Journal of Chromatography, 282 (1983) 249–262
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMSYMP. 182

MOVEMENT OF COMPONENTS IN REVERSED-PHASE CHROMATOGRAPHY

III*. REGENERATION POLICIES IN LIQUID CHROMATOGRAPHY

JOHN FRENZ and CSABA HORVÁTH*
Department of Chemical Engineering, Yale University, New Haven, CT 06520 (U.S.A.)

SUMMARY

The theory of chromatography that assumes equilibrium behavior and Langmuir isotherms was used to analyze various column regeneration schemes and to develop optimal policies for efficient equilibration. Analytical expressions were derived for the volume of solvent required to equilibrate the column with a new mobile phase. The theory permits the selection of optimal regenerant solutions, the choice being made according to the appropriate Langmuir isotherm parameters. The results show that a train of different solvents in series is more effective than any single regenerant. Further, the use of gradient schemes or multi-component regenerants is less efficacious than a regenerant train with square-wave inputs. The theory also shows that for elution of several species from the column the optimal scheme focuses on stripping only the most strongly retained species, as the more weakly bound components are readily removed. The theory allows the calculation of effluent concentration profiles in a straightforward manner that involves only algebraic equations. The assumption of local equilibrium between mobile and stationary phases was confirmed experimentally under conditions typical in high-performance liquid chromatography (HPLC). Thus, this approach can find application in optimizing processes where regeneration is important, *viz.*, in gradient, frontal and displacement chromatography and in preparative and routine analytical separations by HPLC.

INTRODUCTION

Abrupt changes in mobile phase composition frequently occur, but are rarely studied in liquid chromatography. In studies of gradient elution the focus is on the behavior of the eluites, and not on the movement of mobile phase constituents. Frontal and displacement chromatography have received considerable attention as separation processes since they were first categorized by Tiselius[1], but to be practicable these techniques, like gradient elution, require an additional separation step for the regeneration of the column in preparation for the subsequent chromatographic sep-

* For Part II, see *J. Chromatogr.*, 282 (1983) 229.

aration. This process may involve removal of several different species from the stationary phase. Reconditioning of the stationary phase is also important in routine analytical separations, where faithful reproducibility is required, and in preparative separations, where contaminants can reduce the column loadability. Therefore, a comprehensive optimization of chromatographic separations also includes the development of an optimal regeneration policy, particularly in such growing fields of chromatography as on-line process control and preparative separations.

The generalized regeneration scheme involves introduction into the column of a succession of different regenerants of varying composition, until finally the original state of the column is restored and the system is ready for the next chromatographic separation. The simplest example of regeneration is a sudden decrease in the mobile phase concentration of the species to be removed from the column. In this case the eluent is the only regenerant used.

This process, in another context, was analyzed by DeVault[2]. However, his approach was restricted to systems with only one sorbable species and single, sudden changes in composition. A more general treatment of multi-component chromatography at finite concentration —where the adsorption behavior of each component is dependent on the concentrations of all other species present— has been elegantly presented by Rhee *et al.*[3], Helfferich[4] and Helfferich and Klein[5]. These fundamentally identical methods for modeling multi-component chromatography permit the calculation of effluent concentration profiles through algebraic equations for many systems of chromatographic interest for any number of components. The approach assumes equilibrium adsorption conditions for the distribution of material between the two phases throughout the column, an assumption that can hold true in operations with the highly efficient microparticulate packed columns employed in high-performance liquid chromatography (HPLC). It is relatively simple to apply this theory when the adsorption behavior of all components is represented by the Langmuir isotherm[6], a behavior which has been confirmed in liquid chromatography[7].

After examining the applicability of the equilibrium assumption we have applied the theory of multi-component chromatography, as put forth by Helferrich and Klein[5], to the analysis of the factors involved in various column regeneration schemes, and have formulated optimal regeneration policies for HPLC.

THEORETICAL

When the concentration of species i in a mixture is changed from c_i^a to c_i^b at the column inlet, the resulting range of concentrations lying between c_i^a and c_i^b travels through the column with velocities given, in the absence of non-equilibrium effects, by[3]

$$u_{c_i} = \left(\frac{\mathrm{d}z}{\mathrm{d}t}\right)_{c_i} = \frac{u_0}{1 + \varphi\left(\frac{\partial q_i}{\partial c_i}\right)_z} \tag{1}$$

where u_{c_i} is the velocity for the concentration c_i, z is the distance along the column, t is time, u_0 is the mobile phase velocity and φ is the phase ratio of the column, both

of which are assumed to be constant, and q_i is the surface concentration of i in equilibrium with c_i, given for Langmuirian adsorption behavior by

