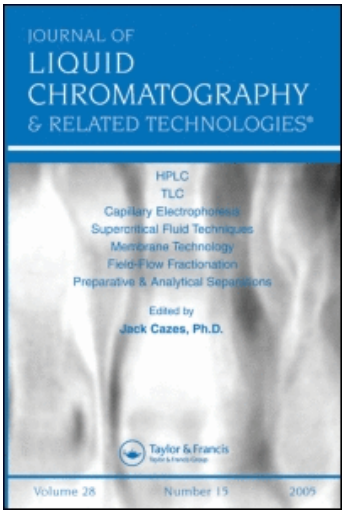

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AN ECONOMIC ANALYSIS OF PERFORMANCE IN PREPARATIVE CHROMATOGRAPHY OF PROTEINS

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SUMMARY

An economic analysis of preparative chromatography of proteins is reported. We present a way to calculate and optimize the efficiency for isolating a desired protein from a given protein mixture with regard to feed, fractionation, product purity, throughput, and operating costs. Evaluation of the overall efficiency for the purification in subsequent steps is also demonstrated.

INTRODUCTION

Performance in chromatographic systems is often measured in terms of either theoretical plates/unit time or resolution/unit time. The concept of "peak capacity," i.e. the maximum number of peaks that a system is

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theoretically capable of producing is also used to specify the performance of chromatographic systems. However, it should be noted that these measures of performance are generally applied to analytical systems where it is the objective to resolve a large number of components in a short time.

The objectives in preparative chromatography are quite different. In preparative separations, a single component is usually being purified and resolution of a large number of components from each other is unimportant. In general, the aim of preparative chromatography is to produce the largest possible amount of product in the shortest possible period of time. So throughput is the most important chromatographic variable in preparative chromatography. The second major variable is system economics. The final goal of most production processes is the profit, which depends strongly on production costs. Consequently, optimization of cost parameters also should be taken into account when setting operational variables for preparative chromatography of proteins.

Chromatography processing costs will be a function of the cost of the system, the loading capacity of the column and the system cycle time. For example, commitment of extensive labor and costly equipment to a lengthy separation on a low capacity column could be economically non-viable. This must be reflected in evaluation of the efficiency of preparative systems.

The efficiency would depend on a wide range of system parameters and operational variables. Because the chromatographic procedures required for purification are sample dependent, the importance of different parameters and variables depends on the given separation problem. In this paper we suggest an economic model for evaluating the efficiency of preparative chromatography columns which is based on feed, product purity, product yield, "clean-up" ratio, throughput and operating costs.

Purification of proteins from complex biological samples generally requires multiple steps. In addition to evaluating the efficiency of individual purification steps, there is also a need for the evaluation of

complete purification processes. This issue will also be addressed in this paper.

THEORETICAL

Efficiency (E) of preparative chromatography will be defined here as the profit achieved per unit time. This concept is reflected in the equation:

$$E = \frac{P}{t} \tag{1}$$

where P is the profit achieved in a chromatographic separation and t is the time of a chromatographic cycle.

Profit may be expressed as:

$$P = V_p - V_o - C \tag{2}$$

where $V_p = v_p m_p$ and is the value of product, v_p is the specific value of product and m_p is the mass of product. The value of initial protein mixture loaded (feed) (V_o) is given by the equation: $V_o = v_o m_o$, where v_o is the specific value of the mixture and m_o is the mass of initial feed. The cost of the chromatographic procedure (C) may be related to the specific cost (value/g) of the chromatographic procedure with respect to product (c) and mass of product (m_p) by the expression $C = cm_p$.

In this manner, efficiency can be written:

$$E = Tp(v_p - c) - \frac{v_o m_o}{t} \tag{3}$$

with $Tp = m_p/t$; where T_p is throughput. The term $(v_p - c)$ is the specific profit separated from the price of feed and is dependent on the separation procedure.

It should be recognized that the mass of product (m_p) from any purification step may be larger than the total mass (m_m) of the desired

protein in the initial sample. This is because resolution may not be sufficiently great to give total purification of the product. It is well established that the resolution of components in a chromatographic system is a function of a number of variables such as theoretical plate number, separation factor, solvent strength, mobile phase velocity, gradient slope, and column dimensions. Although eqn. 3 indicates that the throughput of a system may be increased by increasing m_p, this must not be at the expense of product purity because it reduces v_p exponentially (shown later).

