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TRANSPORT, SPACE, ENTROPY, DIFFUSION AND FLOW

ELEMENTS UNDERLYING SEPARATION BY ELECTROPHORESIS, CHROMATOGRAPHY, FIELD-FLOW FRACTIONATION AND RELATED METHODS

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SUMMARY

We describe here some elements common among separation methods, including the requirement for separative transport, the sensitive utilization of space, and the dissipative (band broadening) roles of entropy, diffusion, and flow. We focus on the need to carefully manage the unceasing competition between separative and dissipative transport in all high resolution methods.

We then examine the more specific roles these elements play in influencing separation in several important analytical separation techniques. We begin with electrophoresis and sedimentation, which are conceptually among the simplest of systems because they require no flow and only a single dimension of space. The conflict between separative and dissipative transport is examined closely for these systems and it is shown that the outcome is a theoretical plate number which can be expressed as a ratio of two energies: a structuring energy $-\Delta\mu^{ext}$, which organizes the separation, and thermal energy RT, which is responsible for its dissipation. It is explained why optimal separation is most often achieved in thin layers or in capillary tubes.

Chromatography and field-flow fractionation are then described as two closely related methods in which flow is powerfully coupled with a simple enrichment process occurring at right angles to flow. Unlike electrophoresis, these systems are intrinsically two-dimensional. It is shown how flow and diffusion processes both assume two diametrically opposite roles: those of aiding separation and simultaneously causing its dissipation. With a few equations it is demonstrated that the dissipative role is best contained by reducing the thickness (diameter) of the system or of certain elements within the system.

INTRODUCTION

With the proliferation and specialization of chromatographic techniques, it is more and more difficult to stay abreast of current advances, and it is increasingly easy to overlook the roots and competing branches of the general separation process, which may offer fresh approaches and solutions to expanding needs. It is the object

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of this paper to visit the roots and follow a few branches of the separations tree to show what marvelous and varied fruits lie around and how they arise. This broad focus is an outgrowth of other studies by the author on the unification of separation science[1–4]; a book on the subject will soon appear[5].

Separation is a process in which components of a mixture are physically removed from one another, each conducted to a place where it can occupy (if resolution is complete) its own region of space. The process can be represented by[1]

$$[a + b + c + \ldots] \rightarrow [a] + [b] + [c] + \ldots \quad (1)$$

where the brackets represent the different regions of space into which the components a, b, c, etc., fall.

The scheme above leads to some general conclusions about separations, valid for any technique. Foremost is the fact that separation requires the transport of components through space to remove them from another's immediate vicinity. Thus transport processes lie at the very heart of separation science.

Separation systems make very intensive use of physical space. The transport and resulting reorganization of components in space leads to a rich pattern of component distribution; this is reflected in the chromatogram or fractogram of the separation. Also driving forces and flow must be thoughtfully oriented in space, and system boundaries must be carefully established to optimally utilize space. Dealing with spatial variables thus assumes great importance.

For most analytical separation systems a principal separation axis can be readily identified. This is the axis along which the separative gradients are generated. This will be the axis extending along a chromatographic column (even if the column is coiled) or along the electrical field lines in electrophoresis. Associated with the principal axis are auxiliary axes of the separation system. The extension of the separation system along these axes defines normally the breadth, width, or diameter of the system. These auxiliary axes, like the principal axis, can have a substantial influence on many aspects of separations. For example, we find quite generally that analytical separation systems tend to be "thin" systems with limited lateral dimensions. The reasons for this will be examined here.

Separative transport, by whatever selective mechanism, generally leads to the formation of concentration pulses that are differently located for different components. These concentration pulses are essentially always out of equilibrium; the narrower the pulse or band, the steeper the concentration gradients and the greater the tendency of those gradients to dissipate spontaneously. This dissipation is thermodynamically driven; it relates to the tendency of entropy to break down all gradients, to maximize dilution, and in the process to thoroughly mix all components[6,7].

Entropy must be considered the single greatest enemy of separations because of its universal inclination to dilute and remix components that have been so carefully isolated in space. Through a variety of mechanisms, entropy relentlessly drives processes that tend to undo separation. Most frequently, entropy exerts itself through the diffusion process. Diffusion drives molecules down concentration gradients and is clearly responsible for band broadening and component intermixing.