$$q_i = \frac{a_i c_i}{1 + \sum_j b_j c_j} \qquad j = 1,2,\ldots, N \tag{2}$$

where a_i and b_i are parameters of the appropriate Langmuir isotherm and are unique to each species in the mixture. When i is the only sorbable species present, eqns. 1 and 2 can be combined to give

$$u_{c_i} = \frac{u_0}{1 + a_i\varphi/(1 + b_i c_i)^2} \tag{3}$$

From eqn. 3, it is evident that the concentration velocity increases with increase in c_i, *i.e.*, higher values of c_i travel faster than lower values. Thus when $c_i^a > c_i^b$, the boundary between the two concentration plateaux spreads out as it travels through the column, giving rise to the familiar diffuse shape that manifests itself as tailing under column overload conditions. On the other hand, when $c_i^a < c_i^b$ the velocity of c_i^b is greater, and mass balance considerations reveal that the resulting self-sharpening front or "step" travels through the column with a velocity given by[3]

$$u_{s_i} = \frac{u_0}{1 + \varphi\left(\frac{q_i^b - q_i^a}{c_i^b - c_i^a}\right)} = \frac{u_0}{1 + \varphi\,(\Delta q_i/\Delta c_i)} \tag{4}$$

Thus, the velocity of a self-sharpening boundary can be found by a single calculation, while a diffuse boundary encompasses a range of concentrations from c_i^a to c_i^b and, consequently, a range of velocities. Thus calculation of the effluent concentration profile can be engineered if only one species is adsorbed by calculation of the relevant concentration velocities.

Eqn. 2 indicates that the adsorption behavior of every component in a mixture is affected by a change in the concentration of any one of them, so that the analysis of concentration velocities and effluent concentration profiles is much more difficult in these systems. Analogous diffuse and self-sharpening boundaries travel through the column, but, in general, the concentration of all components varies across each boundary. The means of overcoming this difficulty[3–5] involves a change from concentration variables to "natural"[5] dependent variables for the system. These variables have the property that only one of them changes across each boundary.

In order to simplify calculations, the time variable is changed to the adjusted time, T, by

$$T = \frac{u_0}{\varphi}\left(t - \frac{z}{u_0}\right) \tag{5}$$

and the adjusted velocity is given by

$$v = \frac{\mathrm{d}z}{\mathrm{d}T} = \frac{\varphi u}{u_0 - u} \tag{6}$$

where $u = \mathrm{d}z/\mathrm{d}t$. With the new variables, eqns. 1 and 4 can be rewritten to yield

$$v_{c_i} = \left(\frac{\mathrm{d}z}{\mathrm{d}T}\right)_{c_i} = \frac{1}{(\partial q_i/\partial c_i)_z} \tag{7}$$

for the adjusted concentration velocity, and

$$v_{s_i} = \frac{1}{\Delta q_i/\Delta c_i} \tag{8}$$

for the adjusted step velocity.

As pointed out above, in order to overcome the difficulty of calculating the concentration and step velocities for a multi-component system, the dependent variable is transformed from the concentrations, c_i, to new variables that are the solutions in h of the Nth order polynomial:

$$\sum_i \frac{b_i c_i}{h \cdot \dfrac{a_i}{a_1} - 1} = 1 \qquad i = 1,2,\ldots, N \tag{9}$$

where N is the number of sorbable components in the system. If the indexing of the species is ordered such that

$$a_1 > a_2 > \ldots > a_i > \ldots > a_N \tag{10}$$

then the h, as defined by eqn. 9, are also ordered as

$$\frac{a_1}{a_1} \leqslant h_1 \leqslant \frac{a_1}{a_2} \leqslant \ldots \leqslant h_i \leqslant \ldots \leqslant \frac{a_1}{a_N} \leqslant h_N \tag{11}$$

Equality in eqn. 11 applies when $c_i = 0$ and either h_i or h_{i+1} equals a_1/a_i. The ratios a_1/a_i have chromatographic significance as the relative retentions or separation factors, since

$$\alpha_{1i} = \frac{q_1/c_1}{q_i/c_i} = \frac{a_1}{a_i} \tag{12}$$

When $c_i = 0$ the root that equals a_1/a_i is termed a "trivial root"[5] of eqn. 9.

As shown by Helfferich and Klein[5], an important characteristic of a "coherent" chromatographic system is that a change in the value of h_i at the column inlet is propagated through the column as a single boundary, either self-sharpening or diffuse. Thus the roots of eqn. 9, called the "H-function", are natural variables for multi-component chromatography, since changes in h_i are physically manifested as distinct composition boundaries, unlike the concentration variables which, in general, change across all boundaries. The restriction of this analysis to coherent systems includes a large part of chromatographic practice. Neglecting deviations arising from kinetic or mass transfer resistances, chromatographic systems tend to coherence as soon as the inlet composition reaches a constant state. Thus, under these restrictions, non-coherence is important in practice only in gradient-type operations, where the inlet composition changes continuously. Even in these systems, if isocratic conditions exist at the start and finish of the gradient, coherent bounds are imposed on the non-coherent zone that passes through the column, and it eventually becomes entirely coherent.