Throughput may also be increased by decreasing cycle time. It will be shown below that cycle time is related to flow rate, gradient slope, recycle time, re-equilibration time and column dimensions. In the case of flow rate and gradient slope, decreasing cycle time by manipulating these variables will be counter-productive if product purity decreases. Obviously, optimizing throughput may be a complex process.

Throughput can be expressed as a function of feed (m_o), mass of impurities removed ($m_o(1-p_o)r$), and loss of desired product ($m_o p_o(1-y)$) as shown in the equation:

$$Tp = m_p/t = \frac{m_o - m_o(1-p_o)r - m_o p_o(1-y)}{t} \qquad (4)$$

where $p_o = m_m/m_o$ and is the purity of initial feed, m_m is the mass of desired protein in the feed, r is the clean up ratio (mass of impurities removed/total mass of impurities in the feed) and y is the yield of desired protein.

Cycle time in gradient elution chromatography may be expressed by the equation:

$$t = t_{eq} + t_e + t_{rc} + t_{reg}/n \qquad (5)$$

where t_{eq} is the equilibration time, t_e is the feeding and elution time, t_{rc} is the recycle time, t_{reg} is the regeneration time and n is the number of separation cycles between two regeneration steps.

After an elution protocol which gives the desired resolution between product and impurities has been established, only recycle time, equilibration time and regeneration time may be manipulated to reduce cycle time [1]. Since recycling, re-equilibrating and regenerating columns are processes not directly involved in the chromatographic process, losses in chromatographic performance while manipulating these variables are remote.

Implementation of preparative chromatography starts with two important steps: column selection, i.e. planning, and optimization. In the column selection stage the following should be decided:

- which type of separation mechanism will be used, i.e. reversed phase, affinity, ion-exchange, hydrophobic interaction, size-exclusion, etc. [2,3],
- what dimension of column will be used, i.e. inner diameter, length, etc. [4], and
- what properties should the sorbent have that will be used, i.e. particle size, pore size, ligand density, etc. [5,6,7,8].

What is decided here determines both the limits in the optimization process and specifications of the instrumentation.

Optimization on a given column involves finding the optimal set of operational (independent) variables, such as:

- feed load [9,10],
- composition of mobile phase and slope of the gradient [10],
- flow rate,
- temperature [12], and
- fractionation.

FRACTIONATION

The complete set of engineered parameters and variables has an affect on the resolution of the desired protein. To achieve maximum throughput and

constant purity in a given preparative chromatography system, resolution of the product protein from impurities should be maximized while holding cycle time at a minimum. In order to make the relation between throughput and and resolution less ambiguous, the clean up ratio (r) is expanded into its component parts:

$$r = \sum_{i}^{n} r_i \tag{6}$$

where r_i is the clean up ratio relative to the i^{th} impurity, and n is the number of impurities. Figure 1 provides an illustration of a mixture in which the desired protein (component 5) is only partially resolved from components 4 and 6 in several of the chromatograms. The various chromatograms in the figure represent what might happen to resolution as particle size is increased from A to D. Although these are synthetic chromatograms and do not represent the actual separation of components from a specific mixture, it is similar to what the authors have observed on columns packed with 10, 20, 50 and 100 μm particle sizes, respectively. It is interesting that all of the components of the mixture other than 4 and 6 are completely resolved from component 5 by all of the columns. This means that in the early stages of most purification procedures where one encounters very crude samples with many components that most columns will give extensive purification. It is also seen that the use of microparticulate columns with large numbers of theoretical plates will not really be necessary in the initial stages of purification.

Resolution of any two solutes in a mixture is given by the equation

$$R_s = 2[(t_{R_2} - t_{R_1})/(\Delta t_{R_2} + \Delta t_{R_1})] \tag{7}$$

where t_R is retention time and Δt_R is peak width in time. When $R_s >$ 1.3 the two components are completely resolved. Obviously this is the case for the resolution of components 1, 2, 3, 7 and 8 from component 5.

