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Can Membrane Cascades Replace Chromatography? Adapting Binary Ideal Cascade Theory of Systems of Two Solutes in a Single Solvent

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Can Membrane Cascades Replace Chromatography? Adapting Binary Ideal Cascade Theory of Systems of Two Solutes in a Single Solvent

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Abstract: It is suggested that the adoption of efficient counterflow cascades may accelerate the continuing encroachment of membranes on chromatography for downstream processing of biologicals, and a specific numerical example is provided to demonstrate their effectiveness. Emphasis is on three-stage ideal cascades, and it is shown that one may begin using the traditional batch operating mode. Conversion to continuous operation is then both simple and straightforward. Membrane cascades are the only means so far available for true continuous downstream processing of therapeutic proteins, which is a natural extension of the continuous upstream processes already beginning to be used for industrial production. Membranes are also attractive for larger entities such as plasmids or viruses whose low diffusivities can severely limit use of chromatographic processes.

Keywords: Membrane filter, diafilters, ultrafilters, cascades

INTRODUCTION

Process chromatography in its many variant forms has become the dominant downstream processing tool for difficult separations, but it is inherently expensive and is not used for commercial-scale separations in any other

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industry. At the same time many other potentially competitive techniques have been developing, and engineers have finally begun to show real initiative for process development in a variety of biological applications (1). Increasingly efficient renaturation of proteins from inclusion bodies shows promise of replacing the capture steps now performed by batch adsorption chromatography in a variety of applications, and crystallization appears to be increasing in importance for finer separations. Membrane filtrations are already providing increased competition to chromatography for the polishing stages of downstream processing, and they are becoming more and more selective, even for such large molecules as proteins (2).

There is also increasing interest in continuous upstream processing for which chromatography is ill suited (3). Use of simulated moving beds, the only continuous process currently available, is both cumbersome and poorly suited to feedback control. To date these devices have been limited to very clean stable systems, for example in the resolution of enantiomers from highly purified racemic mixtures. Finally, there is increasing interest in larger entities such as nucleic acids and viruses, and these have such low diffusivities that the choice of suitable adsorbents is severely limited. Pressure-induced flow across selective membranes, however, can greatly increase transport rates by convection relative to those for diffusion alone (4).

All of this ferment suggests taking a new look at downstream processing, and the purpose of this discussion is to suggest a serious study of membrane cascades. Membrane selectivities are rapidly increasing, and there is now a wealth of practical operating experience available for purposes of preliminary design. Membranes are available for dealing with an extremely wide range of molecular weights, from small monomeric molecules to mammalian cells. Moreover, the technology of dealing with membrane cascades was very highly developed during the 1940s in connection with the effusion process for uranium isotope fractionation (5). There is moreover a wealth of general information about membrane processes (6–8), and there is much directly pertinent prior art of which we cite only repesentrative examples (10–12).

Even very simple counterflow cascades have not been widely used in biotechnology, however, in large part because of control problems and lack of operating experience. This is thus a promising field for research: the long-term prospects look very attractive, but much fundamental research needs to be done before these benefits can be realized. The logical starting point will be the ideal cascade theory of isotope separations, as it will be seen next that these have much to offer for biological applications.

There will however be several basic problems to be overcome if membrane cascades are to be a commercial success for downstream processing. First one must adapt the existing theory, limited to simple binary systems, for the relatively complex biological situations of interest here. The obvious new factor will be the presence of a permeating solvent that makes the new systems multicomponent in nature right from the outset.

Second will be the inevitable presence of permeable contaminants, and it will be important in any final design to see if these can be reduced by acceptable changes in the stream ratios of the cascade.

Underlying all of these problems will be the control aspects since the exit streams from each module of the cascade and the up- and down-flow rates between modules must be changed from stage to stage for maximum efficiency. In addition the modules in each stage can be operated either in parallel or in a cross-flow mode, this latter being a useful feature of all large distillation columns for example. This means that one must consider fairly complex two-dimensional networks. Finally, provision must be made for membrane fouling and the inevitable changes of membrane properties with use and time.

It will therefore be desirable to start with simple prototype systems and then move by degrees to more complex but also more promising situations. Fortunately there are some simple applications where useful results can be obtained rather easily. One can then gain experience and at the same time produce economic processes. There are guides in the literature to aid in this stepwise approach. A logical starting point, used here by way of example, is the whey-derived albumin fractionations of Chaeng and Zydney (2) as the components here are inexpensive and stable, and assays are well established. Moreover one needs only ultrafiltration membranes under situations where sensitivity to minor changes in behavior are probably not very important: one can concentrate here on the solvent problem mentioned above and on developing a reliable control strategy. One can then go on to other well-documented and simple separations such as those already cited (9–12). After that one can begin in earnest on systems where a more complex cascade is really needed.

