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Theoretical Analysis and Critique of the Chromatographic Separation of Macromolecules Using Porous Adsorbents

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Synopsis

A theoretical analysis of the separation of a mixture of macromolecules by a chromatographic column packed with porous adsorbents is given. Fractionation may take place by either exclusion of large molecules from the pores in the column packing or by selective adsorption. A model for packing structure is developed which is used as the basis of a theory of chromatographic separation.

INTRODUCTION

Recognizing that the analysis and separation of mixtures of macromolecules is an important analytical and industrial problem, we have undertaken a program of research to better understand mechanisms of chromatographic separation of such materials and to develop new chromatographic techniques. For reviews of existing methods, see the volumes of Cantow[1] and Determann.[2] Packing columns with adsorbent particles having such porosity as to allow macromolecules to diffuse into them gives a powerful separation technique. Molecules may be separated according to two mechanisms: (1) a molecular sieving action separates according to size, and (2) molecules are separated by adsorptive specificity. It is the purpose of this paper to briefly present an analysis of this technique and indicate its potentialities.

ANALYSIS OF SEPARATION

Methods of analyzing chromatographic separations have been widely developed during the past two decades.[3–5] The analysis of separation of macromolecules by porous adsorbents is basically similar to these analyses, and only a concise summary of the theoretical development[6,7] will be given. Consider a column packed with porous beads and possessing a uniform void fraction α. A dilute solution of a heterogeneous polymer percolates through a column with a flow rate Q and average velocity U. We assume

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that the velocity and concentration are uniform across the cross section. The concentration of species i at position x and time t in the percolating phase is C_i grams (or moles) per unit void volume and, in the dispersed phase, $\bar{C}_i$ grams per unit volume. The value of $\bar{C}_i$ is an average value, for the concentration of a species will vary with bead radius. When there is equilibrium between the dispersed and mobile phases, $\bar{C}_i$ is equal to $\phi_i C_i$, where ϕ_i is a partition coefficient, i.e., a Nernst-like thermodynamic distribution coefficient. The pore volume available to species i per unit packing volume will be taken to be γ_i. To complete the formulation of the problem, we must consider the adsorption process. Let N_i and $\bar{N}_i$ be the concentrations of external and adsorption per unit external and internal surface area. The adsorption isotherms are taken to be linear and of form

$$N_i = \lambda_i C_i \text{ and } \bar{N}_i = \lambda_i \bar{C}_i. \tag{1}$$

By formulating differential mass balances throughout the column and within the packing particles, we may solve for the detailed concentration profiles $C_i(x,t)$ and $\bar{C}_i(x,r,t)$,[3-7] this being done by the method of the Laplace transformation. Generally, the equation obtained for C_i in the

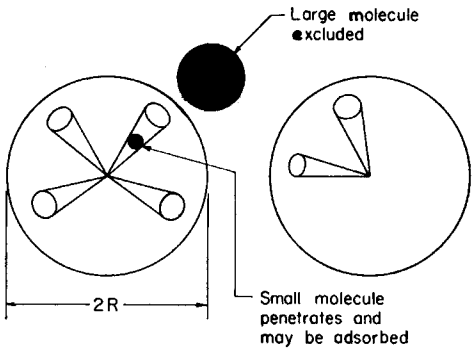

Fig. 1.

Laplace domain is too complex to invert, and one may only obtain approximate inversions by the method of moments. By this procedure, moments of the axial concentration distribution may be obtained, though not the concentration distribution itself. The normalized first moment yields the mean residence time of species i in the column. This result is perhaps more usefully expressed in terms of the elution volume V_i of the species, as follows[6,7]:

$$\frac{V_i}{V_{col}} = \alpha + \gamma_i\phi_i(1 - \alpha) + \lambda_i(1 - \alpha)[\chi^E + \phi_i\chi_i^I] \tag{2}$$

where χ^E is the external surface area per unit packing volume and χ_i^I is the internal surface area available to species i per unit packing volume. For a column packing consisting of impermeable adsorbent beads, γ_i and χ_i^I of eq. (2) are zero. For nonadsorbing porous particles, λ_i is zero.

The higher moments of C_i can be used to analyze the dispersion in the pulse of species i moving through the column. The second moment may be used to evaluate the variance,* and the third moment, the skewness of the curve.

In order to predict the form of an elution diagram, we must know the detailed packing structure. In particular, we must be able to compute the fractional volume and the amount of surface area available to species as a function of molecular size. Different theories of packing structure have been developed through the years for the purpose of analyzing dispersion effects in adsorption columns and packed bed reactors.[8-12] We shall proceed by developing a theory of spherical particles with conical pores which reach inward to the center of the sphere[6,7] (compare Porath[11]). The basis of separation in the conical pore theory is that molecular size limits the depth to which a molecule may diffuse in the porous particle. This gives rise to a molecular sieving effect and also presents an increase in area for adsorption of small molecules. Consider a particle of radius R which contains a series of pores with external radii r_{01}, r_{02} ... (see Fig. 1). A typical pore actually consists of a cone with an altitude of $(R - \epsilon)$