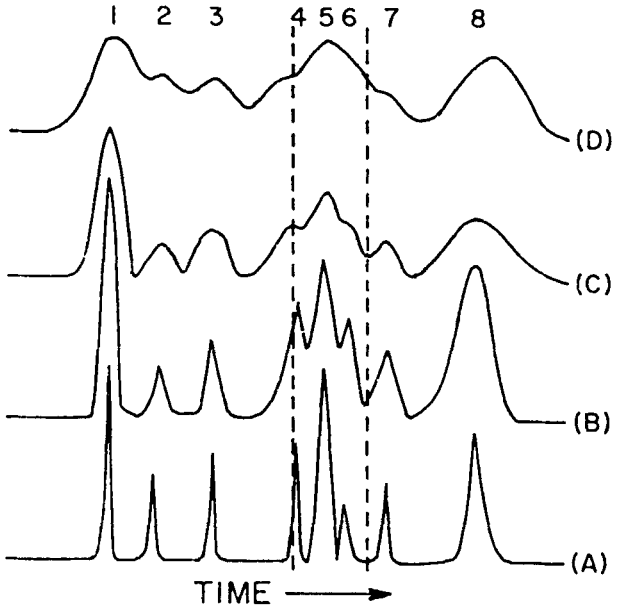

Figure 1. An illustration showing the relative purification of a compound (No. 5) on columns of different resolving power. From D to A resolution is increasing. In this case an increase in resolution decreases the time window of collection, keeping yield of desired protein constant. See future details in text.

Calculation of the amount of components 4 and 6 coeluting with component 5 will be treated below.

The area (A) of any peak in a chromatogram is given by the equation

$$A = K \int_{t=0}^{T} C_c dt \qquad (8)$$

where C_c is the concentration at time t, dt is the time increment, K is a constant relating mass of component to area, and τ is the time at the end of the peak.

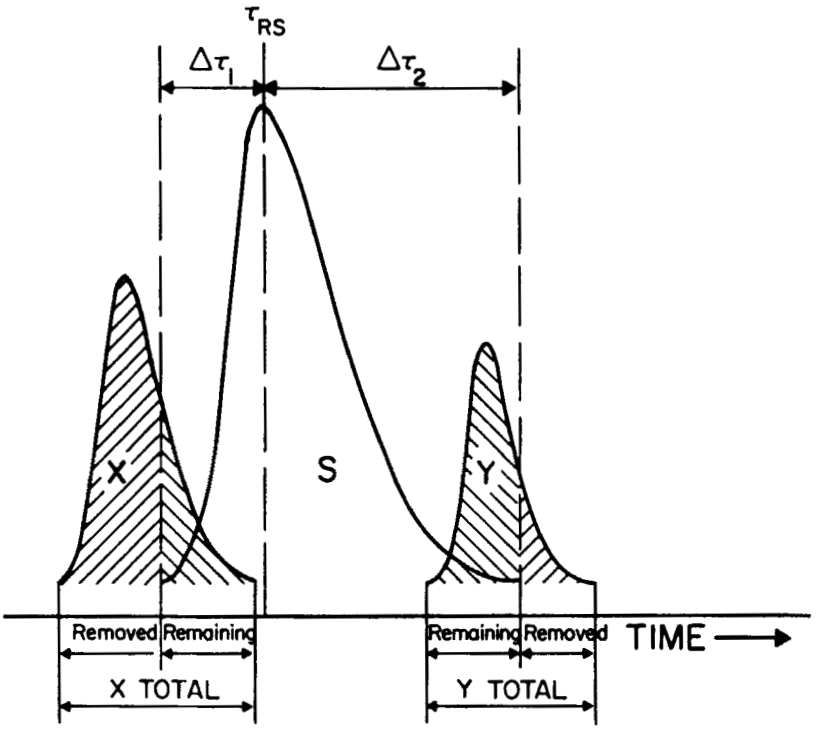

Figure 2. Illustration showing the expansion of clean up ratio into component parts. S is the desired protein; X and Y are impurity components; and $(\Delta\tau_1+\Delta\tau_2)$ is the time window of collection.

An illustration of overlapping peaks is given in Figure 2. It is seen for component X that only a fraction (X removed) of the total mass of X is eliminated when solute is collected between $t_{RS} - \Delta\tau_1$ and $t_{RS} + \Delta\tau_2$. Thus, the relative mass removed of component X would be f_X:

$$f_X = K_X/A_X \int_{t=0}^{t_{RS}-\Delta\tau_1} C_{cX}\,dt \qquad (9)$$

where A_X is the total area for component X. The clean up ratio for component X (r_X) would be

$$r_X = m_X f_X/(m_X+m_Y) \tag{10}$$

where m_X and m_Y are the total mass of component X and Y.

