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The Flow Field-Flow Fractionation Channel as a Versatile Pressure Dialysis and Ultrafiltration Cell

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Abstract

A versatile solute purification cell based on the flow field-flow fractionation channel is described. Equations are obtained for impurity removal, mean solute residence time, and solute throughput. Experiments are described in which methylene blue was purged from a buffer stream and from a stream containing 10% albumin by weight using a 2.65-ml cell. Impurity removals up to about 98 to 99% were observed. Mean albumin residence time in the cell varied from about 10 to 60 min; 90% impurity removal was possible with a residence time of about 25 min.

INTRODUCTION

A continuous solution exchange cell can be readily fashioned from a flow field-flow fractionation (flow FFF) device, or can be constructed along similar lines using modified channel dimensions. The device promises exceptional versatility; by means of flow adjustments it can be operated as a pressure dialysis (diafiltration) cell, a dialysis cell, or as an ultrafiltration unit. It has good throughput, low volume, short solute residence times, and can be scaled to almost any size and capacity.

The cell itself consists of a flow FFF channel (*1–3*). This is a thin

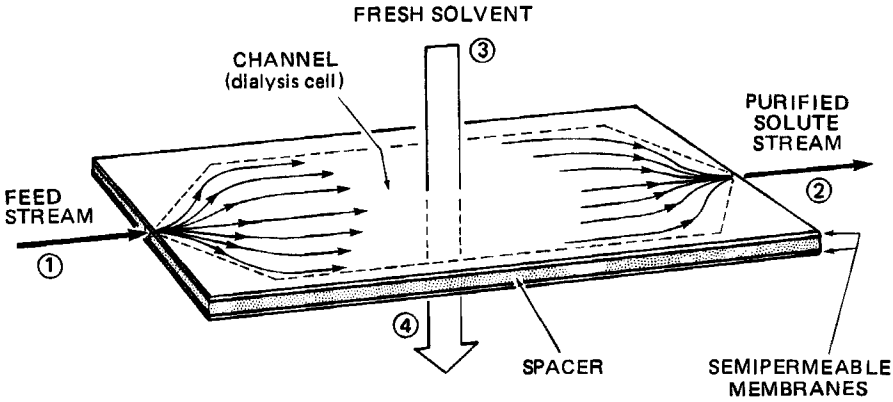

FIG. 1. Flow FFF channel used as a continuous dialysis cell. The relative and absolute flows of the four flow streams are controlled at Points 1, 2, and 3.

sandwich of two rigid (supported) semipermeable membranes with a narrow flow space between. The contaminated solute stream is fed into the flow space at an end of the sandwich and is collected at the opposite end. A fresh solution, of whatever composition desired, is forced in through one face of the sandwich, forcing the original solution (minus the desired solute) out the opposite face. The principles are illustrated in Fig. 1.

The parent technique—flow FFF—is an analytical separation device for macromolecules and particles (*1–3*). It utilizes the cross flow of the channel to force a narrow zone of solute mixture into thin layers against the lower membrane. The layer for each solute has a different mean thickness because of variable back diffusion. The relatively thick layers are displaced rapidly downstream by the axial component of flow, while the thin layers are retained by the regions of semistagnant liquid near the wall. Thus differential displacement occurs and solutes are swept out individually with the column effluent. This methodology has been shown applicable to proteins, viruses, and polystyrene latex beads (*1–3*). In this original mode of operation, it can also be used for *batch* dialysis or ultrafiltration.

Operating in the continuous pressure dialysis or diafiltration mode, the FFF channel will allow the operator to change in any desired way the final composition and concentration of background electrolyte. A salt of one concentration can be almost totally exchanged with another salt or organic material of another concentration. Solute concentration, also, can be varied in any direction if desired (ultrafiltration mode).

Control of these variables is a result of the fact that four separate flow rates can be manipulated in such a way as to achieve the desired purposes. These flow rates are those at the inlet and outlet of the channel (axial) flow stream and the inlet and outlet of the cross-flow stream, as shown in Fig. 1. Three can be manipulated independently and the fourth is then fixed by the condition that the total inflow and outflow of liquid must be equal.