Dissipation is also caused by various flow processes, which we can classify as parasitic forms of flow[1]. One parasitic flow is gravitational convection, where com-

ponents are physically entrained and moved into regions assigned to other components. This also promotes mixing; it is another mechanism by which entropy acts.

In all high resolution separation systems, separative transport and dissipative transport act in relentless competition. I have referred to separation as the art and science of maximizing the ratio of separative transport to dissipative transport[5]. Both of these transport processes must be controlled and manipulated for successful separation. If, in the end, we fail to generate powerful separative transport relative to dissipative transport, we eliminate any possibility that high resolution can be achieved.

EXAMPLES FROM CLASS Sc: ELECTROPHORESIS AND SEDIMENTATION

The above generalities become more concrete if we look at specific classes of separations. We begin with the simplest category, the Sc class, which includes electrophoresis and sedimentation. The "S" means that the system is static, that is, it requires no flow. The "c" expresses the fact that continuous forces (such as electrical) are driving components through the system. These driving forces will act selectively on different kinds of molecules so that some are driven further than others along the principal separation axis[1,5].

The Sc separation is intrinsically a one-dimensional separation. Ideally, there are no gradients in any transverse direction; the system is homogeneous with respect to the auxiliary axes. The separation therefore can be regarded as unfolding solely along the principal separation axis.

Fig. 1 shows such an ideal Sc system. The separative transport due to the application of a driving force (electrical or sedimentation) leads to the isolation of zones while the dissipative process is simultaneously broadening the zones.

The competition between separative and dissipative transport can be more clearly focused if we examine a single zone whose profile is illustrated in Fig. 2. Ideally, the profile is a Gaussian with standard deviation σ, variance σ^2, and effective "width" $w = 4\sigma$. These band width parameters reflect the strength of dissipative transport. If diffusion alone is responsible for such transport, we can relate band width to the diffusion coefficient by the equation

$$\sigma^2 = 2Dt \tag{1}$$

For a uniform system the elapsed time t can be replaced by the distance migrated X divided by the migration velocity U, giving

$$\sigma^2 = 2D\frac{X}{U} \tag{2}$$

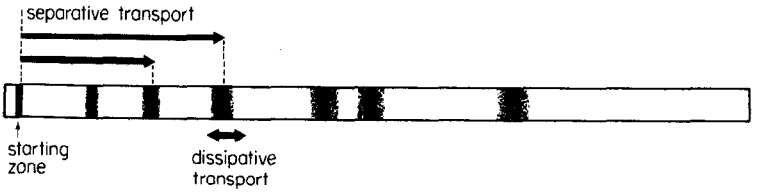

Fig. 1. Illustration of separative and dissipative transport in a simple Sc system such as electrophoresis.

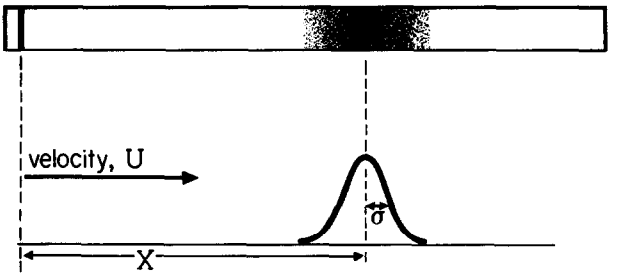

Fig. 2. Gaussian zone formed by dissipative transport.

Here we detour to note that most of the indices used to describe the separation effectiveness of chromatography can also be applied to Sc methods like electrophoresis and sedimentation[8]. These indices include plate height H, number of theoretical plates N, and peak capacity n_c. Since Sc processes can be truly continuous down to the molecular scale, nothing resembling real plates or stages exists along the separation coordinate. However, real plates do not exist in chromatography either; the plate model confuses the true mechanism of such systems[9]. Nonetheless, the plate height, when treated solely as an index to describe separation effectiveness, is useful and, more importantly, universally accepted in chromatography. It is no less useful when applied to techniques like electrophoresis and field-flow fractionation.