The composition of each solution fed to the column can be expressed in terms of h by eqn. 9, together with the rule for trivial roots. In an analogy to the single-species behavior discussed earlier, when a particular root is changed at the inlet from h_i^a to h_i^b, the change is propagated as a self-sharpening boundary or step if $h_i^a < h_i^b$, and as a diffuse boundary if $h_i^a > h_i^b$ (ref. 5). Across either type of boundary only h_i changes its value although, in general, all species concentrations may change. Further, a self-sharpening boundary moves through the column with a speed given by

$$v_{S_i} = h_i^a h_i^b P_i \tag{13}$$

where

$$P_i = \frac{\prod_{j=1}^{i-1} h_j^a \prod_{j=i+1}^{N} h_j^b}{a_1 \prod_{j=1}^{N} a_1/a_j} \tag{14}$$

In direct analogy to the single-species behavior, a diffuse boundary is a continuous change from h_i^a ahead, or downstream, of the boundary to h_i^b behind, or upstream, and the velocity of any h_i within that range is given by

$$v_{h_i} = h_i^2 P_i \tag{15}$$

Eqns. 13–15 suffice to calculate the effluent profile for the coherent portions of any chromatographic process.

Eqns. 13 and 15 indicate that v_{S_i} and v_{h_i} are constant, as long as P_i is constant. Thus, when a single change occurs at the column inlet, the resulting boundaries pass through the column with constant velocities and will therefore not encounter one another. However, two boundaries that start at the column inlet at different times and have different velocities may intesect. Since both are associated with changes in

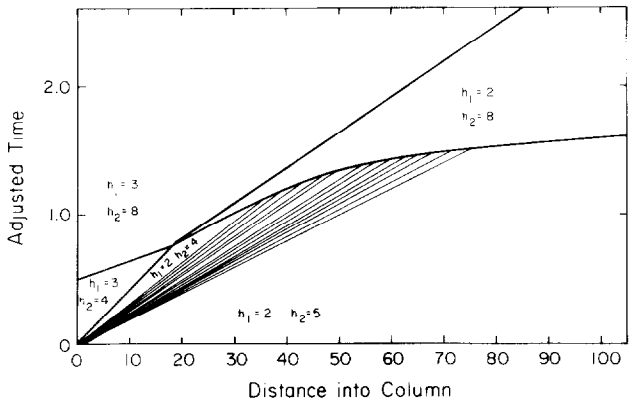

Fig. 1. Distance–time diagram showing the movement of self-sharpening fronts (heavy lines) and a diffuse boundary (thin lines). Boundary velocities were calculated from the changes in h values, shown in different regions of the figure. Intersection of boundaries results in crossover and change of velocity of the boundary, or in combination of the converging boundaries into one.

an H-function root, their velocities will change after intersecting. If the two roots have different indices, they affect one another through the P_i term in the pertinent velocity equation. If the roots have the same index, they combine into a single boundary that involves a change from h_i^a of the earlier boundary to h_i^b of the later one. Fig. 1 is a representation of boundary intersections in the distance–time plane. At time zero, two boundaries arise, a diffuse boundary associated with a change in h_2 from 5 to 4, and a step involving a change in h_1 from 2 to 3. At a later time, the value of h_2 alone is changed from 4 to 8 at the column inlet, and so a single, sharp boundary moves through the column. This boundary crosses the h_1 boundary, and the velocities of both change, as shown by their altered slopes after intersecting. When the two h_2 boundaries intersect, however, they combine into a single front, involving a change in h_2 from 8 to 6.

The analysis of gradients, without periods of constant inlet composition, involves solution of a system of differential equations in h (ref. 5), which, in general, is tractable only by numerical techniques. In our work, such calculations were not carried out, but the passage of the zone of non-coherence associated with the gradient was calculated from the velocities of the coherent boundaries adjacent to the non-coherent zone. This was possible as isocratic conditions were assumed to precede and follow the gradient. These zones were coherent and thus their velocities were calculated as described. They formed bounds on the propagation of the non-coherent zone. In this manner, the time spent in the column by the gradient could be determined, if not the complete concentration profile.