The term r_Y would be computed in a similar manner as r_X using equations 9 and 10.

$$r_Y = m_Y K_Y/A_Y \int_{t_{RS}+\Delta\tau_2}^{t_T} C_{cY}dt/(m_X+m_Y) \tag{11}$$

where t_T is the total elution time for all components.

Combining equations 6 and 10, the clean up ratio (r) can be expressed in the following way:

$$r = \sum_{i=1}^{n} r_i = \sum_{i}^{n} \frac{m_i f_i}{\sum_{j=1}^{n} m_j} \tag{6/a}$$

where m_j is the mass of the j^{th} impurity.

To increase purification requires either an increase in resolution or a decrease in the time window ($\Delta\tau_1 + \Delta\tau_2$) of collection. Although purification is increased by narrowing the collection time window, yield is diminished. Equation 7 indicates that resolution may be increased by increasing the ratio of the distance between peak maxima to peak width. In gradient elution, resolution is generally increased by decreasing gradient slope. The most general technique for decreasing gradient slope is to increase gradient time at a fixed flow rate. Although this is accompanied by some increase in peak width, distance between peaks increases more than band dispersion. Increasing mobile phase velocity at the same fixed gradient time is a second technique for decreasing gradient slope. Increasing flow rate to increase resolution would seem to be counter productive since band dispersion increases with flow rate. Although this is true, there are a number of cases where the distance between peaks increases faster than peak dispersion.

The planning and optimization of system parameters and operational variables in a chromatographic system, should be done in combination with the optimization of the specific cost components (see: Cost Analysis). The profit parameter must be optimized concomitantly with the specific cost. Among all possible ways of operating chromatographic systems there exists one which results in minimum cost and maximum profit with a given set of market conditions [13]. Hence, not only does the throughput function have a maximum, but specific cost and specific profit show optima as a function of any independent variable. Since the specific cost of the feed load is independent of the chromatographic procedure the maximum efficiency would be determined by optimization of the first term in equation 3. Therefore, the maximum efficiency as a function of all independent variables can be expressed by the following equation:

$$\sum_{i=1}^{n} (\partial[T_p(v_p - c)])/\partial X_i = 0 \qquad (12)$$

where X_i is an independent variable and n is the number of independent variables. There are well established methods to determine the optimum values of the variables [14].

PURIFICATION IN SUBSEQUENT STEPS

In many cases it is impossible or not cost-effective to achieve the desired purity in a single chromatographic step, so that several chromatographic procedures have to be applied consecutively [15].

The total efficiency of purification in subsequent steps may be expressed as:

$$E = \frac{\sum_{i=1}^{n} p_i}{\sum_{i=1}^{n} t_i} = \frac{v_n m_n - v_o m_o}{\sum_{i=1}^{n} t_i} - \sum_{i=1}^{n} Tp_i c_i \qquad (13)$$

where P_i is the profit of the i^{th} chromatographic step, t_i is the cycle time of the i^{th} chromatographic step, Tp_i is the throughput of the i^{th} chromatographic step, c_i is the specific cost of the i^{th} chromatographic step, v_n is the specific value of the product of the n^{th} (final) step, m_n is the mass of the product of the n^{th} (final) step, v_o is the specific value of initial feed, m_o is the mass of initial feed, and n is the number of chromatographic steps.

Eqn. 13 shows that the total efficiency of a purification in subsequent steps depends on the difference in value of the final product and the initial feed, the sum of cycle times and the total cost of the chromatographic steps.

Total efficiency is independent of the values of intermediate products. The mass difference between the initial feed and the final product, the sum of cycle times, and the total cost of the procedures depend on the quality of the design and optimization of the total system. If the mass of product of the first separation step is m_1 (see eqn. 4):
where

$$m_1 = m_o[1-r_1(1-p_o)-p_o(1-y_1)] \tag{14}$$

and the purity is p_1 where

$$p_1 = m_m y_1/m_1 = p_o y_1/(1-r_1(1-p_o)-p_o(1-y_1)), \tag{15}$$

then, the mass (m_i) and purity (p_i) of the i^{th} product are given by the equations

$$m_i = m_o \prod_{j=1}^{i-1} [1-(1-p_o)(1-r_j)r_i-p_o y_j(1-y_i)] \tag{16}$$

$$p_i = \prod_{j=1}^{i-1} [p_o y_j y_i/(1-(1-p_o)(1-r_j)r_i-p_o y_j(1-y_i))] \tag{17}$$