For systems that are uniform throughout, the plate height can be defined by[9]

$$H = \frac{\sigma^2}{X} \tag{3}$$

which, with the substitution of eqn. 2, becomes

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

The number of theoretical plates is therefore

$$N = \frac{X}{H} = \frac{X^2}{\sigma^2} = \frac{XU}{2D} \tag{5}$$

The peak capacity n_c is perhaps the most easily visualized separation index[10]. It has some advantages over plate height and number as a universal measure of separation power; for example n_c but not N is applicable to isoelectric focusing techniques[11], as will be explained at the end of this section. The simplicity of the peak capacity is that it is an elementary count of the number of peaks or bands that can be isolated along the principal separation coordinate. The number of bands that can be isolated between the origin and position X is simply

$$n_c = \frac{X}{w} = \frac{X}{4\sigma} = \frac{N^{1/2}}{4} \tag{6}$$

providing all peaks are of equal width w. The last equality in this expression shows that the peak capacity, which is a very "intuitive" index for separation power, is related to the number of theoretical plates, which is less intuitive but more commonly used. Both indices depend on the ratio X/σ, which, as a ratio of separative to dissipative displacement, reflects again the universal contest between separative and dissipative transport.

We now probe more deeply into the underlying driving forces of Sc separation. The movement along the principal separation axis is normally driven by a gradient in chemical potential. For electrophoresis in a homogeneous medium, the chemical potential gradient is simply the gradient in electrical potential energy, proportional to electrical field strength and a component's electrical charge. If we write this chemical potential as μ^{ext}, the driving force per mole becomes

$$\text{driving force} = -\frac{d\mu^{ext}}{dx} \tag{7}$$

The migration velocity U, needed in eqns. 4 and 5, is simply the driving force divided by the friction coefficient f[1]

$$U = -\frac{1}{f}\frac{d\mu^{ext}}{dx} \tag{8}$$

Parameter f, by defining the frictional drag force on a molecule migrating at unit velocity, is fundamental to the description of most transport phenomena[1,12].

By virtue of eqn. 8, the numerator of eqn. 5 can be written as

$$UX = -\frac{1}{f}\frac{d\mu^{ext}}{dx}X = -\frac{\Delta\mu^{ext}}{f} \tag{9}$$

where $\Delta\mu^{ext}$ is the chemical potential drop experienced by a species in migrating distance X.

The denominator of eqn. 5 can also be related to friction coefficient f by means of the Einstein equation[1,13]

$$D = \frac{RT}{f} \tag{10}$$

where RT is the thermal energy.

The substitution of eqns. 9 and 10 back into eqn. 5 yields

$$N = -\frac{\Delta\mu^{ext}}{2RT} \tag{11}$$

which was first obtained as a general expression for Sc-type separations in 1969[8].

Eqn. 11 casts a new light on the contest between separative transport and dissipative transport. We see that the N of eqn. 11 is simply the ratio of two energies. The energy $-\Delta\mu^{ext}$ is the one that drives the separation and leads to the spatial structuring of components. The term RT is thermal energy, which is a thinly disguised form of the entropy effect, acting to undo the separation. The role of RT is most

evident in eqn. 10, which shows that D is directly proportional to RT. A more complete thermodynamic formulation shows that RT in this context is strictly an entropy-related term[1]. Physically, RT can be thought of as representing the strength of Brownian motion, a form of thermal energy that leads to the dissipation of concentration gradients.

Since N is a ratio of structuring energy to dissipative (thermal) energy, it is clear that effective separation can be gained only by having structuring energies that are very large compared with thermal energy. Thus an Sc separation cannot really work unless large forces and large energies are expended on its behalf. Electrophoresis is ideal in this respect because electrical forces are so powerful[1]. However, the separative species must be charged.

If desired, the number of plates for Sc systems can be formulated in terms of mobilities rather than chemical potential increments. Thus for electrophoretic systems, the velocity U in eqn. 5 can be expressed as $E\mu_E$ where μ_E is the electrophoretic mobility, the velocity induced at unit field strength. With this, eqn. 5 becomes $N = XE\mu_E/2D$. Since XE is the voltage V, we have

$$N = \frac{V\mu_E}{2D} \tag{12}$$

This equation is simple to apply if the two transport coefficients μ_E and D are both known, but otherwise eqn. 11 is simpler to use because $\Delta\mu^{ext}$ is calculated from simple electrostatics[1].

