0273-2289/92/3435—0049$02.20

Optimization of Large-Scale Chromatography for Biotechnological Applications

ADELE P. PESKIN* AND SCOTT R. RUDGE

National Institute of Standards and Technology, Chemical Engineering Science Division, 832.01, 325 Broadway, Boulder, CO 80303

ABSTRACT

An economic evaluation of a chromatographic separation is discussed. The effects of particle size, cycle time, solvent, and column costs are analyzed. With small particles (<20 μm), the cost of the packing can be as much as 99% of the total cost of the process, whereas with large particles (>60 μm), resin costs are less than half of the total. A strong optimum is found between 20–40 μm for maximum productivity, using both Gaussian models and the mass transfer model of Lapidus and Amundson to generate peaks. A new compilation of resin costs, column costs, and pump costs is given.

Index Entries: Chromatography; particle size; optimization.

NOMENCLATURE

a	Knox plate height parameter
b	Knox plate height parameter
c	Knox plate height parameter (eq. [5])
c	Concentration in the mobile phase (g/cm^3)
c_i	Initial concentration in mobile phase (g/cm^3)
c_o	Inlet concentration (g/cm^3)
d_p	Resin particle diameter (cm)
D	Dispersivity (cm^2/s)
D_m	Protein diffusivity (cm^2/s)

*Author to whom all correspondence and reprint requests should be addressed.

k_1	Rate constant for adsorption (s^{-1})
k_2	Rate constant for desorption (s^{-1})
K_{av}	Distribution coefficient
L	Column length (cm)
q	Concentration in the resin phase (g/cm^3)
q_i	Initial concentration in the resin phase (g/cm^3)
t	Time for one cycle(s)
t_r	Retention time for a solute(s)
v	Eluent velocity (cm/s)
V	Column volume (cm^3)
z	Distance in the column (cm)
α	Void fraction of column
μ_1	The first central moment (s)
μ_2	The second central moment (s)
σ^2	Variance (s^2)

INTRODUCTION

Chromatography is the workhorse for product isolation in the biotechnology industry. The ability to achieve high purity of a single component in a multicomponent mixture in a chemically and physically "gentle" environment sets chromatography apart as an applied unit operation in biotechnology. Widespread use of chromatography as an analytical tool in product development laboratories also promotes its use in production scale, since regulatory considerations limit processing options. Although there are several rationales for scaling up process chromatography (*1,2*), there are few economic guidelines (*3,4*) for the selection of resin particle size, column length, and eluent velocity.

Numerous transport models are available for predicting chromatographic elution curves for biochemicals (*5–9*). Although these models have been used in the past to evaluate column performance by calculating the adjustable parameters in the models with real elution data, they may also be used to generate simulated elution curves for specific separation conditions. Many of the solutions can be programmed easily on a personal computer and can be used to generate a large number of simulated results in a short time.

ECONOMIC DATA

There are many choices to be made in the design of a large-scale chromatographic system. Costs of different types of chromatographic equipment, such as resins, columns, and pumps, are available through vendor literature. We looked for correlations in cost data with parameters that would physically affect elution profiles of two components for isocratic