In the following section we review the behavior of membrane-based binary splitters and show how they can be modified to deal with biological mixtures. We next show how binary splitters can be connected to form ideal cascades and then concentrate our attention on the simplest of these latter, a three-stage unit. We show that even these very simple and tractable systems can achieve useful separations and that they provide a particularly simple transition to continuous operation: three-stage cascades can be operated in the familiar batch mode and converted to continuous operation simply by flipping a few control switches.

SIMPLE SPLITTERS FOR BINARY AND TERNARY SYSTEMS

Effusion as a Prototype Process

We begin here with the simple binary splitter of Fig. 1, developed long ago for fractionation of isotopes (5), as it provides a convenient point of departure

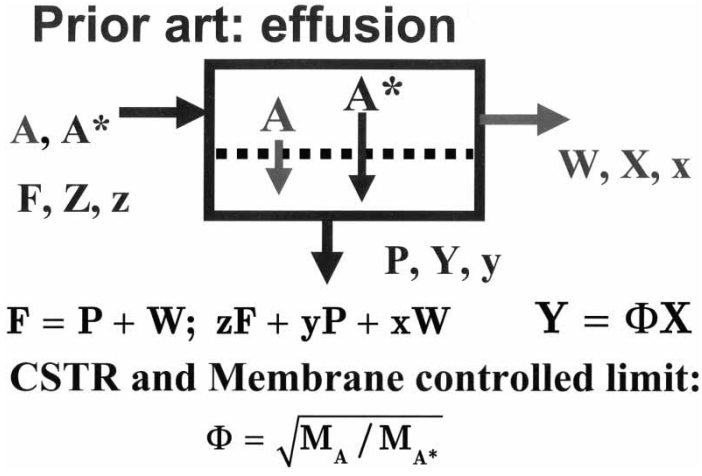

Figure 1. Prior art: effusion.

for our development. Here a mixture of two gaseous isotopic compounds, A and A^*, are to be separated, and A^* is the desired product. The separation is accomplished by forcing the gaseous feed through a micro-porous membrane under conditions of Knudsen flow so that observable species velocities in the membrane are proportional to mean arithmetic molecular speed. For simplicity the gases in the upstream compartment will be considered well mixed, but techniques are available for more complex fluid mechanic behavior. These can be used to advantage during later stages of any design process, including the fractionation of proteins.

Under these conditions molar ratios Y of A^* to A in the permeate are described by

$$Y = \sqrt{M_A/M_{A^*}} \cdot X = \Phi X \tag{1}$$

Here X is the molar ratio of A^* to A in the upstream compartment, and Φ is the *stage separation factor*. The relations between the stream rates and compositions are given by two mass balances:

$$F = P + W; \quad zF = yP + xW \tag{2, 3}$$

Here F, P, and W are the molar stream rates, and z, y, and x are the corresponding mole fractions. The quantities Z, Y, and X are the corresponding mole ratios.

Ultrafiltration-Based Separation

We now consider the separation of a solute pair, *A* and *B*, dissolved in a solvent *I* by differential migration rates across a selectively permeable membrane produced by a transmembrane pressure drop. See Fig. 2. The volumetric flow rate across the membrane per unit membrane area will be defined as the velocity "*v*," and the molar fluxes of solute (4) are given by

$$N_i = c_i S_i v \tag{4}$$

Here N_i is the molar migration rate of species "*i*" per unit area of membrane surface, c_i is molar concentration of species "*i*" at the upstream membrane surface, and the property S_i is known as the sieving coefficient. The sieving coefficient is defined by Eq. (4), but like so many engineering parameters it is a process-dependent quantity and not a state property. Determining its dependence upon both thermodynamic state and operating conditions will be an important part of any serious investigation. In particular we must ultimately be concerned with composition changes across a hydrodynamic boundary layer.