We observe the absence of friction coefficient f from eqn. 11. We note that f cancelled out in the development of eqn. 11 because it had an equal (*i.e.*, inversely proportional) relationship to the two transport terms U, eqn. 8, and D, eqn. 10. In forming the ratio U/D via eqn. 5 to get N, f disappears, but it would be erroneous to think that either transport process occurs without the modulating effect of f, which is proportional to viscosity (Stokes law). This is best visualized by imagining a reference system of fixed f and another system identical except for the fact that f (viscosity) is 100 times larger. With a given chemical potential gradient, the displacement velocity U will be 100 times smaller in the second system, as shown by eqn. 8. However, D will also be 100 times smaller, as shown by eqn. 10. In this molasses-like environment, everything would be equally slowed down – separative transport as well as dissipative transport – with the net result that separation efficiency would not be changed. Clearly, however, separation speed would be sacrificed by a factor of 100. In general, separation time is proportional to friction coefficient f[1]. The value of f consequently has immense practical significance.

We will not discuss at any length isoelectric focusing and isopycnic sedimentation, which are variants of electrophoresis and sedimentation in which steady-state zones are formed by virtue of pH and density gradients, respectively. The plate parameters H and N are inappropriate measures of separation efficiency for these steady-state methods because of the irrelevance of the point of origin measured by X in eqns. 3 and 5. However, peak capacity n_c continues to characterize separation power. (The distance X in eqn. 6 becomes the distance over which the steady-state peaks are distributed, not the distance from the point of "injection.") It has been shown that peak capacities for these steady-state techniques are the same order of

magnitude as those found for normal electrophoresis and sedimentation[11]. This explains the widespread application of these methods.

Sc SEPARATION: FROM ONE TO TWO DIMENSIONS

Sc techniques such as electrophoresis are unusual in that they can be conceptualized as one-dimensional systems. The ideal separation process described above unfolds along one coordinate (the principal separation axis) with no involvement from other coordinates. The one dimensionality does not mean that the other coordinates do not exist; it simply means that there are no gradients found along these auxiliary axes. Thus the secondary coordinates can be ignored without a loss of understanding in describing the ideal Sc process.

While one-dimensional operation is approached by some electrophoresis and sedimentation systems, there are forces at work tending to disrupt this ideal situation. We will describe some of these non-ideal influences in electrophoresis. The first problem is that an electrophoresis system is generating heat because of the dissipation of electrical energy in the system[14–17]. The electrophoretic medium will consequently heat up, but heat losses from the sides will create transverse temperature gradients. The higher temperature found along the centerline of the separation path will be accompanied by a reduced viscosity and this will lead to increased mobility. The charged species will then travel faster in this region than at the edges, leading to a distorted zone. No longer is the system free of gradients along the transverse coordinates.

Other processes can cause non-idealities as well[14–17]. The temperature gradient can induce convective currents, leading to further zone distortion. Also, electroosmotic flow can lead to non-uniform electrophoretic migration in the system.

All of these mechanisms serve to aid the arch enemy of separation: entropy. It does so by allowing the zones to occupy bigger segments of the separation path, becoming more dilute, and therefore overlapping with other zones.

The non-ideal effects described above reduce the plate count N of the system below the value described in eqn. 11. We now must write[1]

$$N = -\frac{\Delta\mu^{ext}}{2\theta RT} \tag{13}$$

where θ is a non-ideal term equal to or greater than unity, which accounts for the loss of separation efficiency.

It is of great practical importance to maintain θ at a level as near unity as possible. This is best achieved (*i.e.*, ideality is most closely approached) by reducing the transverse dimensions of the system. This means that we should utilize thin systems, such as thin strips or narrow-bore capillary tubes. In such systems rapid thermal conduction evens out the temperature profile and reduces zone distortion. The zone distortion that is generated is counteracted by rapid mass diffusion across the thin dimension. In addition, convective effects are suppressed by the high surface area-to-volume ratio of thin conduits[5]. As Jorgenson has found in working with capillary tubes as thin as 25–75 μm[18], it is possible to approach the diffusion-limited ideal described by eqn. 11, which means that θ is approximately unity. We thus chain

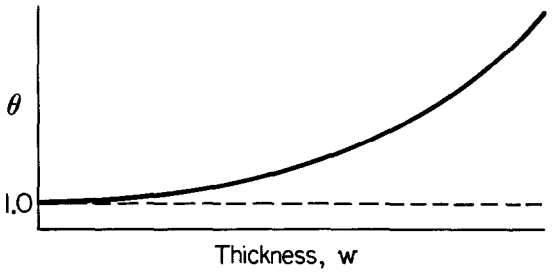

Fig. 3. General trend in which non-ideal term θ increases with thickness w of Sc (*e.g.*, electrophoretic) separation systems.

entropy to its minimal influence, limited to that expressed by ordinary diffusion.

