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Criteria for Concentration Field-Flow Fractionation

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Abstract

Concentration field-flow fractionation (CFFF) is proposed as a possible new subtechnique of field-flow fractionation (FFF). The equations for solute retention in CFFF are obtained as a function of solute distribution and the solute chemical potential increment, $\Delta\mu_c^\circ$, across the flow channel. The minimum practical value of $\Delta\mu_c^\circ$ is related to the necessary increment, ΔC_c, in the concentration of one component of a mixed solvent across the column. This leads to criteria for ΔC_c, for the solvent component's flux, and for the channel width.

The parameters necessary to make the above criteria quantitative are obtained from protein solubility studies and membrane permeability measurements. It is concluded that present FFF channel designs do not meet the criteria of CFFF for the systems studied here, and that some new approach will likely be necessary to implement a practical CFFF system.

INTRODUCTION

In field-flow fractionation (FFF) an external field or influence of some kind is used to force solutes toward one bounding wall of a narrow tube or channel (*1–3*). The wall-hugging layers are of a different thickness for each solute because of the solute's unique interaction with the field and the variable diffusional characteristics. Axial flow, therefore, carries the solutes downstream, each at a different velocity which is a function of the

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layer's thickness. In this way differential migration and, ultimately, separation are achieved.

Fields commonly used are electrical (*4*, *5*), thermal gradient (*6*, *7*), cross-flow (*8*, *9*), and centrifugal (*10–12*). We propose here another "field" of potential interest. This consists of concentration gradients within a solvent. With this field, or gradient, the method can be termed concentration field-flow fractionation (CFFF). The theory of CFFF is simply that a mixed solvent with a concentration gradient of its components across a channel would, much like most other fields, establish an effective chemical potential gradient for the solute. The solute would then accumulate at whichever wall displayed the lowest chemical potential. For instance, a salt gradient in water might encourage proteins to seek the low-salt regions because of chemical forces arising in the salting-out effect. The principles of CFFF are illustrated in Fig. 1.

CFFF is appealing because it would utilize chemical forces rather than physical forces for separation. It would, therefore, display selectivity characteristics different from those of normal FFF, and provide a valuable complementary system to the latter.

Solute distribution in the solvent gradient of CFFF would depend on the same general chemical factors that determine chromatographic retention. The distribution, however, would be realized in only one phase with a gradient rather than in two phases with an interphase. CFFF, therefore, is the FFF method that most closely resembles chromatography, and more than any other FFF method deserves the title *one-phase chromatography* (*13*).

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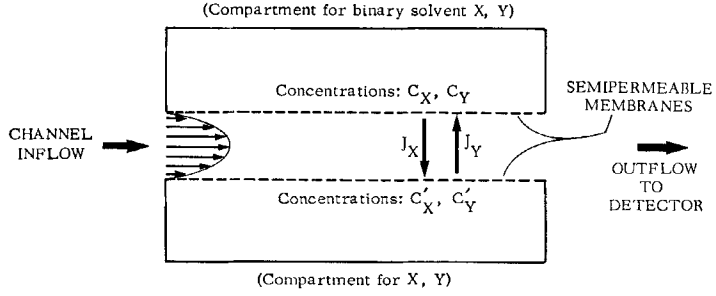

FIG. 1. Schematic edge view of CFFF channel and solvent compartments. The unequal concentration of solvent components, x and y, leads to concentration gradients and diffusional fluxes, J_x and J_y, across the channel. The continuously variable concentration across the channel will attract solute species toward one channel wall, thus giving the basic FFF effect.

Despite the close theoretical parallel with chromatography, CFFF promises to be difficult in its implementation. We intend to establish in this paper the criteria that must be satisfied for a working CFFF system.

We have found that CFFF makes very rigorous demands on solvent fluxes and on membrane and boundary layer permeability in order to function practically. We first establish the major criteria for practicality, and follow this with experiments that yield some of the relevant parameters of the criteria. We suggest approaches and parameters to satisfy these criteria, but we are not able to report an operational CFFF channel at this time.

THEORY

We assume that solute distribution in the CFFF channel is determined by the chemical potential gradient associated with the variable solvent concentration. This neglects any disturbance caused by the diffusional currents of the solvent components. We also assume ideal solutions. All solute concentrations are represented by lower case c's, and solvents by capital C's. The c's are assumed small so that solute–solute interactions are unimportant.