Small cell volume combined with good throughout leads to short residual times for solute in the FFF cell. The actual scale can clearly be adapted over a wide range, at least from microcells of 10 or so microliters to units capable of processing many liters per hour.

Other dialysis and ultrafiltration methods have, of course, demonstrated a high capacity and, between them, a spectrum of capabilities. The hollow fiber bundle is particularly high in surface area and throughput, but under normal ultrafiltration conditions, solute is concentrated and impurity removal is incomplete. Many configurations exist for ultrafiltration units, but each is rather specific and limited in function (*4*). The FFF cell avoids these problems and restrictions because the incoming cross flow can be used both to flush the system with the desired background composition and to dilute or concentrate the solute as desired.

THEORY

Certain restraints obviously exist on the operation and throughput of the flow FFF cell which depend on geometry, type of membrane, degree of purification desired, and so on. The principles of the methodology serve to clarify these restraints and to suggest optimum parameters for channel dimensions and for the rate of cross-flow necessary to maximize throughput. The theory presented here is based on several simplifying assumptions, but it should serve as a general guide for operation of the cell.

Figure 2 shows the idealized pattern of fluid movement and of solute and impurity displacement in the flow cell, viewed from one edge. The fluid at any point is obviously subjected to two flow vectors, a horizontal component from the solute feed solution and a vertical component from the fresh solution entering through the face of the upper membrane. Therefore, all small volume elements of fluid are moving down diagonally to the right, more or less in a direction indicated by the overall "flow vector" shown in Fig. 2. The solution boundary, where fresh solution meets the impure mixture and displaces it downward, lies parallel with the local flow vector at each point. Both the solution boundary and the direc-

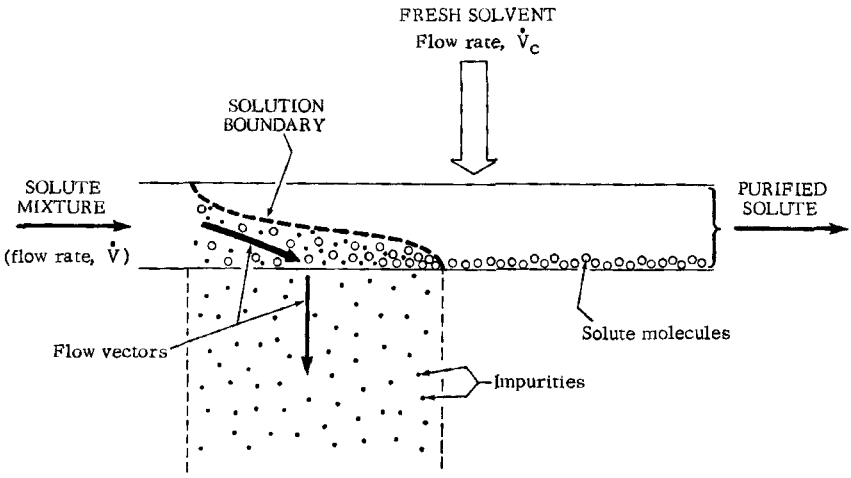

FIG. 2. Edge view of the overall displacement vector and of the idealized solution boundary in the flow FFF dialysis cell.

tion of the local flow vector will bend to the vertical at the membrane interface where horizontal flow ceases. The resulting curvature is shown for the solution boundary; its form has been presented in detail in connection with another of the subtechniques of the FFF method (*5*).

The most obvious requirement for the successful operation of this system is to proportion the flows (and thus the flow vector components) so that the solution boundary is driven into the membrane, rather than being allowed to elute in the (purified) solute stream. This simply requires that the stream of fresh solution at the inlet (upper left-hand corner in Fig. 2) must arrive at the lower membrane before the original mixture with its impurities reaches the end of the channel (at the far right). Both of these arrivals require a flow displacement of one cell volume. Therefore, simply stated, it is necessary that the cross-flow rate (that occurring through the membrane) exceed the channel flow rate: $\dot{V}_c > \dot{V}$. We assume in this section that $\dot{V}$ is constant, which corresponds to the pressure dialysis mode of operation.