We now consider the splitter shown at the top of Fig. 3, and for simplicity we define the upstream compartment to be well mixed. We may then write once more that

$$Y = \Phi X \tag{5}$$

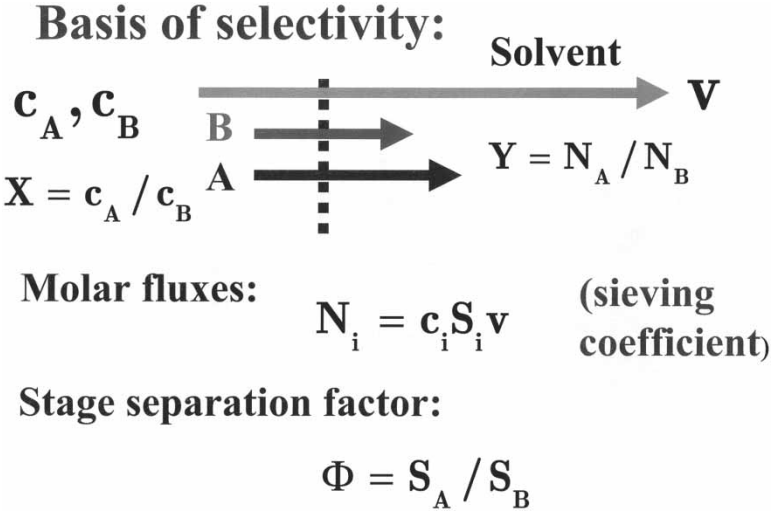

Figure 2. Basis of selectivity.

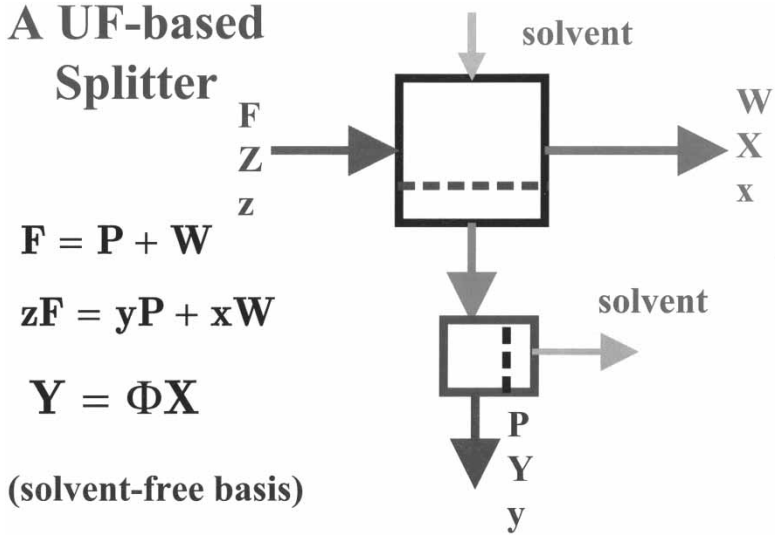

Figure 3. A diafiltration based splitter.

Now however, Y and X are the mole ratios of A, the desired product, to B, on a solvent free basis, and

$$\Phi = S_A/S_B \tag{6}$$

Equations (2) and (3) also hold for this system, but the mole fractions must now also be defined on a solvent-free basis.

We have found that the solvent-free description is an exact analog to that for the effusion cell of Fig. 1. This will permit us to take over essentially all of the effusion literature for our purposes. However, solvent must in general be supplied to our new splitter, as indicated at the top of the diagram, and in part at least will generally have to be removed in the auxiliary splitter at the bottom. The upper splitter thus becomes a simple variant of a conventional diafilter, and the lower one is a simple ultrafilter. The membrane in this ultrafilter must be permeable to solvent but not to the solutes. For such large solutes as proteins this is not a troublesome constraint.

A more serious reservation is the variation of sieving factor with solute concentration levels and operating parameters. The dependence on concentration levels is shown in all systems studied to date, but it does not seem to have been systematically investigated. The dependence on flow regimes and cross-membrane velocity was first investigated formally for gaseous mixtures (5), and a large literature remains to be investigated for liquid systems (6–8).

We shall find that most of the design calculations for our new membrane-based splitter can be solved by analogy to its simple prototype. However, we must then calculate the solvent flows into the diafilter and out through the ultrafilter to complete the design, and we shall also see that these flows can be varied a great deal according to the personal inclinations of the designer and the specific nature of the system. We shall return to this point in our example below.

Cascade Theory

First however we must recognize that a simple binary splitter of the type pictured in Fig. 3 will very often not give as complete a separation as desired. We must then take advantage of counterflow, and we will accomplish this by using a cascade of individual units. This will be a linear cascade such as that pictured in Fig. 4 and qualitatively similar to staged distillation columns. Two fluid streams will enter each stage, one from below as up-flow and another from above as down-flow.