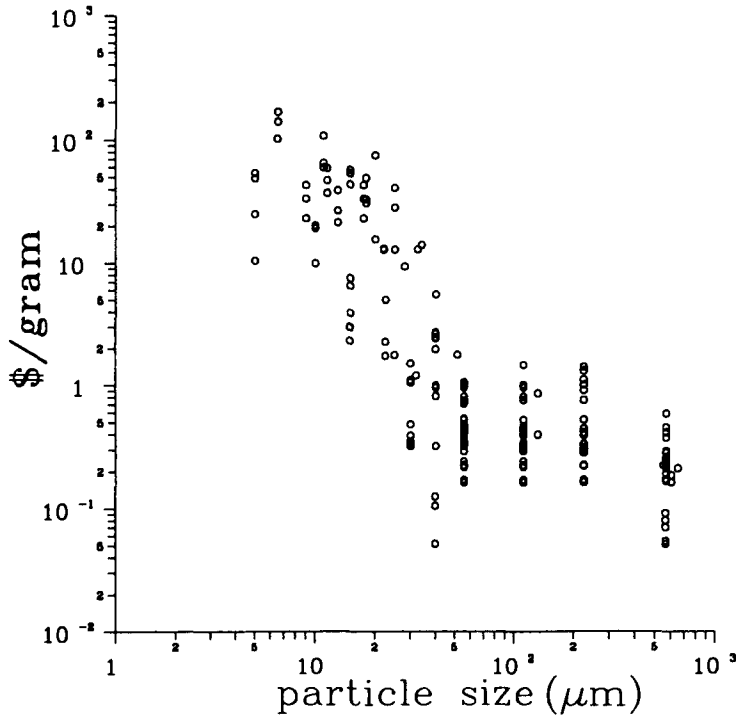

Fig. 1. The cost of chromatography resins as a function of their particle size.

linear chromatography. For chromatography resins, we correlated the cost with particle size; for columns, we correlated the cost with the length and volume.

The cost data for 376 resins are shown in Fig. 1. For particle sizes between 2–100 μm, we found that the price varied with resin particle diameter (d_p) as

$$\text{cost}_{\text{resin}} = 12{,}600\,(d_p)^{-2.5} \tag{1}$$

The resins shown here represent all types of structures, both polymer and silica based, and all types of chemistry, including ion exchange, C_{18}, C_8, phenolic, and affinity resins. The strong correspondence of price to particle size that was found was unexpected (*3*). This implies that the primary problem in producing chromatography resins is sorting them by size. Although the data are scattered, a trend persists with the diameter of the particle.

The costs of chromatography columns are shown in Fig. 2. Although these data were taken from only one source, we think that they represent the cost of a "no frills" column with frits and a fluid distribution system included. We used these data because they spanned an appropriate range of column lengths and volumes. The data are represented by the function

$$\text{cost}_{\text{column}} = 8.57\,(V)^{0.77} \tag{2}$$

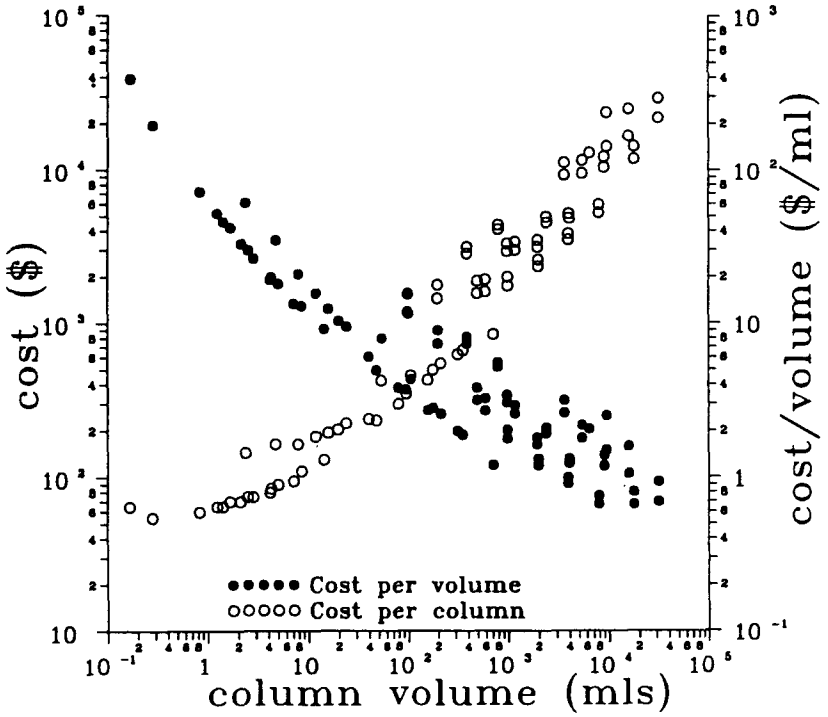

Fig. 2. The cost of chromatography columns as a function of their volume.

where V is the column volume. This result was also unexpected. It had previously been hypothesized that the dimensions of the fluid distribution system, and hence the column radius, would dominate column costs (3).

Since column length, particle diameter, and optimum flow rate all affect the pressure drop across the column, we tabulated the costs of pumps with respect to their maximum operating pressure and their maximum flow rates. These data, which we found to be uncorrelated, are shown in Fig. 3. Pumps appear to be priced based on their relative pulsation, their ability to form gradients, and their capacity for computer control.