The throughput of the i^{th} separation step is Tp_i, where

$$Tp_i = m_i/t_i = m_o/t_i \prod_{j=1}^{i-1} [1-(1-p_o)(1-r_j)r_i-p_o y_j(1-y_i)] \tag{18}$$

Eqn. 18 indicates that the throughput of subsequent steps depends on fractionation. (As was seen earlier, fractionation depends on the resolution of the desired protein.) Assuming that product value is exponentially related to purity and that this reflects the cost of the purification procedure (being generally known as an exponential function of purity), we can write the following equation:

$$c_i = a_i e^{b_i p_i} = a_i \exp[b_i \prod_{j=1}^{i-1} [p_o y_j y_i/(1-(1-p_o)(1-r_j)r_i - p_o y_j(1-y_i))]] \qquad (19)$$

where c_i is the specific cost of the i^{th} step and a_i, b_i are constants.

The specific value of the initial feed load and the final product will depend on the market. By keeping them constant, the maximum efficiency would be determined by calculation of the minimum cost (second term in eqn. 13). The minimum cost of purification in subsequent steps as a function of fractionation is shown in the equation

$$\sum_{k=1}^{n} \frac{\partial \sum_{i=1}^{n} Tp_i c_i}{\partial r_k} + \sum_{k=1}^{n} \frac{\partial \sum_{i=1}^{n} Tp_i c_i}{\partial y_k} = 0 \qquad (20/a)$$

where n is the number of chromatographic procedures. Solution of Eqn. 20/a results in the optimum throughput of consecutive steps. Rearranging eqn. 20/a we may write

$$\sum_{k=1}^{n} \frac{\partial \sum_{i=1}^{n} Tp_i c_i}{\partial r_k} = -\sum_{k=1}^{n} \frac{\partial \sum_{i=1}^{n} Tp_i c_i}{\partial y_k} \qquad (20/b)$$

Eqn. 20/b shows the simple fact that r may be increased at the expense of y and vice versa, i.e., in this way the fractionation has an optimum. Furthermore, in eqn. 20/b it can be seen that the optimum for a process is

not the sum of optima for the individual purification steps, but an overall optimum for the total system.

In the treatment presented above for purification of proteins in subsequent steps, Bellmann's principle of optimality was used to optimize the overall process and to determine the maximum total efficiency [16].

To correctly set the parameters and variables mentioned above, the specific cost components that determine the total specific cost of a given chromatographic procedure should be examined in detail.

COST ANALYSIS

For a more convenient treatment of the cost components, the total specific cost for processing a gram of feed mixture (c_{tot}) will be used instead of total specific cost for a gram of product (c). The relationship between these terms is expressed by the equation

$$c_{tot} = (m_p/m_o)c \tag{21}$$

The total specific cost (c_{tot}) for processing a gram of a mixture is given by the equation

$$c_{tot} = c_{col} + c_{sol} + c_{ins} + c_{mai} + c_{lab} \tag{22}$$

where c_{col} is the cost contribution from the column, c_{sol} is for solvents, c_{ins} for the instrumentation, c_{mai} is the maintenance contribution and c_{lab} is for labor. A further understanding of processing costs may be derived by analyzing each of these variables separately.

1.1 Column Costs. The specific column cost (c_{col}) is given by the equation

$$c_{col} = v_g \cdot C_V \cdot t/t_L \tag{23}$$

where v_g is the volume of column required to chromatograph 1 g of the mixture, C_V is the cost/unit volume of a column, t is chromatographic cycle time and t_L is the average lifetime of a column. The cost/unit volume of column is given by the equation

$$C_V = (H_c + P_f + P_l + P_t)/V_c + s \cdot d_s \quad (24)$$

where V_c is the total volume of the column, H_c is the column hardware cost, P_f is the fixed cost of packing a column (solvents and packing equipment are in this category), P_l is labor cost for packing a column, P_t is the cost of testing a packed column, s is the cost of a unit mass of support and d_s is packing density of the column in g/ml. These terms will vary with the dimensions of the column, support particle size and the way a column is used. For example, end fittings are a major cost that is more a function of diameter than length. Two columns of the same diameter varying 3-fold in volume will not be very different in cost because the major contribution to cost is the machine work that goes into the flanges and fittings at the ends of the column. Reusing columns will also diminish the cost of column hardware; i.e.