However, the need to increase fluid pressure each time it enters a new stage provides a useful opportunity: it permits the designer to change the reflux ratio from stage to stage, and this in turn can provide a considerable increase in efficiency of contacting over a "square" cascade such as distillation. Such modified cascades are generally referred to as "tapered." We

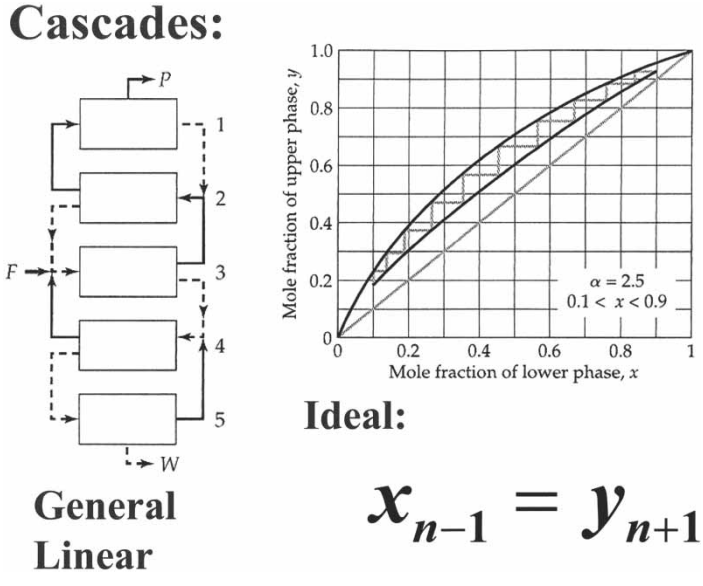

Figure 4. Cascades.

shall adopt here a specific type of taper giving an *ideal cascade* defined by the requirement that all streams entering any given stage must have the same (solvent-free) composition. If "x" refers to retentate and "y" to permeate this requirement takes the form

$$x_{n-1} = y_{n+1} \tag{8}$$

Note that we have numbered the system starting from that producing the desired product as stage "1."

We have thus introduced a novel splitter unit and a novel cascade constraint that permits us to extend cascade theory to efficient operation on ternary systems. The McCabe-Thiele diagram at the right in Fig. 4 shows visually the primary advantage of an ideal cascade: the operating line is everywhere halfway between the equilibrium and 45-degree lines so there is never a "pinch." In addition use of an ideal cascade minimizes the sums of the stage flows required for a given separation.

Once again, however, remember that we must also deal with the solvent and that we shall discuss this requirement in our example.

Three-Stage Ideal Cascades

Here we consider the simplest complete cascade, three stages as shown in Fig. 5. The numbers on this figure refer to our example below, and we

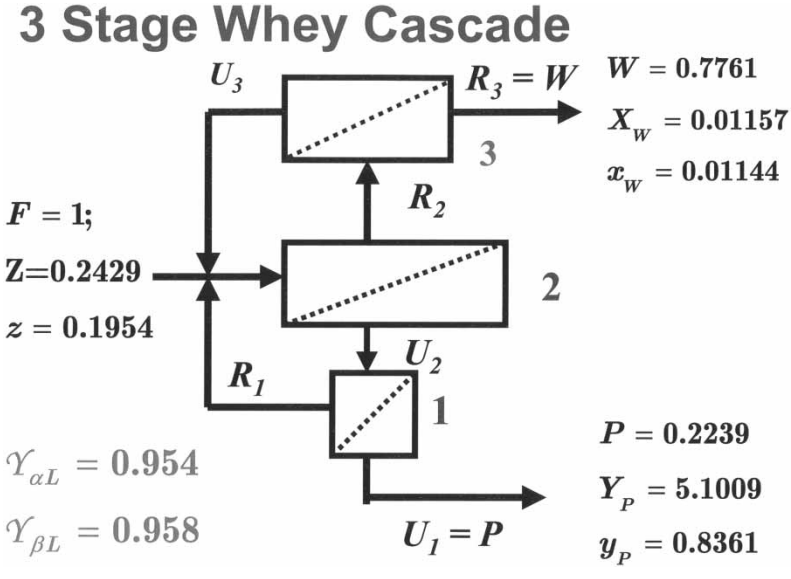

Figure 5. A three-stage whey cascade.