After the boundary trajectories are calculated, the effluent composition profile is known in terms of the h_i values. In order to express this profile in terms of concentrations, the reverse transformation is carried out, as

$$c_i = \frac{\prod_{j=1}^{N} \left(\frac{h_j a_i}{a_1} - 1 \right)}{b_i \prod_{\substack{j=1 \\ j \neq i}}^{N} \left(\frac{a_i}{a_j} - 1 \right)} \tag{16}$$

Calculation of the complete effluent concentration profile gives a description of the effect of a particular regeneration protocol, but the quantity of greatest interest is the overall time required to pass the column from one state to another or, equivalently, the amount of solvent needed to achieve this. This quantity can be expressed in terms of adsorbate and regenerant properties by consideration of the principles outlined above. While regeneration refers strictly to a return of the column to some earlier condition, the treatment here is quite general for changes from one equilibrium state of the column to another. Nevertheless, for convenience, the analysis will be discussed in terms of regeneration, which is the case of most interest.

When regeneration is performed by switching the inlet stream from a solvent containing an adsorbate to the pure solvent alone, a diffuse boundary forms in the column between the adsorbate-rich solution and the pure solvent. This boundary is detected at the column outlet as an exponentially decaying "washout curve". The number of column volumes of solvent required to regenerate (CVR) the column is the volume, in column volume units, for the diffuse boundary or washout curve to leave the column and is given by

$$CVR = \left(\frac{\varphi T_R}{u_0} + \frac{L}{u_0}\right)\frac{u_0}{L} \tag{17}$$

$$= \frac{\varphi}{v_1^b} + 1$$

$$= \varphi a_1 + 1$$

where T_R is the adjusted retention time and v_1^b is the velocity of propagation of the H-function root, calculated for pure solvent through the column. As noted above, this is a trivial root, as $c_1 = 0$, and its travel through the column delineates the end of the diffuse boundary. As a_1 is the initial slope of the isotherm, φa_1 is the retention factor of the adsorbate, and eqn. 17 is formally equivalent to the fundamental retention equation[8] of elution chromatography.

When a plug of regenerant solution is used to speed the removal of the adsorbate from the column, two washout curves or diffuse boundaries arise at the column inlet. One curve corresponds to the decrease in adsorbate concentration in the presence of the regenerant solution and appears at the front of the regenerant plug, and the second marks removal of the regenerant substance by the pure solvent after termination of the plug. The optimal regeneration scheme can be defined as the number of column volumes of effluent required for these boundaries to pass through the column without overlapping one another, a number given by

$$CVR = \varphi\left(\frac{1}{v_A^b} + \frac{1}{v_R^b} - \frac{1}{v_R^a}\right) + 1$$

$$= \varphi\left[\frac{a_1}{1 + b_2c_2} + a_2 - \frac{a_2}{(1 + b_2c_2)^2}\right] + 1 \tag{18}$$

where subscript 1 refers to the adsorbate and 2 to the regenerant. In a similar manner, when plugs of species 2 and 3 are used successively as regenerants, the column volumes of effluent required to regenerate the system is

$$CVR = \varphi\left[\frac{a_1}{1 + b_2c_2} + \frac{a_2}{1 + b_3c_3} - \frac{a_2}{(1 + b_2c_2)^2\,(1 + b_3c_3)^2} + a_3 - \frac{a_3}{(1 + b_3c_3)^2}\right] + 1 \tag{19}$$

In general, when a train of N regenerant plugs in series is employed in the order of decreasing affinity for the adsorbent, the CVR is

$$CVR = \varphi\left[\frac{a_1}{1 + b_2c_2} + a_N - \frac{a_N}{(1 + b_Nc_N)^2} + \sum_{i=1}^{N-1} \frac{a_i}{1 + b_{i+1}c_{i+1}} - \frac{a_i}{(1 + b_ic_i)^2\,(1 + b_{i+1}c_{i+1})}\right] + 1 \tag{20}$$

EXPERIMENTAL

The chromatographic system for examining the nearness of the process to equilibrium consisted of a Model 100 (Beckman-Altex, Berkeley, CA, U.S.A.) solvent-metering pump, a Model 725 (Micromeritics, Norcross, GA, U.S.A.) automatic sample injector, a Supelcosil LC-18 (Supelco, Bellefonte, PA, U.S.A.) 250 × 4.6 mm I.D. reversed-phase, 5-μm particle diameter column, a Model 740 (Kratos, Ramsey, NJ, U.S.A.) multiwavelength UV detector, set at 254 nm, and a Model SDR 306 (Kratos) dual-pen chart recorder. Acetone was obtained from Fisher Scientific (Fair Lawn, NJ, U.S.A.) and distilled water was prepared using a Barnstead still.

Overloaded peaks were injected as 10-μl aliquots, containing the specified mass of acetone, mixed with water. Washout curves were produced by changing the mobile phase via the autosampler bypass switch and the stop-flow technique.