With linear gradients the solute concentration c drops off approximately exponentially with increasing distance from the wall (*3*)

$$c/c_0 = \exp\left[-(\partial\mu^\circ/\partial x)x/\mathscr{R}T\right] \tag{1}$$

In this equation, c_0 is the concentration at the wall, $\partial\mu^\circ/\partial x$ is the chemical potential gradient, $\mathscr{R}$ is the gas constant, and T is the temperature. At a characteristic altitude above the wall, $x = l$, the exponent is equal to -1

$$-(\partial\mu^\circ/\partial x)l/\mathscr{R}T = -1 \tag{2}$$

which gives

$$l = \mathscr{R}T/(\partial\mu^\circ/\partial x) \tag{3}$$

The ratio of l to the channel width (maximum altitude) w is of utmost significance in field-flow fractionation (*2, 3*). This ratio, termed λ, is obtained by dividing Eq. (3) by w:

$$\lambda = \frac{l}{w} = \frac{\mathscr{R}T}{(\partial\mu^\circ/\partial x)w} \tag{4}$$

The quantity $(\partial\mu^\circ/\partial x)w$ is simply $\Delta\mu_c^\circ$, the total increment in chemical

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potential across the channel

$$\lambda = \mathscr{R}T/\Delta\mu_c^\circ \tag{5}$$

In view of the fundamental thermodynamic relationship for ideal solutions

$$(c_w/c_0) = \exp(-\Delta\mu_c^\circ/\mathscr{R}T) \tag{6}$$

quantity $\Delta\mu_c^\circ$ can be expressed as $\mathscr{R}T \ln (c_0/c_w)$, where c_0/c_w is the ratio of concentrations at the opposing channel walls. Therefore, the λ of Eq. (5) becomes

$$\lambda = 1/\ln (c_0/c_w) = 1/\ln \alpha \tag{7}$$

in which the ratio c_0/c_w is represented by α.

The theory of field-flow fractionation expresses the retention ratio R (zone velocity V/mean solvent velocity $\langle v \rangle$) as the following function of λ:

$$R = 6\lambda[\coth (1/2\lambda) - 2\lambda] \tag{8}$$

When Eq. (7) is substituted into Eq. (8), and the coth function is expressed in its basic exponential form, Eq. (8) reduces to the simple form

$$R = \frac{6}{\ln \alpha}\left[\frac{\alpha + 1}{\alpha - 1} - \frac{2}{\ln \alpha}\right] \tag{9}$$

A plot of R vs $\log \alpha$ is shown in Fig. 2.

The curve of Fig. 2 shows that R values remain near unity as the con-

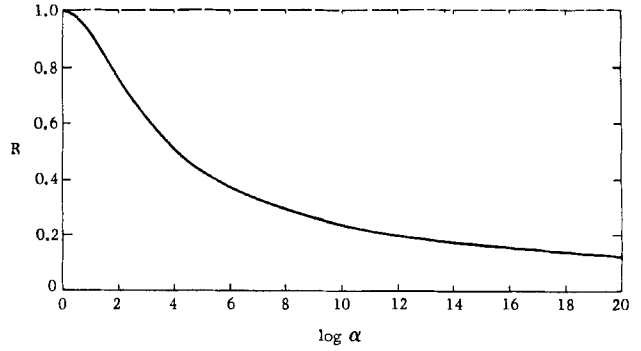

FIG. 2. Retention ratio R versus the logarithm of the ratio α of the concentration of solute at the two channel walls.

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centration ratio α ranges from unity to about 10. This means that all solute zones in this range would travel with or near an inert tracer peak, close to the mean velocity of the solvent. Fractionation would fail to occur in this range because of the nearly equal velocities of solute zones. Only if α exceeded about 10, giving R values under 0.92, would significant differential migration occur. Therefore, the first and most significant criterion for effective fractionation by CFFF is that a solvent gradient must be established of sufficient magnitude to provide a solute ratio of at least 10 to 100 in the CFFF channel, or a $\Delta\mu^\circ$ of about 1.5 to 3.0 kcal, or preferably larger. We investigate the implications of this requirement below.