The degree to which cross flow should exceed axial (channel) flow depends on several factors. First, the solution boundary is diffuse rather than sharp, due to the interdiffusion of components. Thus, at equal flows of the cross and axial streams, impurities that have diffused into the fresh solution will be eluted with the desired solute. A greater cross flow is

needed to sweep this diffuse boundary down through the lower membrane. The required increase in vertical flow depends on the purity desired, and upon relative flow and diffusion rates.

Flow irregularities will also make necessary an increased cross flow to ensure that all stream elements in an unequal set are washed free of impurities before elution.

If the diffusion or effective diffusion of solute across the thin layer were rapid enough so that instantaneous mixing could be assumed, the removal of impurity could be approximated by the exponential expression found for perfectly mixed cells (*6*)

$$(c/c_0) = \exp(-\dot{V}_c/\dot{V}) \tag{1}$$

where c is the concentration in the channel effluent stream and c_0 is that in the channel feed stream. A removal rate slightly higher than that suggested by Eq. (1) is expected because of the incomplete mixing—or residual stratification of the type suggested in Fig. 2.

Another important variable that is influenced by the different flow rates is the mean residence time of solute in the channel. Unstable solutes, particularly, must always be processed as rapidly as possible.

Mean solute residence time in the channel, t_r, is equal, in the ideal case, to the residence time of discrete solute peaks injected at the head of the channel. The latter residence time has been characterized thoroughly in the original flow FFF studies (*1–3*). To a good approximation we can use

$$t_r = (w^2/6Df)\dot{V}_c/\dot{V} \tag{2}$$

where w is the width between membranes and D is the solute diffusion coefficient. The function f is normally close to unit. This equation shows that t_r is influenced by the flow ratio, $\dot{V}_c/\dot{V}$, but not appreciably by the absolute magnitude of the flows, providing they are at a constant ratio. More importantly, t_r can be decreased markedly by reduction in w. Other reasons exist for seeking minimal w values, as will be seen shortly.

In the following treatment we will assume that the rates of cross flow and channel flow remain in constant proportion to one another at a level fixed by purity requirements, solute residence time, etc.

Upon first consideration, it appears that there is no limit to throughput in the FFF cell if one simply keeps increasing the cross and axial flows in proportion. However, as with any semipermeable membrane method, concentration polarization and other transport processes limit the rate of purification.

With increasing cross flow, the solute is compressed to an increasing

degree against the lower membrane. Its concentration distribution in the ideal (dilute) case is (*2*)

$$c = c_0 \exp(-xU/D) = c_0 \exp(-x/l) \tag{3}$$

where c_0 is the concentration at the lower membrane, U is the cross flow velocity, and D is the diffusion coefficient. Mean layer thickness l changes in inverse proportion to U. Therefore, with increasing U, l decreases and wall concentration, c_0, increases proportionately. This increases the resistance to cross flow and the back pressure. It may also encourage precipitation and solute leakage through the membrane.

Another factor enters here. With increasing compression of the solute layer, its mean axial displacement velocity relative to that of the solvent decreases because solute resides increasingly in the almost stagnant flow regime next to the wall. (This phenomenon, acting selectively, is responsible for separation in flow field-flow fractionation.) A lower relative velocity means that more solute must pile up in any given region to maintain (or accommodate increased) steady-state throughput.

In the ideal case, then, a doubling of both flow velocities will double throughput, but it will quadruple the solute concentration at the lower membrane; it will double once because of layer compression, and once more because of the solute buildup caused by relative velocity retardation and increased throughput. The actual (nonideal) case will reflect these general trends. Experimentally, then, there will be a finite limit to throughput for any given cell.

Now let us examine the consequences of reducing—say by a factor of 2—the thickness, w, of the flow gap while maintaining both volumetric flows constant. The layer thickness remains constant because of the constant cross-flow velocity, U. However, with w reduced by a factor of 2, the average axial flow velocity will be doubled. The mean axial solute velocity, by comparison, quadruples. It doubles once because the mean carrier velocity doubles, and it doubles again because the flow of the narrower channel sweeps out the boundary layer more effectively. Therefore, there is an effect opposite to buildup, namely dilution, at the membrane. This occurs by a factor of 4, and results from the fourfold speedup noted above and the concommitant fourfold reduction in the mean solute residence time in the cell. This allows an increase in throughput. More precisely, with each halving of the gap width, w, and quartering of wall concentration, c_0, throughput can be increased (using a simultaneous increase in the two flows) by a factor of 2, before c_0 returns to its original value. In general, then, we are guided by a reciprocal relationship for

throughput, T:

$$T = \text{const.}/w \tag{4}$$

This line of reasoning points to decreasing gap width, w, as one of the major factors in optimization. A minimum value of w is also optimum for FFF separations.