note that stage "1," that producing the product, is now at the bottom of the figure. We introduce this simplest complete cascade because we believe it is all that we should deal with until enough process development has taken place to design more complex systems with confidence, and we concentrate our attention here on the stage-to-stage recursion relationships. We will avoid blending streams of differing *solute* composition in accordance with ideal cascade theory and therefore require that

$$X_1 = Y_3 = Z \tag{9}$$

Here X_1 is the composition of the retentate, R_1, rising in the diagram from stage "1," Y_3 is the composition of the permeate or ultrafiltrate, U_3, descending from stage 3. Using the definition of stage separation factor we can complete the specification of terminal stream compositions:

$$Y_P = Y_1 = \Phi Z; \quad X_w = X_3 = Z/\Phi \tag{10, 11}$$

More generally

$$Y_n = \Phi X_n = \sqrt{\Phi} Y_{n+1} \tag{12, 13}$$

as numbered in the figure. We now go on to complete the mass balances for the system as a whole:

$$F = P + W; \quad zF = y_P P + x_W W \tag{14, 15}$$

or

$$z = \theta y_P + (1 - \theta) x_W \tag{16, 17}$$

It now only remains to calculate the two remaining intermediate compositions by making similar balances about the top and bottom stages. We now illustrate these procedures by examining a specific example.

Example: Fractionation of Lactalbumins

Assume by way of example the system described in Table 1 and use α-lactalbumin as the product in a mixture with β-lactalbumin:

$$\Phi = 21 \quad \text{and} \quad Z = 0.1071/0.441 = 0.2429 \tag{18}$$

These conditions correspond to the system of Cheang and Zydney (2) for their 30 KDa membrane, and they permit comparison with the results of these investigators. Note that the β-lactoglobulin exists primarily as a dimer.

Table 1. Whey isolates

Protein	100 KDa membrane S	100 KDa membrane Φ	30 Kda membrane S	30 Kda membrane Φ	Conc. g/L	Mol. Wt, KDa	Conc. mm
α-lact.	0.67	—	0.23	—	1.5	14	0.1071
β-lact.	0.16	4.2	0.011	21	8.0	18	0.441
BSA	0.009	74	0	∞		67	

System Mass Balances

We begin by defining the input to the system using a solvent-free feed rate of one milli-mole per minute (abbreviated as mmols/min) (Fig. 6). Then in these units:

$$F = 1; \quad Z = 0.1071/0.441 = 0.2429;$$
$$z = 0.2429/1.2429 = 0.1954 \qquad (19,\ 20,\ 21)$$

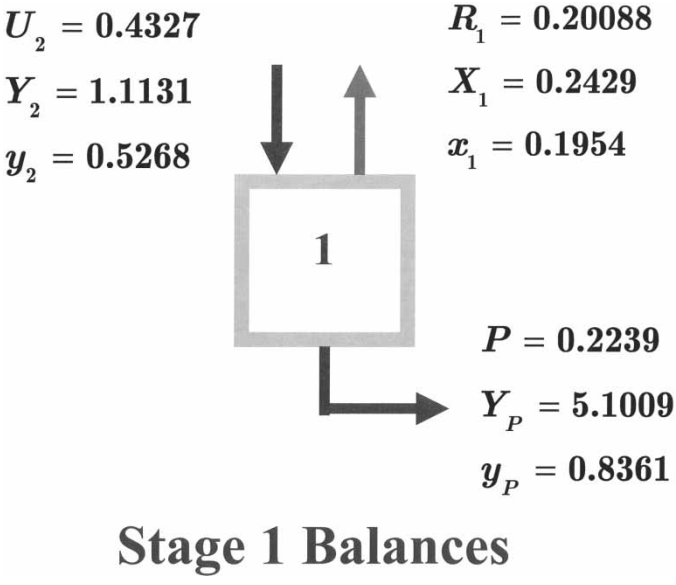

Figure 6. Stage 1 mass balances.

We next note that for an ideal cascade

$$Y_3 = X_1 = Z = 0.2429; \quad y_3 = x_1 = z = 0.1954 \qquad (22,\ 23,\ 24)$$

and

$$X_w = X_3 = Y_3/21 = 0.01157; \quad x_3 = x_W = 0.01144 \qquad (25,\ 26)$$

while

$$Y_P = Y_1 = 21X_1 = 5.1009; \quad y_P = 0.8361 \qquad (27,\ 28)$$

We are now ready to calculate the α-lactalbumin yield, and this requires making two mass balances on the cascade. We shall follow convention in writing one for total moles and the other for α-lactalbumin, all on a solvent-free basis:

$$F = P + W; \quad zF = y_P P + x_W W \qquad (29,\ 30)$$

These equations can be combined to give

$$z = y_P\theta + x_W(1-\theta); \quad \theta = P/F \qquad (31)$$

$$\theta = \frac{z - x_W}{y_P - x_W} = \frac{0.1954 - 0.0144}{0.8361 - 0.0144} = 0.2239 = P \qquad (32,\ 33)$$

$$= 1 - W; \quad W = 0.7769$$

This quantity is known as the fractional cut for the separation. The yield of α-lactalbumin is then

$$\Upsilon_\alpha = \frac{\theta y_P}{z} = (0.2239 \cdot 0.8361)/0.1954 = 0.958 \qquad (34)$$