Because of the non-ideal effects, θ increases with the thickness of the system, at first slowly and then dramatically. This is illustrated schematically in Fig. 3. As a result of the strong influence of the lateral dimensions of electrophoretic systems on separation efficiency, it has been difficult to scale up electrophoresis to such a level that useful preparative work can be done.

CLASS F(+) SEPARATIONS: CHROMATOGRAPHY AND FIELD-FLOW FRACTIONATION

Chromatography and field-flow fractionation (FFF) are more closely related to one another than either is to electrophoresis[1,5]. Both techniques fall in a category of separation methods designated by F(+). These symbols signify two essential elements of the separation: flow (F) along the principal separation axis and a selective enrichment process exerting its influence in a direction perpendicular (+) to the flow axis[1]. The two methods differ in detail because the enriching influence utilizes continuous (c) field-derived forces in FFF, whereas discontinuous (d) phase-derived forces are used in chromatography.

Chromatography and FFF are inherently two-dimensional systems. This is so because the essential enrichment process occurs at right angles to the principal separation axis (*e.g.*, across rather than along the interface between stationary and mobile phases). The enrichment process is absolutely required and must be taken into account to even conceptualize the separation. Therefore even under ideal circumstances these F(+) techniques cannot be considered to be one-dimensional systems. (However, the second dimension may be only a few micrometers across, allowing the systems to function like they are one-dimensional.)

By now we might surmise that the flow process has multiple roles in separation systems. We have seen that flow can be parasitic and dissipate a separation, but in the F(+) techniques flow creates a new coordinate (the principal separation axis) along which separation can be expressed[1]. Flow coupled with the enrichment process leads to differential transport along this axis. In roles such as these (and there are several in separations), flow is essential. Without flow, chromatography would reduce at best to a weak two-phase extraction system in which a maximum of two components could be separated.

While flow is essential in a number of separation processes such as chromatography, flow is not selective. Flow cannot separate on its own; it must be combined

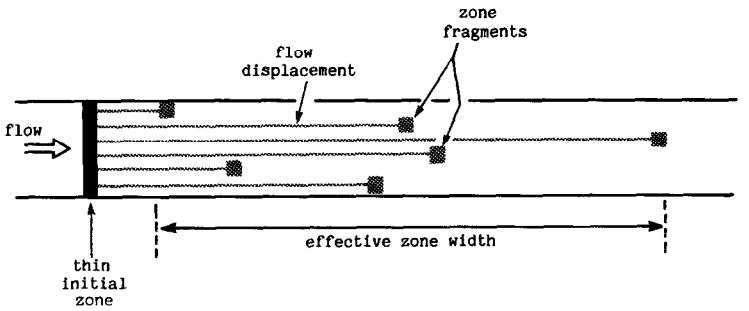

Fig. 4. Illustration of the disruption and dispersion of a thin zone by arbitrary non-uniform flow.

with a selective influence such as phase distribution forces in chromatography or selective interactions with a field in FFF. Once this combination with selective forces is made, flow becomes a powerful agent for generating separation.

While the flow phenomenon has its essential side, it has also, as suggested earlier, its dissipative side. The latter is very difficult to avoid. In chromatography and FFF, flow aids entropy by breaking up narrow component bands and dispersing them along the flow coordinate. This is shown schematically in Fig. 4.

The basic problem is that, with few exceptions, flow in confined spaces is non-uniform. This non-uniformity is generated by viscous drag at walls, at particle surfaces, and at all other solid boundaries. Thus, as we move across the transverse coordinate of a separation system, the local flow velocity invariably undergoes substantial change. The different flow velocities in different transverse regions serve to carry various components along at unequal velocities, thus breaking up the bands. The fragmentation of a thin band by this mechanism is illustrated in Fig. 4. The small fragments are further dissipated by diffusion, leading to a large overall entropy gain.