RESULTS AND DISCUSSION

HPLC columns and equipment have been employed[9] to satisfy the model assumptions and to verify the predictions of the theory of multi-component chromatography. Other attempts to apply the theory to predict breakthrough curves in large adsorption columns met less success, owing to the significant mass transfer and kinetic resistances inherent in this equipment[10,11]. In order to assess the contribution of these factors, a series of experiments, such as shown in Fig. 2, were carried out. Increasing amounts of acetone were injected into a reversed-phase chromatographic system with distilled water as the mobile phase. As expected[12], when the resulting

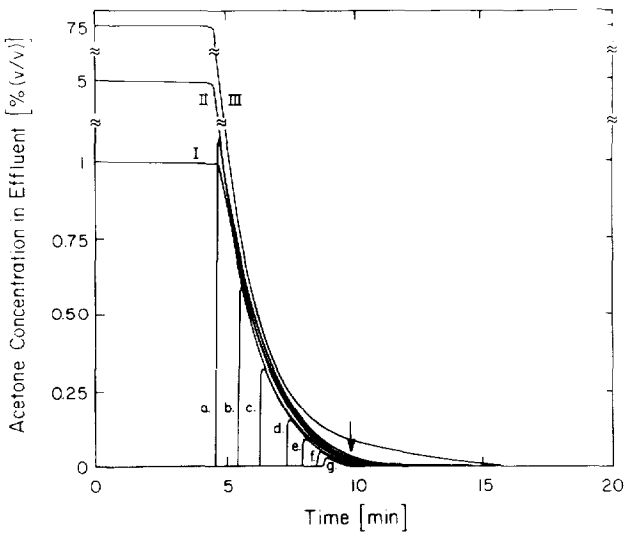

Fig. 2. Elution and washout profiles in non-linear chromatography of acetone. Column, 5-μm Supelcosil LC-18 (25 × 4.6 cm I.D.); mobile phase, distilled water; flow-rate, 1 ml/min; detection, absorbance at 254 nm. Initial concentration of washout curves: (I) 1%; (II) 5%; (III) 75% (v/v) acetone. Mass of acetone injected in 10 μl to produce overload peaks: (a) 8; (b) 4; (c) 2; (d) 0.8; (e) 0.4; (f) 0.16; (g) 0.08 mg. The arrow indicates the retention time of 3 μg of acetone, within the linear portion of the acetone adsorption isotherm.

profiles, marked by letters a–g, are superimposed, as in Fig. 2, their diffuse rear boundaries coincide. The arrow marks the position of the peak maximum, determined for the injection of 3 μg of acetone, in the linear region of its adsorption isotherm. The elution profiles shown in Fig. 2 all skew towards the front, as expected for non-linear Langmuirian isotherms. The termination of the diffuse boundaries near the retention time of the infinitesimal peak provides evidence that the system is near equilibrium. If serious non-equilibrium processes existed, the rear boundary of these peaks would broaden and the tail of the peak would extend beyond its position determined in the linear domain. Further confirmation was obtained by equilibrating the column with an acetone–water solution and switching the inlet stream to pure water in order to wash out the acetone. This change at the column inlet produces a diffuse boundary between the acetone solution and the pure water, as is also shown in Fig. 2. The diffuse boundaries, labelled I–III, follow the rear boundaries of the elution peaks well, further suggesting that non-equilibrium effects are minor. The only appreciable deviation occurs at the low concentration end of the boundary between 75% acetone and water. This may be related to slow desolvation of the alkyl chains at the surface[13], but it is a very small effect, contributing only at the 0.1% level. Similar behavior was found at both higher and lower flow-rates. The lack of any significant flow-rate effect inspires confidence that kinetic resistance may be neglected in this analysis.

As the assumptions underlying the theory appear to be satisfied under conditions employed in HPLC, the predictions of the theory concerning column regeneration were investigated. Fig. 3 shows the dependence of the number of column volumes of effluent needed for regeneration (CVR) as a function of regenerant Langmuir

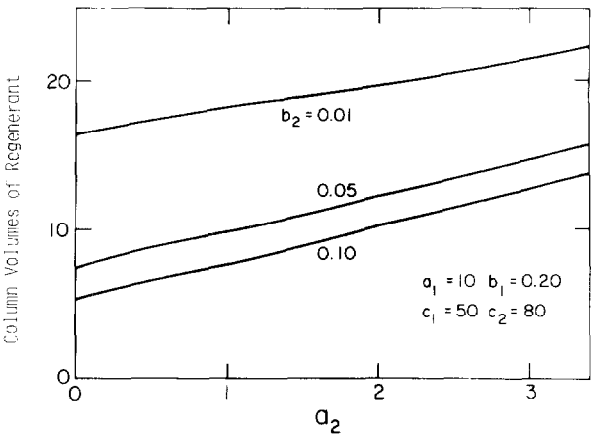

Fig. 3. Dependence on regenerant Langmuir parameters a_2 and b_2 of the number of column volumes of effluent required to regenerate a column after saturation with an adsorbate at a normalized concentration of 50. For the adsorbate, $a_1 = 10$ and $b_1 = 0.20$.