The yield of β-lactalbumin, obtained with a purity of 0.988 is

$$\Upsilon_\beta = (1-\theta)x_3/z = (1 - 0.2239) \cdot 0.989/0.8046 = 0.954 \qquad (35)$$

These are the key findings of the example, but it remains to be seen what is needed to obtain them.

Stage Mass Balances

We now calculate the intermediate stream rates and compositions that will be needed later in calculating solvent flows (Fig. 7). We begin by writing from Eqs. (13) and (24) that

$$X_2 = \sqrt{21} \cdot x_W = 4.58 \cdot 0.01157 = 0.05302; \quad x_2 = 0.05035 \qquad (36)$$

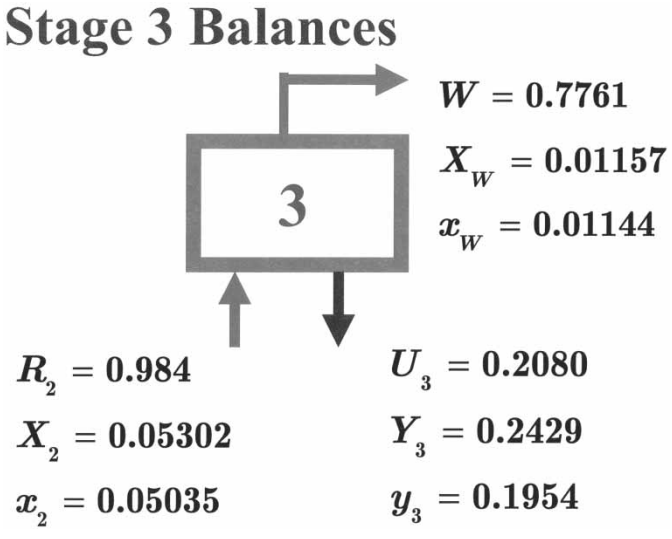

Figure 7. Stage 3 mass balances.

while

$$Y_2 = Y_P/\sqrt{21} = 5.1009/4.5825 = 1.1131; \quad y_2 = 0.5268 \tag{37}$$

Compositions are now complete, and it remains to calculate the (solvent-free) stream rates. We begin with stage 1 and write

$$U_2 = R_1 + P; \quad y_2 U_2 = x_1 R_1 + y_P P \tag{38, 39}$$

It follows that

$$0.5628 U_2 = 0.1954(U_2 - 0.2239) + 0.8361 \cdot 0.2239 \tag{40}$$

or

$$(0.5268 - 0.1954)U_2 = 0.1872 - 0.1954 \cdot 0.2239$$

$$U_2 = \frac{0.1872 - 0.04375}{0.5268 - 0.1954} = 0.4327 \tag{41, 42}$$

Then

$$R_1 = 0.4327 - 0.2239 = 0.2088 \tag{43}$$

We now turn our attention to stage 3 and write

$$\begin{aligned} R_2 &= U_3 + W = U_3 + 0.7761; \\ 0.05035R_2 &= 0.1954U_3 + 0.01144 \cdot 0.7761 \\ &= 0.1945(R_2 - 0.7761) + 0.00888 \end{aligned} \qquad (44,\ 45,\ 46)$$

Then

$$R_2 = \frac{0.1954 \cdot 0.7761 - 0.0089}{0.1954 - 0.05035} = 0.984 \qquad (47,\ 48)$$

$$U_3 = 0.984 - 0.7761 = 0.2080$$

This completes specification of all streams on a solvent-free basis.

Solvent Flows

We now reach a point of great flexibility: there is no a priori requirement for using any particular solute concentration or even to use the same concentrations in all stages. Only the ratio of the two proteins is important for operating on a solvent-free basis. Moreover, since the only streams returning to the feed stage, retentate from stage 1, and ultrafiltrate from stage 2, have the same solvent-free composition as the feed, one can even operate completely in a batch mode. We return to this point shortly.

This flexibility relaxes constraints on solute concentrations, and one possible strategy presents itself immediately: always operate at the maximum concentration permitted by the nature of the system under study. Factors affecting the choice of permissible concentration include viscosity and the presence of low-molecular weight impurities. The choice of concentration is then an economic one and can be different for each stage. However, there is an advantage to simplicity so we will assume identical concentrations for all streams in our numerical example. Moreover, we shall take those of Cheang and Zydney to simplify comparison with their paper.