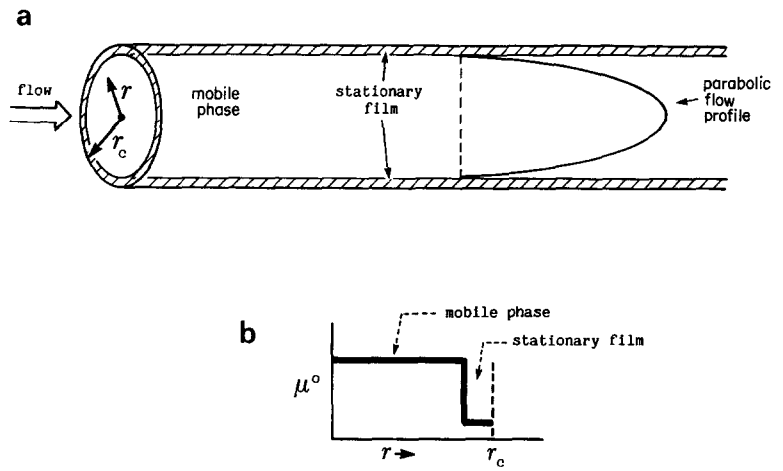

Fig. 5. Capillary chromatography column showing (a) radial coordinate system and parabolic flow profile and (b) profile (with discontinuity) in chemical potential μ° across tube radius.

CAPILLARY CHROMATOGRAPHY

The above principles can be illustrated by considering the simplest chromatographic system, a capillary column (see Fig. 5a). The concepts are the same whether the mobile phase is a gas, a liquid, or a supercritical fluid. If we follow a line across the radius of a capillary tube and into the stationary phase coating its wall, we encounter a sudden (discontinuous) change in the phase-based chemical potential μ° at the interface, as illustrated in Fig. 5b. The step height at the discontinuity is different for different components, thus giving selectivity in the concentration distribution along this secondary axis. The system is obviously two-dimensional because no separation could be imagined if the enrichment axis was unavailable to couple with the flow axis.

The flow profile in a capillary tube is parabolic or bullet-shaped. If transport occurred mainly by flow, the parabolic flow profile would contribute greatly to band broadening by converting a thin initial zone into a broad parabolic zone (see Fig. 6). Separation would be impossible under such circumstances.

Separation in capillary tubes becomes possible only because substantial diffusional transport is present to complement flow transport. Diffusion is almost universally thought to aid entropy (*i.e.*, faster diffusion yields greater entropy) but here we find a clear exception. The mechanism is one in which diffusion degrades the immediate transverse gradient (*i.e.*, reduces transverse non-equilibrium) to cause an increase in local entropy but in so doing the overall entropy generated by non-uniform flow (coupled with some diffusion) is reduced.

The mechanism of band containment can be explained in molecular terms. The beneficial diffusion acts, as noted, along the radial coordinate. Without this diffusion, molecules are trapped in little volume elements of fluid and are carried at different velocities to different positions by the parabolic flow. If molecular diffusion is rapid, molecules can diffuse radially into other streamlines and eventually assume something closer to an average velocity than to the more extreme velocity typical of a fixed streamline. By diffusing back and forth across the various streamlines of the parabola and into the stationary film, the molecule is effectively undergoing a kind of random walk in which it jumps back and forth between higher than average streamlines and slower than average streamlines (dyn). For very rapid diffusion, the random walk is one having many steps which reduces the fluctuations caused by the excessive occupancy of high or low velocity streamlines. Therefore high diffusion coefficients lead to thin sample zones with minimal overlap.

While diffusion here assumes the unlikely role of helping us contain band

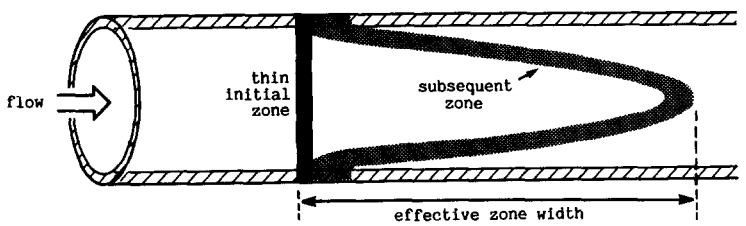

Fig. 6. Broadening of thin zone in capillary chromatography by non-uniform (parabolic) flow.