The chemical potential of dilute solute in a mixed solvent is a function of composition. In a binary solvent we have $\mu^\circ = \mu^\circ(C)$, where C is the concentration of one of the two components—usually the minor one (such as salt in an aqueous solution). The chemical potential increment, $\Delta\mu_c{}^\circ$, across a CFFF channel is $(\partial\mu^\circ/\partial C)\Delta C_c$, where ΔC_c is the increment in the concentration of the solvent component between channel walls. Quantity $\Delta\mu_c^\circ$ must equal or exceed a certain minimum value, $\Delta\mu_c^\circ$ (min) as noted above:

$$(\partial\mu^\circ/\partial C)\Delta C_c \geq \Delta\mu_c^\circ(\text{min}) \tag{10}$$

Equation (10) can be rearranged to specify the following criterion for ΔC_c:

$$\Delta C_c \geq \Delta\mu_c^\circ(\text{min})/(\partial\mu^\circ/\partial C) \tag{11}$$

This requirement on ΔC_c is least severe for solutes whose distribution is affected strongly by small differences in solvent concentration—that is, for solutes with large $\partial\mu^\circ/\partial C$ values. In general, such a strong solvent sensitivity is confined to macromolecular solutes (*14*). Thus this methodology, like most other FFF techniques, is most promising for macromolecules and small particles.

The most direct method for generating solvent gradients is through the use of a channel bounded by semipermeable membranes, as suggested by Fig. 1. Solvent compartments above and below the channel, each with a different concentration, provide the necessary concentration gradient.

Two important (and as we shall see, difficult) requirements are associated with this arrangement. First, it is necessary to replenish fresh components to the solvent compartments as rapidly as they are depleted by the diffusional intermixing. Second, the system must be designed so that an excessive part of the total concentration increment between solvent

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compartments is not dissipated in boundary layers and membranes. Enough must remain to provide the ΔC_c increment for the channel as specified by Eq. (11).

The quantitative meaning of the first requirement can be easily calculated. The absolute magnitude of the flux of the solvent component across unit area of the channel is simply $D\,dC/dx$, where D is the binary diffusion coefficient for the two solvent components. The total flux, J, across membrane area A is therefore

$$J = AD\Delta C_c/w \tag{12}$$

Inasmuch as ΔC_c must satisfy Eq. (11), the flux must exceed a certain minimum value which is found to be

$$J \geq AD\Delta\mu_c^\circ(\min)/(\partial\mu^\circ/\partial C)w \tag{13}$$

The second requirement can be treated by assuming that the solvent components must diffuse through the layers of a sandwich consisting of the two membranes, two boundary layers, and the CFFF channel. For each layer, i, the steady-state flux density is

$$(J/A) = P_i\Delta C_i \tag{14}$$

where P_i is the permeability and ΔC_i the concentration increment of layer i. For the CFFF channel, $P_c = D/w$. Equation (14) yields $\Delta C_i = (J/A)(1/P_i)$ for each layer. The total concentration increment, ΔC_{tot}, is the sum of all such terms:

$$\Delta C_{tot} = \sum \Delta C_i + \Delta C_c = \frac{J}{A}\left(\sum \frac{1}{P_i} + \frac{w}{D}\right) \tag{15}$$

where the summation is for all layers except the CFFF flow channel, which is accounted for separately in the last term. The fraction of ΔC_{tot} found in the CFFF channel is

$$\frac{\Delta C_c}{\Delta C_{tot}} = \frac{w/D}{\sum (1/P_i) + w/D} \tag{16}$$

Since the minimum workable ΔC_c is specified by Eq. (11), we have the following criterion for the total concentration increment between compartments:

$$\Delta C_{tot} \geq \frac{\Delta\mu_c^\circ(\min)(\sum (1/P_i) + w/D)}{(w/d)\partial\mu^0/\partial C} \tag{17}$$

If ΔC_{tot} is fixed, then the channel width, w, necessary to satisfy Eqs. (11)

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and (16) is

$$w \geq \frac{D\Delta\mu_c^\circ(\min) \sum (1/P_i)}{\Delta C_{tot}(\partial\mu^\circ/\partial C) - \Delta\mu_c^\circ(\min)} \tag{18}$$

In summary, to make CFFF function practically, we must satisfy the following criteria: (a) it must be possible to make the increment in concentration of the solvent component in the channel equal or exceed the value given in Eq. (11), and that in order to sustain this criterion it is necessary that (b) the flux of solute must equal or exceed the value expressed in Eq. (13), and (c) the total concentration difference between reservoirs must satisfy Eq. (17) or channel width w must be governed by Eq. (18).