$$H_c = H_i/n_p \quad (25)$$

where H_i is the initial cost of the column and n_p is the number of times it will be packed. Assuming that a column will be reused many times, the hardware contribution to column cost will be much less than the labor of packing (P_l) and testing (P_t) a column. For example, packing and testing are the dominant costs in analytical columns. It has also been found in small preparative columns (<100 ml volume) that packing a column twice as large does not require twice the labor. As column volume increases it is likely that P_l/V_c and P_t/V_c will decrease. At the present time there is insufficient literature on packing preparative columns for a quantitative relationship to be established between V_c, P_l and P_t.

The specific cost of packing material (s) is composed of three components;

$$s = s_s + s_d + s_i \quad (26)$$

synthesis of the basic support material (s_s), derivatization of the support matrix with stationary phase (s_d) and particle sizing (s_i). It is often the case in the synthesis of a support material that a broad range of particle sizes is produced. Since narrow particle size distribution is required for chromatography, support materials must be subjected to a sizing process before use. The distribution of particle sizes within a synthetic lot of support materials influences both s_s and s_i.

Particle sizing may be achieved by a variety of techniques such as elutriation, air classification and sieving. It is generally accepted that there is a disproportionate increase in the production cost of smaller particle size supports. Although elutriation provides particles of narrow size distribution it is a method of relatively low throughput. Air classification is the most practical technique for obtaining narrow particle size distribution in the range from 5-30 μm. In contrast, sieving is of high throughput and low cost in the production of supports over 40 μm. Beyond 60-80 μm the cost of sizing supports is almost independent of size.

Loading capacity of a support material may be specified in several ways. The volumetric term (v_g) used in equation 23 is defined here as the volume of column required to chromatograph 1 g of sample. When peak width has increased more than 10% beyond an analytical load, columns are considered to be overloaded. Column geometry, equilibration kinetics, efficiency with which the sample is distributed on the column and other operational factors all influence v_g. In contrast, static loading capacity (L_c) measurements in grams of protein adsorbed/ml of support measure the intrinsic loading capacity of a packing material without regard to operational parameters. As the intrinsic loading capacity (L_c)increases, it is apparent that the

volume (v_g) of a support required to separate 1 g of a sample will decrease. With high performance ion exchange supports it has been found [17] that the dynamic loading capacity of a support is approximately 10% of the intrinsic loading capacity, i.e.:

$$v_g = K_l/L_c \tag{27}$$

where K_l is a constant relating v_g to L_c.

Combining equations 24, 25 and 27 and substituting into equation 23 produces the expression

$$c_{col} = \frac{tK_l}{t_L L_c}\left[(H_i/n_p + P_f + P_l + P_t)/V_c + d_s(s_s + s_d + s_i)\right] \tag{28}$$

1.2 Solvent Costs. It is now well established that the separation of macromolecules in surface mediated modes such as ion exchange, reversed phase, hydrophobic interaction and bioaffinity require gradient elution. Linear gradients are the most common. Occasionally, initial and final solvent holds and special recycling techniques are also used. In addition, special solvent washing or cleaning steps are used at intervals in the separation of a large number of compounds. A general expression for specific solvent costs (c_{sol}) would be

$$c_{sol} = c_A + c_B + c_{R1} + c_{R2} + c_{CL} \tag{29}$$

The cost of the initial A solvent (c_A) will be

$$c_A = F_A \cdot c_{vA}(t_A + t_G/2)v_g/V_c \tag{30}$$

where F_A is the flow rate of A in ml/min, C_{vA} is the average cost/unit volume of solvent A, t_A is the initial time held at pure A, and t_G is gradient time. The same general expression would apply to solvent B

$$c_B = F_B \cdot c_{vB}(t_G/2 + t_B)v_g/V_c \tag{31}$$

where t_B is time held at pure B at the end of the gradient, F_B is the flow rate of B and c_{vB} is the cost/volume of B. In those cases where a linear programmed recycle of two solvents is used, the equations