MODEL FORMULATION

Gaussian Peaks

Initially, we assumed that eluted peaks followed a Gaussian curve, which follows the equation

$$(c / c_o) = h_{max} \exp \{ [(t - t_r)^2 / 2\sigma^2] \} \quad (3)$$

where c is the solute concentration, c_o is the injected concentration, h_{max} is the peak maximum, t_r is the retention time, t is the time, and σ^2 is the variance in the peak. This form is easily integrated with respect to time.

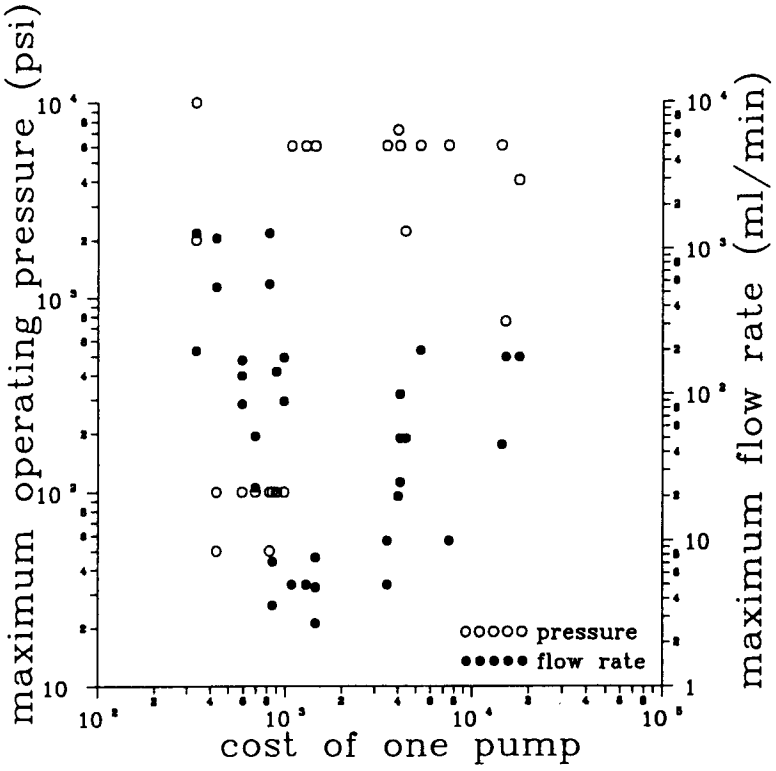

Fig. 3. The cost of high-pressure liquid pumps.

For two different components (represented by two different retention times), a cumulative mass of eluted component could be calculated, and a product cut time could be determined by requiring a particular purity, in this case, 99%. The retention times were calculated from the equation

$$t_r = (L / v)\,[\alpha + (1 - \alpha)K_{av}] \tag{4}$$

where α is the column void fraction (0.33), L is the column length, v is the superficial velocity of the eluent, and K_{av} is a linear equilibrium distribution coefficient. We studied two components with distribution coefficients of 9 and 10, with 9 being the component of interest.

The variance was estimated with the Knox equation (*10*),

$$\sigma^2 = \{d_p\,(\mathrm{a} + \mathrm{b}/\nu + \mathrm{c}\nu)\,[\alpha + (1 - \alpha)K_{av}]^2 L/v^2 \tag{5}$$

which relates plate height to particle size and eluent velocity. Here, ν is $v\,dp/D_m$, D_m is the molecular diffusivity (approx 5×10^{-7} cm^2/s for proteins), and a, b, and c are constants, which we have chosen as 1, 2, and 0.5, respectively (*10*). These constants give a minimum plate height at $\nu = 2$. The Knox equation gives a good first approximation to variance in chromatographic peaks (*11*). Although this equation is empirical, it represents actual chromatographic experience, including such effects as axial diffusion and eddy diffusion or backmixing.