Throughput will also increase in rough proportion to the membrane area. Its increase with increasing breadth (side-to-side distance) is clearly a result of an increase in flow capacity. Increasing path length, L, will increase capacity in a way dictated by the following argument. If U is maintained constant while L changes, then the layer thickness remains constant in accord with Eq. (3). The volumetric cross flow must, therefore, increase in proportion to the increase in L (that is, it will vary in direct proportion to membrane area, which in this case is proportional to L). The axial flow rate, being fixed at a constant fraction of the rate of cross flow, will also increase in proportion to L. Thus, throughput will gain in direct proportion to the length of the dialysis cell.

Our solute throughput equation can now be reformulated as

$$T = \text{const. } aL/w \tag{5}$$

where the constant increases with the square root of the concentration of incoming solute and depends also on the allowable back-pressure. Throughout this treatment, of course, we have assumed that wall concentration and the resulting back-pressure and risk of precipitation are the limiting factors to increased throughput.

Equation (3) suggests the thin sandwich configuration for purification efficiency. For best throughput, membrane area (aL) is to be maximized and width (w) minimized. Indeed, w has been reduced to 0.025 to 0.050 cm in practical flow FFF cells. Much greater reductions in w are undoubtedly possible, and these can be combined with arbitrary increases in membrane area for increased throughput.

EXPERIMENTAL

Preliminary experiments have been run to demonstrate purification capability. The FFF cell and ancillary apparatus are the same as those used in previous flow field-flow fractionation studies (*1–3*). The cell dimensions are 42.2 × 1.65 × 0.038 cm, giving a volume of 2.65 ml. Rigid membrane channel walls were constructed by casting cellulose acetate membranes on two rigid, porous polypropylene plates, as discus-

sed elsewhere (*1–3*). The membranes were tested and confirmed for 100% retention of small protein molecules such as albumin before the following studies were made.

Methylene blue (MW 320) was chosen as the indicator to demonstrate the removal of small molecules such as salt ions and organic solvents from macromolecules in the cell. An acetate buffer solution (0.2 *M* sodium acetate adjusted to pH 5.4 with acetic acid) containing 6.1×10^{-5} *M* methylene blue was fed into the inlet end of the dialysis cell by a metering pump (Laboratory Data Control) at a flow rate of about 5.6 ml/hr. A Nupro needle valve placed at the outlet was used to maintain a constant axial flow rate. The cross-flow stream, consisting of pure acetate buffer, was provided by a separate pump. The cross flow was changed stepwise from about 5 to 100 ml/hr. After each change of cross-flow rate, a time of 2 hr was allowed to reestablish steady conditions. The volumetric flow rates of both axial and cross-flow streams were then measured. The concentration of methylene blue in the exit stream was derived from its absorbance relative to that of pure acetate buffer at a wavelength of 600 nm.

In order to determine the effect of high protein concentration on the rate of dialysis, 100 g of bovine serum protein (Miles Laboratories) was added to 1 liter of the methylene blue–acetate buffer solution described above. The resulting solution was then fed into the cell. By following the same procedure as before, the concentration of methylene blue in the stream exiting from the dialysis cell was measured. The adsorbance due to albumin was subtracted out from the total adsorbance measured. The axial flow rate in this study was 18 ml/hr.

RESULTS AND DISCUSSION

The capability of the cross flow in purging methylene blue from the axial stream is illustrated in Fig. 3. The percentage removal is calculated from the measured concentrations of methylene blue in the feed stream (c_0) and outlet stream (c). The figure shows this percentage to rise steadily with the increased ratio of cross flow to channel flow. This result is expected because the flow ratio indicates the number of times that the incoming solution is effectively removed and replaced by fresh solution during passage of the solute through the channel (see Eq. 1).