$$c_{R1} = F_1 \cdot c_{v_1} \cdot (t_R C/2) v_g/V_c \tag{32}$$

and

$$c_{R2} = F_2 \cdot c_{v_2} \cdot (t_R C/2) v_g/V_c \tag{33}$$

may be used. The terms C_{R1} and C_{R2} represent the costs of recycle solvents 1 and 2 respectively while t_{RC} is recycle time, F_1 is the flow rate of solvent 1, F_2 is the flow rate of solvent 2, c_{v_1} is cost/volume of solvent 1 and c_{v_2} is the cost/volume of solvent 2. Regeneration or cleaning costs are treated with the equation

$$c_C = F_C \cdot c_{vC}(t_c/n) v_g/V_c \tag{34}$$

where it is assumed that a column may be cleaned with a single solvent, C_{vC} is the cost/volume of solvent, F_C is flow rate of cleaning solvent, t_C is the time required to clean the column, and n is the number of column cycles between cleaning.

1.3 Instrument Costs. Although the major cost in the acquisition of preparative scale systems is instrumentation; column hardware, plumbing systems, and electronic components last for many years. The specific instrument costs (c_{ins}) may be calculated from

$$c_{ins} = 0.9\ I_i(t/t_I) v_g/V_c \tag{35}$$

where I_i is the initial instrument cost, t is cycle time and t_I is the lifetime of the instrument. It is assumed that 10% of the potential operating time of the system will be spent in maintenance and repair of the system. For example, cleaning and replacing columns is considered maintenance and repair.

1.4 Maintenance Costs. Using the value of 10% for maintenance and repair and assuming that it may be accomplished by a single individual, greater than 2 hr of labor a day must be charged to the system. Since it is probable that the time required to maintain and repair systems of 100 ml and several liters of column volume will almost be the same, one can write

$$c_{mai} = (W + I_i)0.1(t/t_I)v_g/V_c \quad (36)$$

where W is total labor cost of maintaining the system. It is possible that larger systems will be more economical because throughput increases while maintenance costs remain relatively the same.

1.5 Operational Costs. Labor costs for operating the system will depend to a large extent on the degree of automation in the system. At any rate, the amount of time required to operate both a large and small system should be relatively the same. As in the case of c_{lab}, this again favors larger systems.

DISCUSSION AND CONCLUSIONS

We have presented an economic treatment for the evaluation of a preparative chromatographic procedure in the isolation of proteins. It is based on "efficiency" in accordance with chemical engineering sciences that takes both technical and economic sides of production into account. The major technical parameter, throughput, is combined with the economic parameters (cost and profit) to produce equations that may be used to optimize a chromatographic procedure. These equations show that the overall efficiency of a process depends on the throughput and the cost of the individual purification steps if the unit mass values of initial feed and final product of desired purity are kept constant. Throughput and cost of individual purification steps are expressed as functions of the fractionation (i.e. clean up ratio and yield

of desired protein) and the purity of product after each step. These expressions were used to optimize the overall process using Bellmann's principle of optimality. As expected, the optimum for the total process is not the sum of the optima for the individual purification steps.

To facilitate the practical application of this economic treatment a detailed cost analysis is presented. This cost analysis treats specific column, solvent, instrument, maintenance, and labor costs for processing one gram of feed. The economic performance of a chromatographic system was shown to be dominated by three factors: the cycle time to column lifetime ratio (t/t_L), the column volume required to process a gram of sample (v_g), and the total volume of the column (V_c). The cycle time to lifetime ratio expresses the reciprocal of the number of chromatographic cycles for the column. It is seen that even very expensive columns can have high economic performance if the cycle number is sufficiently high. Obviously, with respect to cost the most efficient system will be one which produces the largest amount of product (with the largest clean-up ratio and yield of desired protein) and requires the smallest specific column volume (v_g) and cycle time (t). The column volume (V_c) seems to be inversely proportional to the specific cost components. But the column cost, the initial instrument cost, the labor cost of operating and maintaining the system may be disproportional to column volume (or system size), i.e. if the capacity of an instrument is two times larger than that of another, its value is not twice that of the other. Hence the specific cost of column, instrument, and maintenance can be expressed in the following way:

$$c = f(V_c^{-\beta}), \text{ where } 0 < \beta < 1 \tag{37}$$

Good agreement was found between the results presented above and a function

$$c = \alpha K_p^{-\beta} \tag{38}$$

of general validity in process scale operations showing the relationship between specific cost and production capacity (18). The term K_p represents the processing capacity of a unit operation and the terms α and β are constants specific to the process.

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