Mass Transfer Models

Mass transfer theory was used to obtain a more realistic view of the dispersion within the chromatographic column. A mass balance over the column combined with a mass balance describing the mass transfer on and off resin particles within the columns is taken from Lapidus and Amundson's 1952 paper (*3*):

$$D\,(\partial^2 c / \partial z^2) = v\,(\partial c / \partial z) + (\partial c / \partial t) + (1 / \alpha)\,(\partial q / \partial t) \tag{6}$$

$$(\partial q / \partial t) = k_1 c - k_2 q \tag{7}$$

$$\begin{aligned} c &= c_o(t) \quad z = 0,\ t > 0 \\ c &= c_i(t) \quad t = 0,\ z > 0 \\ q &= q_i(t) \quad t = 0,\ z > 0 \end{aligned} \tag{8}$$

For the case:

$$c_i = q_i = 0,\ c_o(t) = c_o,\ D = 0$$

(axial diffusion neglected):

$$(c / c_o) = \exp(-kz / v\alpha)\,[\exp(-k_2\,(t - z / v))\,I_o\,(2\{k_1 k_2 z\,(t - z / v) / \alpha v\}^{1/2}) + k_2 \int_0^{t - z/v} \exp(-k_2 s)\,I_o\,(2\{k_1 k_2 z s / av\}^{1/2})\,ds] \tag{9}$$

We have used eq. (9) to generate chromatographic peaks for two solutes with capacity factors ($K_{av} = k_1/k_2$) $K_{av} = 9, 10$ as before. The solution is for step changes in concentration at the column inlet. Peaks were generated from the difference between two closely timed step changes, the time difference representing the time of an injection (*12*). This time was not varied, to represent large or small sample volumes. There is no reason this could not be done, however. These equations are very stiff, owing to the high solvent linear velocity compared to the rate of mass transfer. In order to solve these equations, an approximation for $I_o(x)$ that is valid at large values of x was used:

$$I_o(x) \cong [\exp x / (2\pi x)^{1/2}]\,\{1 - [(-1) / 8x] + [(-1)(-9) / 2!(8x)^2] - [(-1)(-9)(-25) / 3!(8x)^3]\} \tag{10}$$

The chromatographic peaks generated from the Lapidus and Amundson model are not restricted, as in plate theory, to a Gaussian curve. The model does not, however, relate solute concentration to particle size directly. Therefore, to compute solute dispersion as a function of particle size for our economic model, we referred to moment theory to relate d_p to k_2. As the injection time of the protein solution decreases to 0, the first and second central moments (*13*) of eq. (6) are:

$$\mu_1 = (L / v)\,[\alpha + (1 - \alpha)\,(k_1 / k_2)] = t_r \tag{11}$$

and

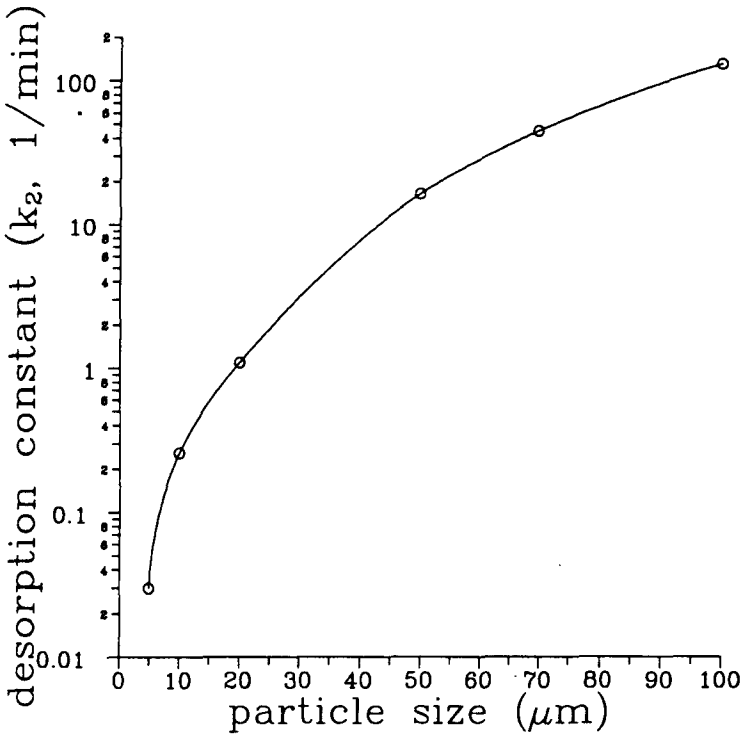

Fig. 4. The variation of k_2 with particle diameter.

$$\mu_2 = 2(1-\alpha)(k_1 / k_2^2)(L / v) = 2\sigma^2 \quad (12)$$

Equations (11) and (12) may be solved for k_1 and k_2 by combination with eq. (4) and (5). By calculating the mass transfer coefficients in this way, we effectively reintroduce axial diffusion and mechanical backmixing, which are represented as the "b" and "a" constants in eq. (5).