The near coincidence of the two sets of data in Fig. 3 demonstrates that the percentage removal is not seriously affected by the presence of albumin in solution under our experimental conditions. Also, the percentage

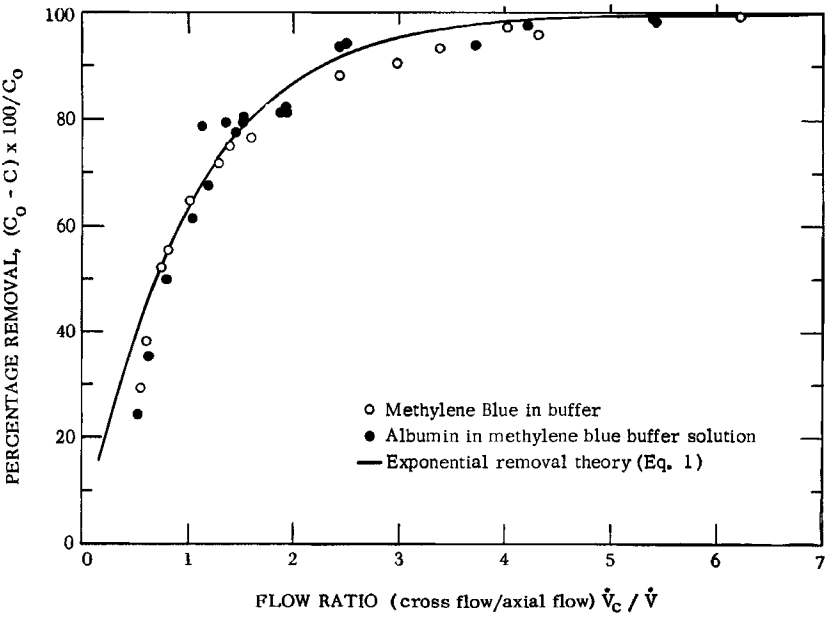

FIG. 3. Percentage removal of methylene blue from channel flow stream as a function of flow ratio. In one case (open circles) the channel feed contains only methylene blue (6.1×10^{-5} *M*) in sodium acetate buffer at pH 5.4. In the other case (filled circles) the feed is identical except for the addition of 10% by weight of albumin. In the protein-absent case, the channel flow rate, $\dot{V}$, averaged 5.6 ml/hr and the cross-flow rate was varied from 3.5 to 95 ml/hr. The albumin runs were made with $\dot{V} = 18$ ml/hr and $\dot{V}_c$ from 10 to 100 ml/hr. The line expresses the percentage removal according to Eq. (1).

removal does not seem to be reduced by overall flow rate increases under these conditions in that the axial flow rate was more than three times larger (18 vs 5.6 ml/hr) with the protein solution than with the reference solution.

While detailed theoretical curves making allowance for finite diffusion rates have not been developed to describe the results of Fig. 3, we found the percentage removal to increase less rapidly with flow ratio than was expected. If there were instantaneous and complete mixing of all fresh solution from the cross-flow stream with each volume element of liquid during its passage through the channel, the methylene blue ratio should decrease according to the exponential expression of Eq. (1). The present cell should, in theory, provide a more complete removal because of incomplete mixing, as noted in the theory section. In fact, however, the

percentage removal approaches that predicted by the exponential expression only in some cases when $(\dot{V}_c/\dot{V}) < 3$, and falls slightly below the predicted level for higher flow ratios. The reasons for this are not entirely clear. Experiments with different cell geometries would perhaps yield evidence on this matter.

Despite the theoretical discrepancy, Fig. 3 illustrates the fact that rather complete removal of the impurity can be achieved under reasonable conditions. Solvent and small molecule/ion substitution can, of course, be achieved with equal efficiency. In fact, we can think of impurity removal as a substitution process when using the present method because there is a gradual but almost total exchange during flow through the cell of the contaminated solvent with fresh solvent containing the desired constituents.

We now use Eq. (2) to calculate the mean residence time, t_r, for albumin in the cell. For this calculation we use $D = 6.8 \times 10^{-7}$ (*7*). Figure 4 shows the results plotted as a function of the flow ratio, $\dot{V}_c/\dot{V}$, over the range employed with the albumin experiments. The t_r values are in all cases less than 1 hr, and in the best cases they are about 10 min. While these values are already very good, they could be reduced substantially by reductions in w, as suggested by Eq. (2).