As pointed out by these authors the use of diafiltration inherent in our process is an important purification step: it removes low-molecular weight impurities. Thus one will frequently be able to use higher than feed concentration in all stages, including the feed stage. This latter fact is because the composition within a well mixed stage is that of the exit from the stage, x_2 in our nomenclature, not the feed composition z. Diafiltration through this stage will have removed a large fraction of low-molecular weight impurities originating in the feed.

Solvent Flows for Uniform Solute Concentration

Remembering that our basis is one milli-mole (mmol) of protein feed per minute we may now calculate total stream rates, as suggested in Fig. 8, through our system. Beginning with the combined streams to the feed stage, 2, we may write that the molar rate of protein transport into stage 2 is

$$M_F = F + U_3 + R_1 = 1 + 0.2080 + 0.2088 = 1.4168\,\text{mmols/min} \quad (49)$$

and the total molar concentration is

$$c_{tot} = 0.548\,\text{mmols/L} \quad (50)$$

Then the volumetric flow rate of solution to stage 2 is

$$Q_F = 1.4168/0.548 = 2.585\,\text{L/ min} \quad (51)$$

The corresponding flows of retentate and ultrafiltrate are

$$Q_R = 0.984/0.548 = 1.796\,\text{L/ min}$$

$$Q_u = 0.437/0.548 = 0.797\,\text{L/ min} \quad (52,\ 53)$$

We next write for the rate of protein transport across the stage 2 membrane

$$M = Avc[x_{\alpha L}S_{\alpha L} + (1 - x_{\alpha L}S_{\beta L})] = 0.4327\,\text{mmols/ min} \quad (54)$$

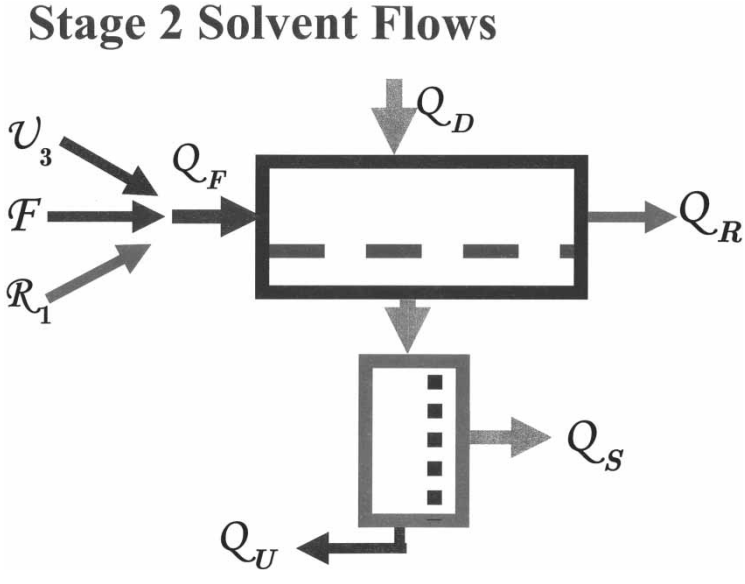

Figure 8. Stage 2 solvent flows.

or

$$Q_D = Av = \frac{0.4327}{0.548 \cdot 0.1264} = 6.247\,\text{mmols/min} \tag{55}$$

It follows that the amount of solvent that must be removed by the secondary membrane is

$$Q_S = Q_D - Q_U = 6.247 - 0.797 = 5.5\,\text{L/min} \tag{56}$$

Flows across the other two stages can be calculated similarly.

Note once again however that these are only representative numbers to illustrate the procedures that must be followed. Control of solvent flows must be determined by the designer to suit the system and process requirements.