parameters, as calculated from eqn. 18. Concentrations were made dimensionless in these calculations by normalizing to unit concentration. At the regenerant concentrations examined, *CVR* is essentially a linear function of a_2, the initial slope of the regenerant isotherm. It is a stronger function of b_2, the parameter that indicates the strength of the regenerant in suppressing the isotherm of the adsorbate. Fig. 3 readily shows that a combination of low a_2 and high b_2 is optimal for decreasing the regeneration time.

For most substances, there may be a coupling between a_i and b_i, so it is more enlightening in this investigation to formulate a relationship between the parameters to mimic more nearly the array of regenerants available for chromatography. Fig. 4 shows such a relationship, together with the dependence of *CVR* on the adsorption properties of the regenerant for two different adsorbates. These curves exhibit a minimum, indicating that the selection of the regenerant based on its Langmuir param-

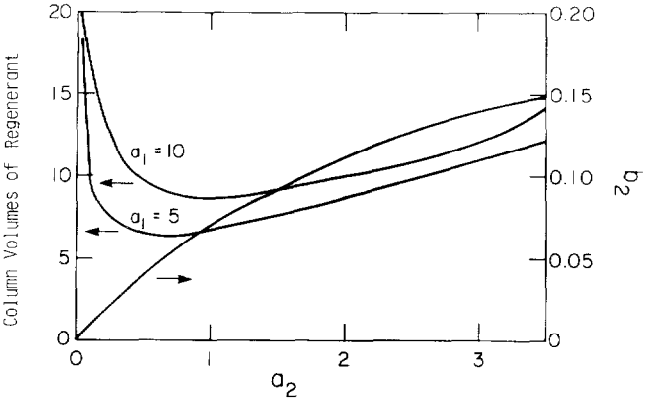

Fig. 4. Dependence of *CVR* on regenerant adsorption properties (left ordinate) and simulated linkage between regenerant Langmuir parameters (right ordinate) for adsorbates with $a_1 = 10$, $b_1 = 0.20$ and $a_1 = 5$, $b_1 = 0.167$. The regenerant concentration is 80.

eters is an important part of the optimization scheme. On this basis, the regenerant should have relatively high b_2 in order to be effective at suppressing adsorption of the substance to be removed from the column, and it should also have low a_2 so that it is quickly removed from the column. As these demands are in conflict when there is a coupling between the parameters, an optimal regenerant can be determined that is different for each adsorbate.

The concentration of the regenerant plug also plays a role in the optimization of the operation, as shown in Fig. 5. The regeneration volume decreases with increasing regenerant concentration, as calculated from eqn. 18 and shown in Fig. 5. This is because the regenerant is more effective at high concentrations in removing the adsorbate from the surface without itself being appreciably more difficult to remove. The saving in regeneration time with increasing regenerant concentration is greatest at lower concentrations, and little gain in efficiency is achieved by raising the concentration of regenerant beyond a certain value. However, the total amount of regenerant pumped into the column, given by the volume of the plug times its concentration, increases linearly at high concentrations, as also shown in Fig. 5. This behavior may be significant in larger scale operations, where solvent demands must be taken into account in the optimization protocol.

Figs. 3–5 were calculated by assuming that the regenerant was pumped through the column as a square-wave plug. However, other shapes for the inlet regenerant concentration profile may be considered in formulating an optimal regeneration policy. Accordingly, Fig. 6 shows a linear gradient-type of input (A) from the regenerant to pure solvent and the resulting outlet profile (B). The square-wave inlet profile (C) is also depicted along with its corresponding outlet concentration profile (D). Comparison of Fig. 6B and D indicates that in both cases complete removal of the regenerant occurs at the same time. The profile resulting from the gradient input is broader and declines less steeply. The conclusion is that less regenerant contacts the stationary phase in a gradient rather than square-wave input operation and that the regeneration process is therefore less efficient. This inefficiency is particularly pro-