broadening, it must not be forgotten that longitudinal diffusion proceeding at right angles to lateral diffusion is acting simultaneously and in a more normal fashion as a source of band broadening. The hero/villain role of diffusion is reflected in the form of the plate height equation for the mobile phase[9]

$$H = \frac{2D_m}{\langle v \rangle} + \omega \frac{r_c^2 \langle v \rangle}{D_m} \tag{13}$$

in which $\langle v \rangle$ is the average flow velocity, r_c is the tube radius, and ω is a constant. The first term accounts for longitudinal diffusion while the second describes the non-equilibrium effects arising from non-uniform flow. The mobile phase diffusion coefficient D_m shows its two-faced nature by appearing in the numerator of the first term and the denominator of the second. A similar pair of terms arises for the stationary phase. Since the influence of velocity v on H is quite opposite in the two terms, the optimization of resolution requires an intermediate velocity, as is well known.

More importantly for our purposes, H in the crucial non-equilibrium (second) term increases with the square of r_c. This reflects the fact that for increased tube radius, the molecules have further to diffuse and correspondingly less success in overcoming the non-uniform flow effects. Clearly we are best served by thin tubes, a conclusion identical to that reached for electrophoresis, but for the somewhat different reasons we have outlined. The requirement for thinness is most stringent for liquid mobile phases because of their small diffusion coefficients. The diffusion process is so slow in liquids that tubes as thin as 10–20 μm are needed for truly effective operation. This dimensional requirement is so extreme that it has hindered the development of a capillary technology applicable to liquid chromatography[19–21].

For all perpendicular flow or F(+) systems, equations of the above form govern H. The underlying diffusion and flow phenomena are basically of the same kind as we have described above for capillary columns. For example, in a packed column there are some regions where the flow velocity is higher than in other regions because the particles are spaced randomly in the packing matrix and the channels between them are unequally sized and thus unequally susceptible to flow. Thus there is some critical diffusion distance between these channels in the packed bed that is scaled to particle diameter. The plate height equation for a packed column therefore has many of the same elements as for a capillary column, including terms of the form shown in eqn. 13. However, band broadening in packed columns is complicated by the irregular flow pattern, which gives rise to eddy diffusion and its rather complicated coupling with the non-equilibrium term, which is much like the second term on the right of eqn. 13. A full discussion of this phenomenon is beyond the scope of the present paper[5,9].

As indicated above, the non-equilibrium term (the second on the right of eqn. 13) is of the same form in packed and capillary columns, but the diffusion distance r_c for a capillary tube must be replaced by some multiple of the particle diameter which, as noted above, expresses the diffusion distance in packed columns. Consequently, analogous to the requirement for working with thin (small r_c) tubes in capillary chromatography is a parallel requirement to work with small diameter packing particles in packed columns. Thus while packed columns can be fairly large in di-

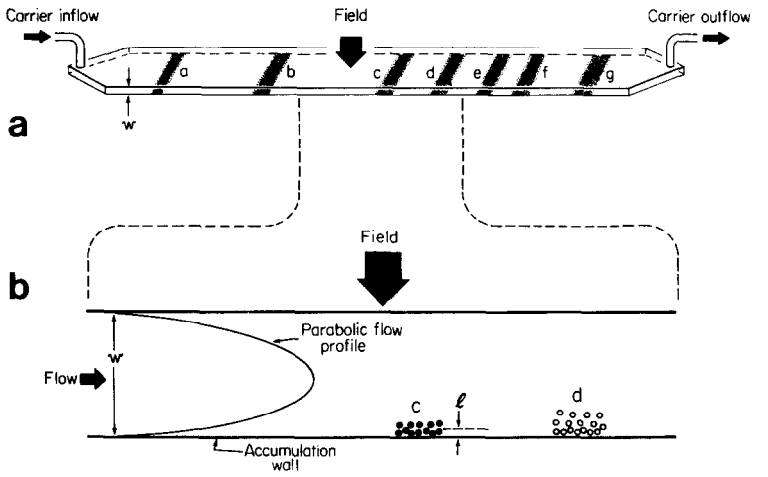

Fig. 7. The operation of an FFF system is illustrated by diagrams of (a) a ribbon-shaped FFF channel on which components a–g are being separated and (b) a magnified edge view in which the formation of thin component clouds near the accumulation wall is shown.

ameter, the basic units making up that diameter —the particles and the channels between them— must be thin, in conformity with the other separation techniques under discussion.