Batch Operation

One may operate this system as a batch process in which feed from a storage tank (at left in Fig. 9) is introduced to an appropriately sized UF module and the two output streams are fed to two additional tanks, one for the α-L rich stream, the ultrafiltrate, and one for the β-L rich stream, or retentate Then, at one's leisure one can process these two intermediate streams. One thus

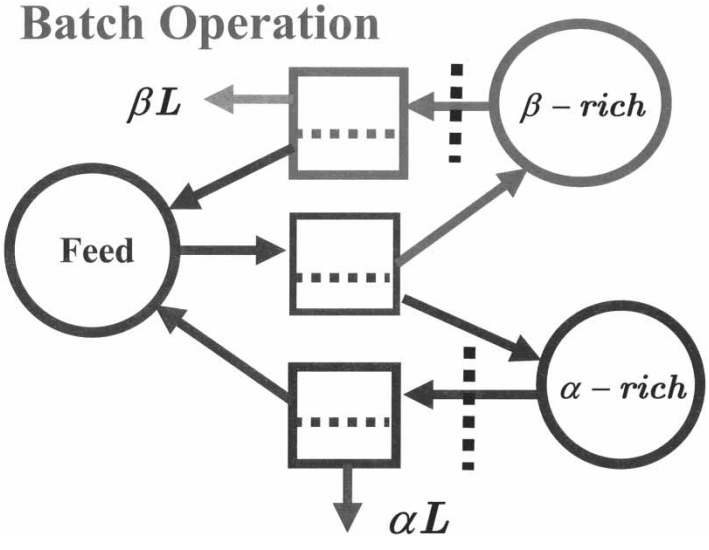

Figure 9. Batch operation.

sends the ultrafiltrate of the β-L rich tank back to the feed tank and sends the retentate out as purified β-*L*. Correspondingly one sends the retentate from the α-*L* tank to the feed tank and the ultrafiltrate out as purified α-*L*. In this way the overall process is broken down into three simpler components each closely related to a standard diafiltration.

Converting to continuous operation only requires increasing the flow rates from the three tanks to maintain their volumes constant. Each can moreover function as a surge tank to absorb inevitable fluctuations in flow.

The next major problems, which we shall not discuss here in any detail, are those of actual operation. Suitable pumps must be found capable of independently controlling the many process streams, and a control strategy must be developed to synchronize their operation if continuous processing is to be successful. The controllers in turn require both protein and solvent flows as input, and this requirement in turn suggests that one must measure both flow rate and protein concentration and obtain protein flows by multiplying these two. It will be desirable to work out most of these problems in batch operation.

Simpler Alternates

The careful reader might well ask how the above cascade compares with the simpler two-stage systems of Chang and Zydney. What these authors show is essentially a modification of our Fig. 5 in which no recycle is returned to the feed. This is *qualitatively* equivalent to discarding streams U_3 from stage 3 and R_1 from stage 1, and the result is a loss of yield for both product streams. It is this avoidance of "loose ends" that has always been the advantage of reflux cascades. A major advantage of the above development is to show that proper choice of stream rates can make it possible to return blend these streams and return the blend as feed: the batch process just described. Difficulty of process control has always been a problem for biological membrane processes, and it is this author's belief that the next commercial step will be the properly designed batch process. Only when it has been thoroughly debugged will it be sensible to "throw the switches" and go to a truly continuous process.

These comment should not be construed as a criticism of Cheang and Zydney for whom this author has the greatest respect. It was simply not their purpose to produce an optimized process.

CONCLUSIONS

It has been shown that binary ideal cascade theory can be extended to systems of two solutes in a single solvent.

The basic unit or module in the modified cascade is a novel splitter consisting of a diafiltration unit combined with an ultrafiltration unit operating on the permeate from the diafilter. The diafiltration membrane is selective for one of the two solutes, and the ultrafiltration membrane passes only the solvent.

It has been shown how the filtration rates through these membranes can be controlled so that individual modules can be combined and operated to conform to ideal cascade theory for fractionation of the two solvents from one another.

An example is provided using experimental data in the reviewed literature for the simplest case of a three-stage cascade.

It is shown that a three-stage cascade can be operated in batch mode, a new development.

NOMENCLATURE

F	molar rate of protein feed to system, M/t, Eq. (6)
$\mathcal{F}$	volumetric total feed rate, ℓ^3/t, Fig. 8
"i"	denotes any species
P	molar rate of protein in product stream, M/t, Eq. (6)
$\mathcal{P}$	volumetric total product rate, ℓ^3/t, Fig. 8
W	molar rate of protein in waste stream, M/t, Eq.
$\mathcal{W}$	volumetric total waste rate, ℓ^3/t, Fig. 8
Q_i	miscellaneous volumetric flow rates, ℓ^3/t, Fig. 8
X_i	mole ratio of desired to undesired solute in retentate from stage "i", dmls, Eq. (1)
x_i	corresponding mole fraction
Y_i	mole ratio of desired to undesired solute in permeate from stage "i", dmls, Eq. (1)
y_i	corresponding mole fraction
Z_i	mole ratio of desired to undesired solute in retentate from stage "i", dmls, Eq. (1)
z_i	corresponding mole fraction

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