It was mentioned earlier that the FFF channel can be operated either in the ultrafiltration mode, pressure dialysis mode, or dialysis mode. The dialysis mode is achieved by simply eliminating the cross flow. In this mode our apparatus would resemble the thin-layer microtubular continuous-flow countercurrent device described by Zeineh et al. (*8*). It is felt, however, that the cross flow is a positive feature that promotes solution exchange and composition control without any serious disadvantages. The throughput and removal figures presented here for our initial cell compared to those for the above-named dialysis cell tend to confirm this conclusion.

Our preliminary experiments were all run in the diafiltration or pressure dialysis mode in which albumin emerged from the cell unchanged from its original concentration. This mode requires that Flows 1 and 2 (Fig. 1) be equal; both are designated by $\dot{V}$. It is a simple matter, however, to change either the outlet or inlet $\dot{V}$ value in order to change the emerging solute concentration. Either higher or lower concentrations can be reached. The concentration of the outgoing stream relative to its incoming (feed) value, c_{out}/c_{in}, is given simply as the ratio, $\dot{V}_{in}/\dot{V}_{out}$, of initial to outgoing channel flow rates. With channel flows controlled in this manner, the FFF cell appears capable of yielding the entire range of effects provided by

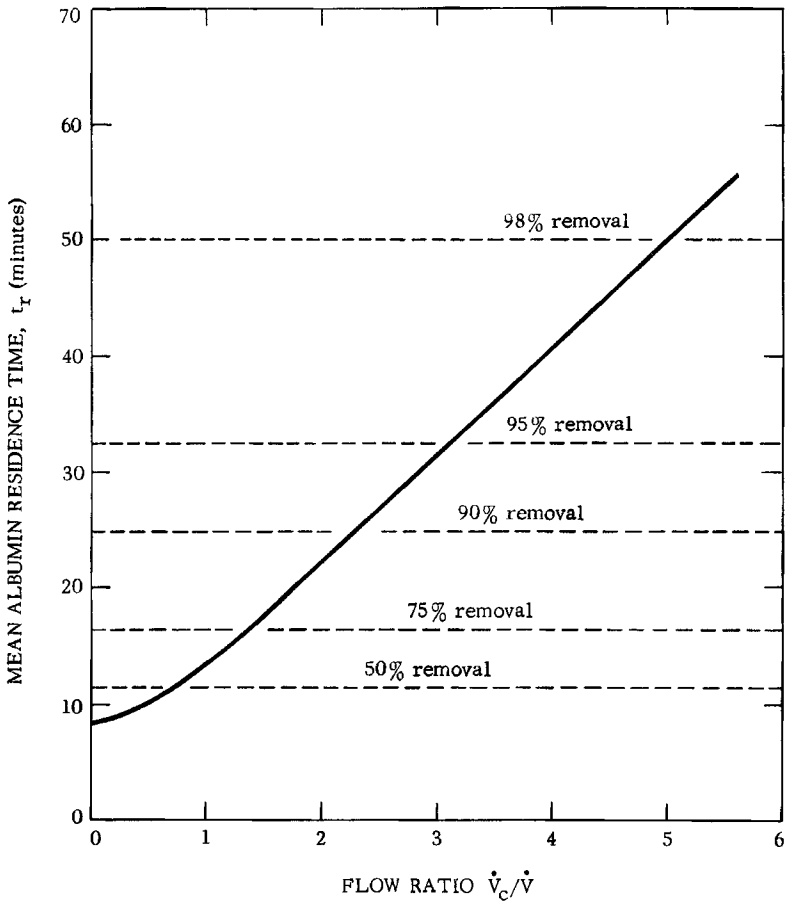

FIG. 4. Mean residence time of albumin in the FFF cell as a function of the flow ratio. This curve is calculated from Eq. (2) which has been demonstrated as valid in corresponding flow FFF experiments. Channel flow rate, $\dot{V}$, is 18 ml/hr. Comparison with Fig. 3 shows that 90% impurity removal is achieved at $(\dot{V}_c/\dot{V}) \sim 25$ min. This and other removal percentages are shown in the figure.

ultrafiltration and dialysis, but with more versatility in the control of the background solution and of residence times.

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