EXPERIMENTAL

Two types of experiments were done. First, the solubility of hemoglobin and fibrinogen in various salt solutions and in ethanol was measured in order to determine the effect of salt or alcohol concentration on protein distribution and chemical potential. In this way it is possible to obtain the increment, ΔC_c, in the concentration of salt or alcohol necessary to develop an effective CFFF system. For this purpose, 4 g hemoglobin and 0.15 g fibrinogen were dissolved in separate 100 ml volumes of Trizma buffer solution [containing 0.01% by weight of tri(hydroxymethyl)amino methane and 0.2% sodium acetate, the mixture then titrated to pH 5.5 with acetic acid]. The solutions were divided into stoppered flasks to which measured quantities of various salts and of ethanol were added. The mixtures were shaken gently for 4 hr, filtered, and the concentration of the remaining protein solution was measured at 280 nm against a blank solution.

Next, experiments were done on membrane permeability to determine if the necessary increment, $\Delta C_c(\min)$ could be developed in the channel. The experimental arrangement of Fig. 3 was used for this purpose. The solvent compartments, cut from Plexiglas, were 42 × 2.8 × 1.6 cm. The pumps maintained continual flow at about 50 l/hr. The total volume of each flow system (reservoir + compartment + flow lines) was 1000 ml. A 1 M salt or n-propanol solution was fed into the upper reservoir; distilled water occupied the lower reservoir. The rate of permeation of salts was determined by measurements in the lower compartment using a Barnstead Model PM70CB conductometer. A gas chromatographic unit was used to determine the permeation of n-propanol.

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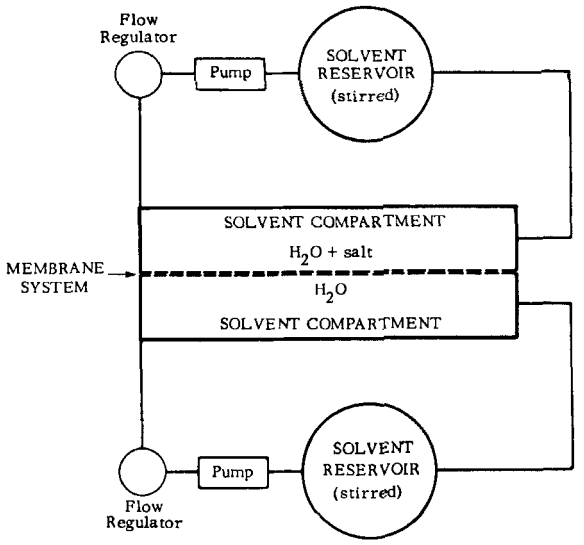

FIG. 3. Schematic diagram of system used for membrane permeability studies. Several salts as well as ethanol were used, each at an initial concentration of 1 mole/l. The rate of permeation into the H_2O compartment was measured by conductometry for the salts and gas chromatography for ethanol.

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The membrane or membrane sandwich was clamped between the two compartments. The membrane materials were obtained from diverse sources: the regenerated cellulose from Arthur H. Thomas Co.; the reinforced cellulose in wood fiber from Union Carbide; and the cellophane from Dr. Donald Lyman of the University of Utah. Solute materials were obtained as follows: fibrinogen from Sigma Chemical Company, and α-, β-, and γ-globulin, along with cholesterol and hemoglobin, from Mann Research Laboratories.

RESULTS AND DISCUSSION

The protein solubility studies in various salt and alcohol solutions established values for the concentration drop required across the channel for effective CFFF. Plots of $\ln c$ vs C for proteins in salt solutions are shown in Fig. 4. The slopes of the lines equal $-(1/\mathscr{R}T)(d\mu^\circ/dC)$, as can be seen by taking the derivative $d \ln c/dC$ of the thermodynamic expression, $\ln c = \ln c_0 - \Delta\mu^\circ(C)/\mathscr{R}T$. With these slopes, values of $d\mu^\circ/dC$