For a given particle size, we found very little change in k_2 over a wide range of velocities, from 0.004 to 0.1 cm/s. The variation that we found between d_p and k_2 is shown in Fig. 4, in which k_2 is given in units of min^{-1}.

For both the Gaussian model and the mass transfer model, we calculated the cutoff time for a recovered product of 99% purity for particle sizes from 5 to 100 μm, for column lengths of 5–100 cm, and for eluent velocities of 0.004–0.1 cm/s. We defined productivity as the amount of product recovered at 99% purity divided by the cutoff time. Families of curves were generated for each particle size, for 100, 200, and 300 cycles/resin lifetime. A typical curve, generated with the mass transfer model, is shown in Fig. 5. Over the range of velocities, the lowest velocities give too long a cycle time, and the highest too much dispersion in the peaks to be optimal. We used these data to select an economic optimum for each particle size.

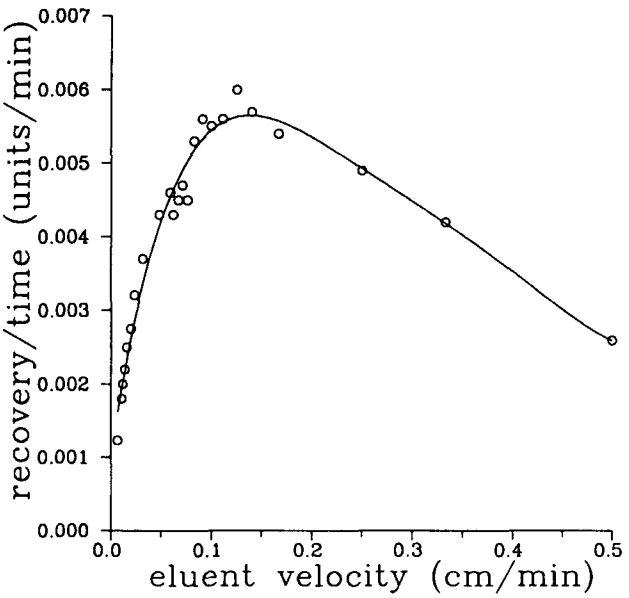

Fig. 5. A typical productivity vs eluent velocity curve, for a 70-μm particle in a 10 cm long column. The maximum from this curve is evaluated in the economic model, and compared to maxima from other column lengths and particle sizes.

ECONOMIC MODEL

The cost of the process was calculated by accounting for operating costs (primarily solvent), capital costs (columns, pumps, detectors, injectors, and filters), and packing costs. The final equation for cost per column lifetime has the form:

$$\begin{aligned}\text{Cost} &= v \times t \times \text{cost}_{\text{solvent}} \\ &\quad + (\text{cost}_{\text{column}} + 25{,}000) \times (t / 10) \\ &\quad + V \times (\text{cost}_{\text{resin}} / \#\text{cycles}) \times 1.1 \times 0.67 \times 3.5 \end{aligned} \tag{13}$$

which is similar to previous models (*3*). Productivity, given in units of product per dollar per year, is then calculated from the cost:

$$\text{Productivity} = (\text{units of product / time}) \times (1 / \text{cost}) \tag{14}$$

We have assumed that column peripherals, such as detectors and filters, cost $25,000. Solvent and utilities (on a solvent basis) were assumed to cost $0.005/mL, based on a liter of HPLC grade water. Capital equipment is depreciated over 10 yr, with no salvage value. Resin is depreciated over its lifetime in cycles and also has no salvage value. Labor costs are estimated as 2.5 times the cost of the packing (*14*). The calculations were based on a nominal 10 cm^2 cross-sectional area column. Taxes, return on investment, and overhead were not included.