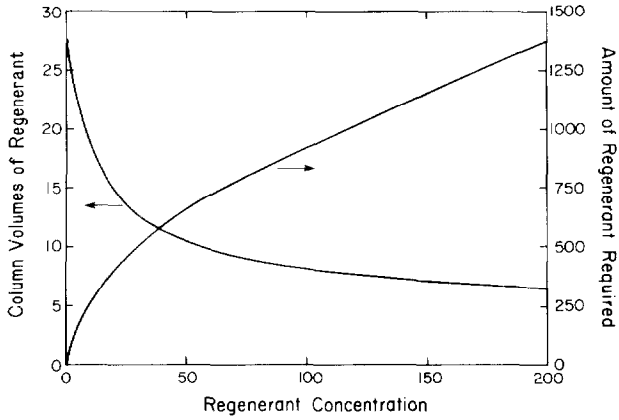

Fig. 5. Dependence of regeneration volume on concentration of the regenerant solution (left ordinate) and on amount of regenerant employed in the process (right ordinate). The latter, dimensionless quantity is calculated as the product of the volume of regenerant solution used and its concentration. For the adsorbate, $a_1 = 10$, $b_1 = 0.20$, $c_1 = 50$, and for the regenerant, $a_2 = 1$, $b_2 = 0.071$.

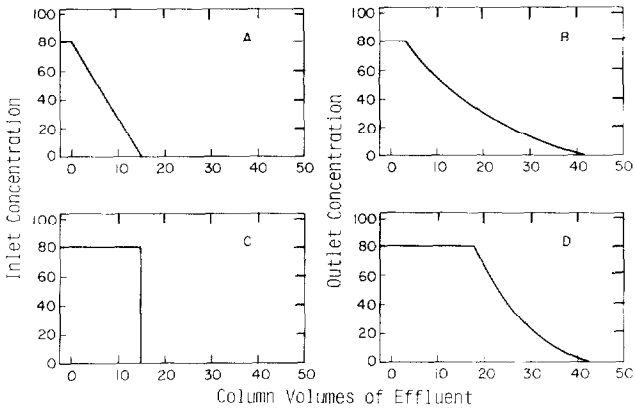

Fig. 6. Inlet and outlet concentration profiles for gradient-type (A, B) and square-wave (C, D) regeneration schemes. The diffuse boundary, measured at the column outlet, is shown in (B) for the gradient and (D) for the square-wave operation. Regenerant properties as in Fig. 5 with a concentration of 80.

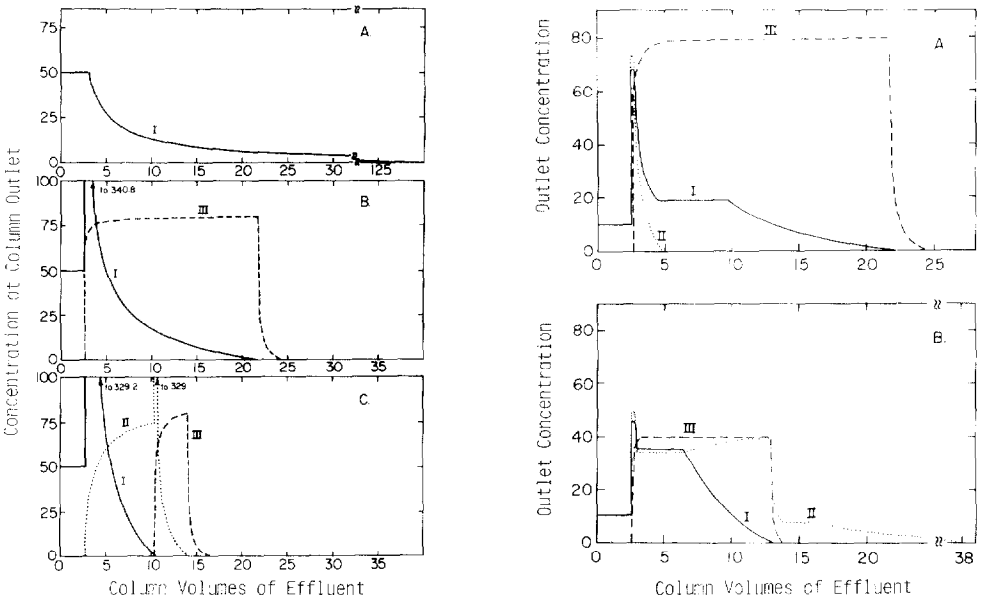

Fig. 7. (A) Washout curve for the adsorbate, component 1, with the mobile phase solvent as the only regenerant. (B) Outlet concentration profile following use of a plug of component 3 to act as the regenerant. I and III are the adsorbate and regenerant profiles at the column outlet, respectively. (C) Regeneration of the column by a regenerant train of components 2 and 3 in series. Curve I is the concentration profile of component 1 in the effluent, II is that of 2 and III is the profile of 3. In all cases $a_1 = 50$, $b_1 = 0.238$, $c_1 = 50$, $a_2 = 10$, $b_2 = 0.20$, $c_2 = 80$, $a_3 = 1$, $b_3 = 0.071$ and $c_3 = 80$.

