such a system would generate 36% of the number of theoretical plates per unit length of the electrophoresis analog, or 360 000 plates for a 1-m FFF channel. This number would increase if a higher electric field strength (still only a few volts) or a longer channel were used.

The above calculations show that the theoretical limits of resolution are comparable in electrical FFF and electrophoresis operated at similar field strengths. However, the remarkable potential of electrical FFF has never been realized. Below we examine some of the reasons that may contribute to this discrepancy.

First of all, we note that the equations for electrophoresis (eqn. 12) and for electrical FFF (eqn. 20) are minimum plate height values, accounting only for band broadening for molecular diffusion in the case of electrophoresis and by molecular diffusion and non-equilibrium in the case of electrical FFF. Both methods will be subject to additional band broadening mechanisms. For both, resolution will be lost because of the finite width of the injected sample. Electrophoresis will suffer a further degradation of resolution if temperature gradients develop across the capillary and non-equilibrium effects are thus encountered. Electrical FFF, by contrast, should not be noticeably affected by temperature gradients. Both methods, but especially electrophoresis, will be diminished in effectiveness by non-equilibrium effects attendant to the adsorption of components at the walls.

Electrical FFF clearly has some unique problems in reaching its high theoretical resolution. First of all, the sample material, compressed into a layer approximately 1 μm thick, will become highly diluted as it emerges from the channel, thus pushing the limits of detectability. Second, any surface roughness with a relief comparable to the magnitude of l will disturb the normal migration pattern and cause band broadening. Third, specific interactions with the accumulation wall of the electrical FFF channel will perturb both migration and band broadening. (The latter point might be highly relevant because the membrane walls so far utilized in electrical FFF have shown a tendency, based on evidence from flow FFF, to interact abnormally with macromolecules). Finally, we observe that membrane-based channels are difficult to fabricate into uniform flow conduits as needed for FFF.

The above problems are all compounded by the paucity of scientific studies devoted to electrical FFF. This neglect is surprising in view of the theoretical results (summarized above) that show that electrical FFF has the inherent capability to become a versatile high-resolution electrically driven technique serving a role complementary to that of electrophoresis.

While the FFF equations shown above are applicable to the normal mode of operation of electrical FFF with typical macromolecules as solutes, the high resolution and speed of another FFF technique, flow FFF, in separating cell-sized particles[15] suggests that electrical FFF might also be highly effective for larger biological and non-biological particles.

ELECTRICAL SPLITT SEPARATION

Separation in split-flow thin (SPLITT) cells represents another potential option in harnessing electrical forces[16]. This approach, which we shall call E-SPLITT separation, is particularly interesting because of its capability for continuous (thus preparative) separation, and because of the high speed of the separation.

The E-SPLITT separation cell resembles an FFF channel with flow splitters inserted at one or both ends. Despite continuous flow in the system, the mechanism of separation is fundamentally more like that of electrophoresis than FFF. Specifically, in the transport mode of E-SPLITT operation, a continuous sample stream entering on one side of the splitter is compressed (to a controllable level) by a larger stream of carrier entering at the other side of the splitter (see Fig. 4). If the flow-rate of the carrier stream is adjusted to a sufficiently large value, the sample stream can be compressed to a thickness of only 10 or 20 μm. From this ultrathin sample lamina the charged components are driven differentially across the channel by the applied electrical field. Components with high mobility (component A in Fig. 4) are driven far enough in the short transit time through the channel that they emerge beneath the outlet splitter. Less mobile species (component B) emerge above the outlet splitter in a separate stream. Adjustments in both the electrical field strength and in the outlet stream splitting ratio can fine tune the cutting point (the outlet splitting plane) between the collected species. Each of the fractions collected can be subjected to further processing in subsequent SPLITT cells. In theory it should be possible to isolate narrow cuts after passage through several such cells operated under different conditions.