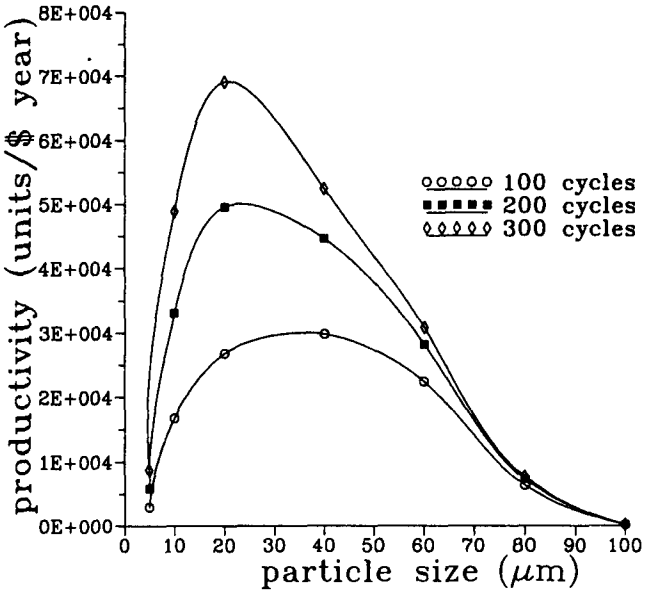

Fig. 6. The optimum particle size for 100, 200, and 300 cycle resin lifetime, assuming Gaussian peaks.

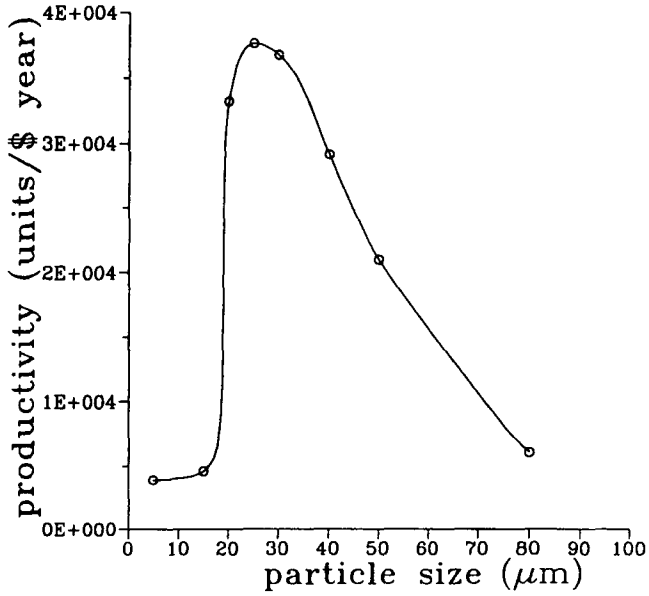

Fig. 7. The optimum particle size for 100 cycle resin lifetime, calculating the peaks from Lapidus and Amundson's nonequilibrium theory.

We then sorted through the productivity data generated for each resin particle size and found the point that gave maximum productivity (units of product per year) per dollar invested. The results as a function of particle size are given in Fig. 6 for Gaussian peaks and in Fig. 7 for mass transfer peaks. Both methods indicate an optimal productivity between 20–40 μm.

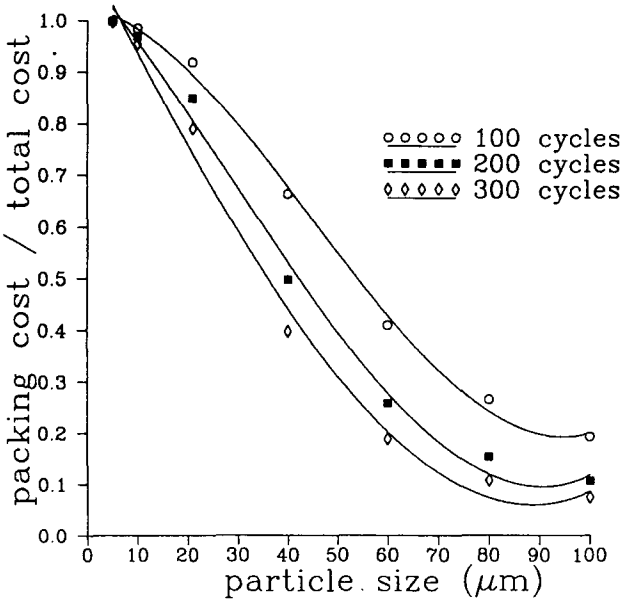

Fig. 8. The cost of resin as a percentage of total cost, as a function of particle size. Small particles dominate the economics of chromatography.