Fig. 8. Effluent concentration profiles for multi-component adsorbate (A) and regenerant (B). In (A) the adsorbate consisted initially of components 1 and 2, each at a concentration of 10. The regenerant was the same as in Fig. 7B. In B, the same adsorbate as in A was followed by a plug, containing components 2 and 3, each at a concentration of 40.

nounced because the regenerant is less effective in removing the adsorbate at low concentrations. These results can be generalized to show that a plug, such as that in Fig. 6C, is the optimal input shape for the regeneration process.

The course of regeneration by various schemes is illustrated in Fig. 7. The washout curve for a strongly retained substance is shown in Fig. 7A. As the pure mobile phase solvent is a weak eluent for this adsorbate, over 100 column volumes of solvent must be pumped into the column to remove it completely. Injection of a plug of regenerant with the characteristics given in the caption reduces the regeneration volume by about 80% and results in the outlet concentration profile shown in Fig. 7B. The solid curve marked I is the profile of the adsorbate, component 1, and the profile labelled III is for the regenerant, component 3. At the front of the regenerant zone is a spike in adsorbate concentration, a feature that is exploited in certain trace-enrichment procedures[14]. The dramatic decrease in *CVR* is shown to be a consequence of the power of the regenerant to strip the adsorbate rapidly from the column. A further decrease in regeneration time is achieved by using two plugs of different regenerants, pumped into the column in series, as shown in Fig. 7C. In this case, a regenerant, component 2, the effluent profile of which is labelled II and which has adsorptive properties intermediate between those of I and III, provides an even faster removal of the adsorbate. This regenerant is then stripped efficiently from the column by the less retained component 3. The consequence of this behavior, as predicted by eqn. 19, is that shorter regeneration times can be attained by increasing the number of species employed in a regenerant train pumped into the column, as long as the individual regenerants are positioned in the train in the order of their decreasing affinity for the stationary phase.

The regeneration of a column loaded with two adsorbates is shown in Fig. 8A and B. In Fig. 8A the regeneration scheme is similar to that employed in Fig. 7B, but the column is loaded with both species 1 and 2 and the concentration of component 1 is lower. Nevertheless, the time required for regeneration is identical. This is explained by noting that two diffuse boundaries form at the front of the regenerant plug. In the first boundary, the concentration of 2 goes to zero, and in the second the concentration of 1 vanishes. The regeneration time is determined largely by the time taken by this boundary to traverse the column. As it travels independently of the earlier boundary, its velocity, and therefore the regeneration time, is independent of the composition of the solution to be removed from the column. Therefore, in designing a regeneration policy only the adsorption behavior of the component with the highest affinity for the stationary phase must be taken into account. The result shown here also indicates that the regeneration time is independent of the concentration of the adsorbate, as also shown by eqn. 20.

Fig. 8B shows the same regeneration scheme, carried out with a regenerant solution containing both components 1 and 2, so that 2 is common to both the adsorbate and regenerant solutions. When this mixed regenerant is used, the removal of the key adsorbate, component 1, is faster, but the removal of component 2 from the surface by the pure solvent is slow enough to make the overall process less efficient. Comparison with Fig. 7C shows that a regenerant train is much more efficient than the mixed regenerant for rapid stripping of an adsorbate from the column.

CONCLUSIONS

The theory of multi-component chromatography of Helfferich and Klein[5] provides a means to analyze regeneration schemes quickly and to develop an optimal policy for efficient column equilibration. The theory shows that, given a choice of regenerants, one of them is the most effective as a regenerant for the removal of a particular adsorbate. Use of the optimal regenerant results in large savings in equilibration times. Even greater efficiency is obtained by using a train of regenerants, pumped into the column in order of decreasing affinity for the stationary phase. This scheme of regeneration in series yields superior results to the use of mixed regenerants. When a mixture of compounds must be removed from the column, the optimal overall regeneration policy is governed only by that for removal of the most strongly adsorbed component.

The theory assumes that kinetic and mass transfer resistances are negligible in the system, assumptions that hold true for state-of-the-art HPLC columns. Only algebraic equations need be solved in order to calculate effluent concentration profiles, and analytical expressions for the time required for regeneration are readily derived. Modeling the regeneration process by this theory offers a useful tool for the optimization of column reconditioning practices in such applications as routine analyses and preparative separations, especially by gradient elution, frontal and displacement chromatography.

ACKNOWLEDGEMENTS

This work was supported by grants Nos. CA 21948 and GM 20993 from the National Cancer Institute and National Institute for General Medical Sciences, U.S. Public Health Service, Department of Health and Human Services.

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