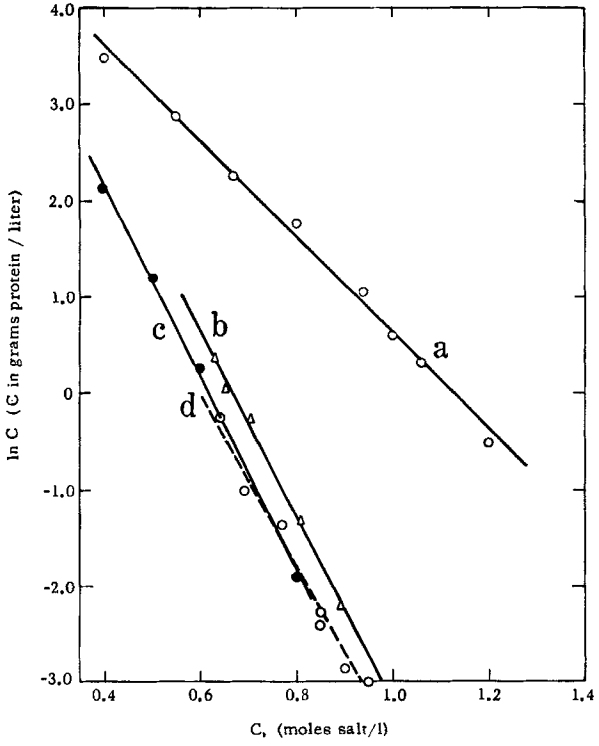

FIG. 4. Plots of ln protein concentration versus salt concentration in the salting-out region. The plots are: (a) hemoglobin in Na_2SO_4; (b) fibrinogen in $(NH_4)SO_4$ at pH 6.6; (c) hemoglobin in sodium citrate; (d) fibrinogen in $(NH_4)SO_4$ at pH 6.0. Curves b and d are from the data in Ref. 15.

can be obtained; these are shown in Table 1 [the fibrinogen plots are from the literature (*15*)]. Values of $d\mu^\circ/dC$ for ethanol solutions are also shown; these were obtained using a graph similar to that in Fig. 4.

Also shown in Table 1 is the minimum ΔC_c which, according to Eq. (11), yields a significant CFFF effect assuming that $\Delta\mu_c^\circ(\min)$ is 1.5 kcal/mole. The $\Delta C_c(\min)$ is seen to be ~ 1 mole/l for salts and generally > 10 moles/l for ethanol. These concentration increments are fairly large and would be difficult to maintain in a typical CFFF channel, as we shall now discuss.

We have also calculated for presentation in Table 1 the minimum flux or throughput, $J(\min)$, of the various salts and of ethanol needed to maintain the above $\Delta C_c(\min)$ values. Equation (13) was used for this

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TABLE 1

Parameters for Protein and Chloresterol Solubility in Various Salts and Ethanol. Quantity $\Delta C_c(\min)$ Is the Minimum Usable Salt or Alcohol Concentration Increment in the Column, and $J^*(\min)$ is the Minimum Solute Flux for a Channel of 25 cm Area and 0.05 cm Width. Other Parameters Are Defined in the Text

Protein	Aqueous solvent component	$\partial\mu^\circ/\partial C$ $\left(\frac{\text{cal-l}}{\text{mole}^2}\right)$	$\Delta C_c(\min)$ $\left(\frac{\text{moles}}{\text{l}}\right)$	$J(\min)w/A$ $\left(\frac{\text{moles}}{\text{cm-sec}}\right)$	$J^*(\min)$ $\left(\frac{\text{moles}}{\text{sec}}\right)$
Hemoglobin	Na_2SO_4	970	1.6	0.0014	0.68
Hemoglobin	Na citrate	1960	0.76	—	—
Fibrinogen[a]	$(NH_4)_2SO_4$ (pH 6.6)	1870	0.80	0.0008	0.42
Fibrinogen[a]	$(NH_4)_2SO_4$ (pH 6.0)	1760	0.85	0.0009	0.45
α-Globulin	Ethanol	191	7.8	0.0068	3.4
β-Globulin	Ethanol	0.76	1970	1.70	860
γ-Globulin	Ethanol	41	37	0.032	16
Chloresterol	Ethanol	34	44	0.038	19
Fibrinogen	Ethanol	93	16	0.014	7.0

[a]Data from Ref. 15.

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purpose. Diffusion coefficients for these calculations were obtained from the literature (*16*): for salts in aqueous solutions we use $D = 1.05$, 0.91, 0.80, and 0.58×10^{-5} cm^2/sec for $(NH_4)_2SO_4$, K_2CO_3, Na_2CO_3, and $MgSO_4$, respectively; and 0.87 and 1.0×10^{-5} for *n*-propanol and ethanol, respectively.

The results are shown in the last two columns of Table 1. The last column is the flux calculated for a channel having specific dimensions: the membrane area A is 25 cm^2 and the channel width w is 0.05 cm. Such a channel would clearly be feasible based on our previous success with other FFF channels of only slightly different dimensions, and it could possibly be altered even more in the direction of reducing J. These dimensions, however, provide a good starting point for estimating the feasibility of CFFF in light of the solvent flux criterion. As the last column in the figure shows, the $J(\min)$ value [given as $J^*(\min)$ to designate the specific case] is ~ 0.5 mole/sec for salts and > 3 moles/sec for ethanol. The salt transport is clearly most feasible, but even this represents a loss from the upper compartment of about 1800 moles/hr, or 200 to 300 kg/hr, a truly excessive amount. Clearly, for this system, the dimensions of the CFFF channel would have to be modified drastically to yield a practical system. This would require an extensive redesign of the basic channel configuration.