DISCUSSION

The work presented here indicates that a 20–40 μm particle is a good target for scale-up of chromatographic processes. We think that this is a valid, if not general, conclusion, subject to the constraints and assumptions in our work. Scenarios in which smaller or larger particles would prove more economic are certainly imaginable. For instance, separations in which the distribution coefficients for two components were more similar (9.9 and 10, for instance) or much higher (90 and 100, for example) would probably favor smaller particles. However, should a separation warrant smaller particles, alternative column chemistry should also be considered.

The cost of the resin as a fraction of the total cost is shown in Fig. 8. Below 20 μm, the cost of the packing dominates the economics. Therefore, our results for small packings are not sensitive to the assumptions made on the cost of the peripherals or solvent. However, for large particles, these assumptions are critical. This indicates that the chromatography process designer must pay more attention to the selection and cost of the capital and operating costs when working with larger particles.

The results also show that the maximum in productivity shifts slightly towards smaller resins as the useful number of cycles increases. A column that will be expected to run for a great number of product campaigns should contain the smallest particles in the process.

Finally, more complex chromatographic modes remain to be analyzed. Concentration-dependent (nonlinear) chromatography, gradient elution, and moving port or recycle chromatography are all important operations that deserve attention.

For a current economic data base, 20–40 μm particles have some economic advantages over expensive 2–10 μm particles and sluggishly performing 80–100 μm particles. The models used to reach this conclusion are preliminary in nature, but yield consistent results, which is promising. Recommendations for specific processes require specific data on solute properties, but the same general algorithm may be followed to determine a target resin size for scale-up as has been presented here.

ACKNOWLEDGMENTS

The authors thank Robin Stewart, Mike Jones, V. G. Pangarkar, Geoff Slaff, and Robert Cooley for helpful comments during the preparation of this manuscript. This article was presented at the 13th Symposium on Biotechnology for Fuels and Chemicals in Colorado Springs in May, 1991.

REFERENCES

1. Rudge, S. R. and Ladisch, M. R. (1985), *Separation, Recovery, and Purification in Biotechology, ACS Symposium Series 314,* J. A. Asenjo, J. A. and Hong, J. eds., pp. 122–152.
2. Yamamoto, S., Nomura, M., and Sano, Y. (1987), *J. Chromatog.* **409,** 101–110.
3. Mazsaroff, I. and Regnier, F. E. (1986), *J. Liq. Chromatog.* **9(12),** 2563–2583.
4. Ladisch, M. R. (1987), *Adv. Biochem. Eng.*, Bungay, H. R. and Belfort, G., eds., J. Wiley and Sons, NY, pp. 219–237.
5. Lapidus, L. and Amundson, N. R. (1952), *J. Chem. Phys.* **56,** 984–988.
6. Rasmusson, A. and Neretnieks, I. (1980), *AIChE. J.* **26(4),** 686–690.
7. Thomas, H. C. (1944), *J. Am. Chem. Soc.* **66,** 1664–1666.
8. Whitley, R. D., Van Cott, K. E., Berninger, J. A., and Wang, N-H. L. (1991), *AIChE. J.* **37(4),** 555–568.
9. Carta, G. (1988), *Chem. Eng. Sci.* **43(10),** 2877–2883.
10. Knox, J. H. (1977), *J. Chromatog. Sci.* **15,** 352–364.
11. Rudge, S. R. (1989), Applied electric fields in the size exclusion chromatography of proteins, PhD Thesis, Purdue University, IL.
12. Lightfoot, E. N., Sanchez-Palma, R. J., and Edwards, D. O. (1962), *New Chemical Engineering Separation Techniques,* Schoen, H. M., ed., Interscience, NY, pp. 161–163.
13. Kuchera, E. (1965), *J. Chromatog.* **19,** 237–248.
14. Kelly, J. J., Wang, G. Y., and Wang, H. Y. (1985), *Separation, Recovery and Purification in Biotechnology, ACS Symposium Series 314,* Asenjo, J. A. and Hong, J., eds., pp. 193–207.