FIELD-FLOW FRACTIONATION

Field-flow fractionation (FFF) is, as noted, closely related to chromatography[1,22–24]. However, there is a fascinating difference in the way band broadening by differential flow is controlled. This is best explained by reference to diagrams of an FFF channel system as illustrated in Fig. 7.

The upper diagram in Fig. 7 (labelled a) shows a group of components, a–g, being separated on a thin ribbonlike FFF channel. This thin flat channel configuration is generally preferred for FFF operation. The channel is free of packing so it most resembles the capillary tubes used in chromatography. However, in the light of the discussion above of chromatography, this resemblance poses a basic dilemma whose solution is very uncharacteristic of chromatographic systems.

The roots of the dilemma are this: FFF is designed to separate macromolecules and colloids, which require a liquid carrier. Diffusion coefficients in liquids are low, as we have noted, but they are extraordinarily low for such macromolecular materials. Therefore we project, based on our discussion of capillary columns, that we would need a very thin channel, preferably under 10 μm, in order to allow this sluggish diffusion to offset non-uniform flow effects. However, channels of ribbonlike structure, which must be very uniform for FFF operation, are difficult to build in thicknesses of 100 μm, let alone 10 μm.

Our solution to this potentially difficult problem is unsuspectedly easy because it takes advantage of the fundamental mechanism of FFF. This mechanism is explained in the lower diagram (Fig. 7b), which shows a magnified edge view of the

FFF channel. This diagram shows that the molecules of the two components are pressed into a thin cloud against one channel wall, the accumulation wall. The effective mean thickness of the cloud for component c is shown as l in the figure.

A parabolic flow profile controls the flow distribution in an FFF channel, just as it does in capillary chromatography. However, in FFF channels the parabolic profile loses much of its effectiveness in tearing bands apart. This is because the component molecules are hugging the wall and are thus hidden from the effects of flow over all but a very thin liquid lamina extending upwards *ca.* $2l$ into the channel. While the non-uniform flow remaining within this lamina is destructive, its effects are ameliorated by the thinness of the component clouds and the ability of the molecules within them to undergo a rapid random walk by diffusion over the thin lamina. Consequently, band broadening is contained not by the thinness of the channel but by the thinness of the component clouds within the channel. Typically, distance l is 0.1–0.01 or less times that of channel thickness w. With a channel thickness of 100 μm, l is accordingly in the range 1–10 μm and the effective diffusion distance, *ca.* $2l$, is 2–20 μm. This is adequately thin to realize the rapid diffusion necessary to overcome the effects of non-uniform flow.

Not surprisingly, the plate height equation for FFF resembles that of its cousin, capillary chromatography. For FFF we have[25,26]

$$H = \frac{2D}{\langle v \rangle} + \omega \frac{l^2 \langle v \rangle}{D} \tag{14}$$

This equation is identical to eqn. 13 except that l replaces tube radius r_c as the approximate scale length over which lateral diffusion must occur. We have, of course, replaced mobile phase diffusion coefficient D_m by the generalized symbol D since there is no stationary phase diffusivity from which D must be distinguished. We note also that the dimensionless parameter ω will have a somewhat different dependence on retention, giving different values in the two expressions. The details need not concern us here.

Since the D values for the macromolecular materials studied in FFF are extremely small, the first term of eqn. 14 tends to be negligible, leaving the second (non-equilibrium) term on the right as the most significant term. This term is highly sensitive to the cloud thickness parameter l, as suggested in the above discussion. The requirement to make this parameter small gives FFF its characteristic thinness, a characteristic which, as we have seen, it shares with most other analytical separation techniques. However, in the case of FFF, the thickness parameter l is not a fixed property of the separation system; its value is modulated up and down in response to variations in the field strength. More details on this and other characteristics of FFF can be found in the cited literature.

CONCLUSIONS

We have attempted to show how certain basic elements underlie all separation processes. By describing the way in which these elements assume their various roles in some major separation systems, it is hoped that separation concepts will be simplified, relationships between methods will be clarified, and a wider appreciation of

the entire discipline of separations will be gained. The practical spinoff should be a better understanding of the capabilities and limitations of all methods and increased skills in choosing those techniques which offer the best solutions to specific problems.

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