The experiments dealing with membrane permeation utilized both single membranes and double membranes in order to isolate the effects of boundary layer permeability. The results were not conclusive because the flux was more than twice as great in the single as in the double membrane system. This suggests that a liquid layer or perhaps some air bubbles were incorporated between the membranes, giving extra resistance to permeation. Inasmuch as the ratio never fell below 2, we assumed that the boundary layer resistance was unimportant and we used, therefore, twice the resistance of the single membrane system. While some error—at very most a factor of 2—may have been incurred in this procedure, it would not change our conclusions in any significant way.

The permeation results as derived from the single membrane systems are summarized in Table 2. Along with the estimated 2-membrane permeability, $P = \Sigma(1/P_i)$, we have compiled values of w(min), the minimum channel width, which, according to Eq. (18), leads to a concentration drop across the channel of sufficient magnitude to give a CFFF retention effect. Quantity $\Delta\mu°$(min) was assumed to be 1.5 kcal/mole, as before, and ΔC_{tot} was given the value of the concentration of the salt at saturation, or the

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TABLE 2

Permeability Results for Various Salts and *n*-Propanol in Several Membranes. The Permeability, P, is equal to $1/\Sigma(1/P_i)$ in the Text, and Represents the Value Estimated for Two Membranes in Contact, as Derived from Single Membrane Experiments. The Minimum Channel Width, w(min), Is the Value Derived from Eq. (18) as Being Necessary to Realize a CFFF Effect

Membranes and thickness	Solvent components / Parameters	$(NH_4)_2SO_4$	K_2CO_3	Na_2CO_3	$MgSO_4$	*n*-Propanol
Regenerated cellulose, 0.0074 cm	$P \times 10^7$ (cm/sec)	2.0	1.9	1.6	1.1	8.2
	w(min) (cm)	12	6.9	150	47	0.58
Regenerated cellulose, 0.0170 cm	$P \times 10^8$	9.2	9.8	8.0	2.9	—
	w(min) (cm)	26	13	300	170	—
Cellophane, 0.0030 cm	$P \times 10^7$	2.6	2.9	2.9	0.95	—
	w(min) (cm)	9.3	4.4	84	53	—
Reinforced cellulose in wood fiber, 0.0122 cm	$P \times 10^8$	9.8	9.8	7.2	2.3	—
	w(min) (cm)	25	13	340	220	—

concentration in moles/l of pure *n*-propanol, since the latter is totally miscible with water. This procedure leads to the highest conceivable C_{tot} values.

The w(min) values shown in Table 2 show again that CFFF would be difficult to realize experimentally using conventional channels. The most favorable values are ~ 1 cm, whereas normally w is ~ 0.25 mm for FFF. The above results can be summarized in relationship to the three criteria stated at the end of the *Theory* section. For our most favorable systems the ΔC_c(min) values that must be reached to fulfill Criterion (a) are ~ 1 mole/l, a value readily obtainable in theory. However, such a value applied to a channel of normal dimensions would lead to a salt or alcohol flux of several hundred kg/hr, a value too high to readily satisfy the practical requirements of Criterion (b). Finally, the channel width, w, needed for Criterion (c) is ~ 1 cm, beyond the present practical range. We conclude, therefore, that CFFF would require a drastic revision in the channel system or the basic approach, and/or in the use of solute–solvent–membrane systems that are much more favorable than the present ones.

It is important to emphasize that the experiments done here apply to very specific solute–solvent–membrane systems, and in no way delineate the possible scope of development of CFFF for other systems. The results are merely suggestive as far as the question of the general applicability of CFFF is concerned. The results, however, are sufficiently negative that they suggest the desirability of a radically different approach.

Such approaches undoubtedly exist. For instance, the solvent concentration gradient could possibly be established by an external field. Strong sedimentation forces, for example, are routinely used to create gradients in salt and sucrose solutions in gradient centrifugation methods. The gradients in the present case may require that the second solvent component be of high molecular weight. Interfering effects (such as the direct coupling between the solute and the field) would, of course, have to be accounted for.

CFFF may be the most difficult of all the known FFF systems to implement. Its unique retention mechanism, however, would justify future efforts to design a practical CFFF system. Hopefully, this work has contributed toward that goal.

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