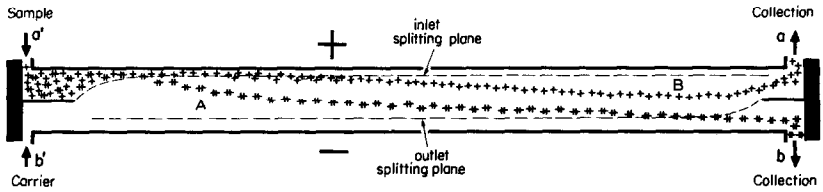

Fig. 4. Diagram of electrical SPLITT cell performing continuous separation in transport mode.

The E-SPLITT cell can also be operated in the equilibrium mode. Here the pH is chosen such that some species go to the anodic wall and others to the cathodic wall. The pH can be adjusted in subsequent cells to isolate a narrow range of PI values.

Separation in SPLITT cells is generally rapid because of the short transport path (a few hundred μm) needed to achieve separation. Even if several linked SPLITT cells are used, the total processing time need not exceed a few minutes.

In the transport mode illustrated in Fig. 4, separation occurs by virtue of differential displacement across channel thickness w. The sharpness of the separation, as in any electrophoretic displacement, is determined by the maximum number of theoretical plates achievable during transport and by any other band broadening factors including the thickness of the initial sample lamina. As indicated earlier, the incoming sample thickness can be arbitrarily adjusted, but there is a tradeoff between the thinness of the sample lamina and the total sample throughput. Band broadening sources other than initial sample thickness and diffusion are expected to be negligible if the channel is properly constructed.

The diffusion limit to resolution is defined by eqn. 13, which is the expression used to describe the efficiency of ideal CZE. If 25 V are applied across such a SPLITT cell and z ranges from 1 to 5, then between 500 and 2500 theoretical plates could be realized for the system. This obviously does not yield the high level of resolution

available in CZE, but it has the potential to be highly effective as a preparative method. We have carried out preliminary experiments in the equilibrium mode of operation showing[17] that proteins with different pI values can be separated with a channel potential of only 1 V. Many improvements are still needed for routine and effective operation.

While E-SPLITT is a much newer technology for continuous electrical separation than deflection or free-flow electrophoresis[19,20], it should not only provide higher separation speed but, because of the nature of the profile of flow in thin flat cells, a higher resolution as well[21].

ISOELECTRIC FOCUSING

Zone formation and resolution in IEF is governed by a mechanism highly dissimilar to that of CZE. In IEF, stationary component bands form at different positions according to the isoelectric points of the components[3,4]. The final distribution of bands is independent of how or where the components begin their migration. The concept of a narrow initial zone evolving continuously into resolved bands as in CZE is inapplicable. The operational definition of plate height as $H = \mathrm{d}\sigma^2/\mathrm{d}X$ is meaningless because variance σ^2 is determined by quasi-equilibrium conditions and not by initial zone width or time-dependent diffusion as expressed by eqn. 3. While plate height parameters are accordingly inapplicable to IEF, the peak capacity, n_c, a broader index of separation power applied to CZE, IEF, FFF, and related methods, is a valid measure of performance.

The peak capacity is simply a count of the number of individual components that could theoretically be resolved in the course of separation[2]. (Because of statistical peak overlap, the actual number of resolved components is almost invariably less than n_c by a substantial margin[18].) For electrophoresis the maximum peak capacity can be estimated as[2]

$$n_c \approx \left(\frac{-\Delta\mu^{ext}}{32RT}\right)^{\frac{1}{2}} = 0.25N^{\frac{1}{2}} \tag{22}$$

This equation shows that for N values lying in the vicinity of 10^5 or 10^6, the peak capacity can reach levels as high as several hundred.

When one accounts for the mechanism of stationary band formation in isoelectric focusing, the peak capacity is found to be approximated by[2]

$$n_c \approx \left(\frac{-\Delta\mu^{ext}_{max}}{8RT}\right) \tag{23}$$

an expression almost identical to eqn. 22. (Here $-\Delta\mu^{ext}_{max}$ is the chemical potential drop for a species driven to an equilibrium position at the opposite end of the separation path.) Thus, despite the drastically different mechanism of the two separation methods, the peak capacities depend on the same factors and are of the same order of magnitude. This is borne out by abundant experimental work, which shows that both IEF and CZE are capable of resolving very large numbers of components.

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