* From the second moment, the variance is found to be:

$$\sigma_i^2 = \sigma_{1i}^2 + \sigma_{2i}^2 = \frac{2E_i x}{U} + \beta U x \tag{F-1}$$

where

$$\beta = \frac{\left\{\frac{2K_i^2}{k_i\alpha} + \frac{2\phi_i K_i^2}{\bar{k}_i} + \frac{2\phi_i\gamma_i(1-\alpha)\left[1 + \frac{K_i}{\gamma_i(1-\alpha)}\right]^2}{\theta_i\alpha}\right\}}{\left[1 + \frac{K_i}{\alpha} + \frac{\phi_i\gamma_i(1-\alpha)}{\alpha} + \frac{\phi_i\bar{K}_i}{\alpha}\right]^2} \tag{F-2}$$

This equation is of the van Deemter type.[3-5] Here, E_i is the eddy dispersion coefficient; θ_i is the coefficient of interphase mass transport between the dispersed and mobile phase; K_i and $\bar{K}_i$ are given by

$$K_i = \lambda_i\chi^E(1-\alpha) \text{ and } \bar{K}_i = \lambda_i\chi_i^I(1-\alpha); \tag{F-2a,b}$$

and k_i is the adsorption rate constant defined by

$$\frac{\partial n_i}{\partial t} = k_i\left[C_i - \frac{n_i}{K_i}\right] \tag{F-3}$$

where n_i is $N_i\,\chi_E\,(1-\alpha)$. A similar expression defines $\bar{k}_i$. The quantities E_i and θ_i (and therefore β_i) depend upon flow rate, the former because it represents dispersion due to flow around the packing beads. θ_i is given by

$$\frac{1}{\theta_i} = \frac{1}{\kappa_i} + \frac{h_i}{15D_p}. \tag{F-4}$$

Here $15D_p/h_i$ represents mass transport resistance within the bead,[6,7] where penetration to a depth $h_i \leq R$ may occur; D_p is the intraparticle diffusivity; and κ_i is the mass transport coefficient from the interface to the bulk of the mobile stream. This quantity is dependent upon the detailed hydrodynamics of the mobile phase.

and a volume of $\pi r_{0\alpha}^2 (R - \epsilon)/3$. A molecule of diameter d_i can only penetrate to a depth at which the pore diameter is approximately equal to d_i. The volume of the cone frustum available to the molecule is then just the difference between the total pore volume $\pi(R - \epsilon)r_{0\alpha}^2/3$ and the volume of that part of the pore from which the molecule is excluded, i.e., $\pi(R - \epsilon - h_i)[(d_i/2)]^2/3$. It may be shown that

$$\gamma_i = \frac{V_{Fi}}{4/3\ \pi R^3} = \sum_{\alpha=1}^{n} \left(\frac{r_{0\alpha}}{2R}\right)^2 \sqrt{1 - \left(\frac{r_{0\alpha}}{R}\right)^2} \left[1 - \left(\frac{d_i}{2r_{0\alpha}}\right)^3\right] \tag{3}$$

To complete our analysis, we must evaluate the variation of external and internal surface area available when pores are introduced. The external surface area of a particle of radius R is simply $4\pi R^2$ minus the area of the apertures caused by the pores. The quantity χ^E, the external surface area per unit packing volume, is for a sphere with n pores of varying radius:

$$\chi^E = \frac{3}{R}\left[1 - \frac{1}{4}\sum_{\alpha=1}^{n}\left(\frac{r_{0\alpha}}{R}\right)^2\right] \tag{4}$$

The internal surface of a pore available for adsorption is simply the area of the frustum of a cone. A cone of base radius $r_{0\alpha}$ and altitude $(R - \epsilon)$ has a surface area of $\pi(R - \epsilon)r_{0\alpha}$. If h_i is then the depth to which a molecule of species i may penetrate, it follows that χ_i^I, the internal surface area available to species i per unit peaking volume, in a particle with a distribution of pore sizes is:

$$\chi_i^I = \frac{3}{4R}\sum_{\alpha=1}^{n}\left(\frac{r_{0\alpha}}{R}\right)\sqrt{1 - \left(\frac{r_{0\alpha}}{R}\right)^2}\left[1 - \left(\frac{d_i}{2r_{0\alpha}}\right)^2\right]. \tag{5}$$

We of course regard this to some extent as just an approximate but reasonable model allowing calculations. If we take all the pores to have the same value of $r_{0\alpha}$, it becomes a two-parameter model.

SEPARATION OF COMPLEX MIXTURES

The main point that we wish to make is the ability of chromatographic columns packed with porous adsorbents to separate complex mixtures. This may best be shown by the following example. Consider four monodisperse species of polyisobutylene A, B, C, and D, with molecular weights of 28,000, 196,000, 364,000, and 532,000 dissolved in benzene. The radii of gyration, $\sqrt{\overline{s_i^2}}$, were computed[7] from

$$[\eta] = \Phi' \frac{(\overline{s_i^2})^{3/2}}{M_i} = KM_i^{\alpha} \tag{6}$$

where Φ' is Flory's universal constant, 3.1×10^{24}, when $[\eta]$ is in dl/g $\overline{s^2}$ is in cm^2. For A, B, C, and D we obtain radii of gyration of 5.46, 14.4, 19.7, and 23.8×10^{-7} cm. The solution is percolated through a 20-

cm-long, 2-cm-diameter column, packed with spherical particles 50 microns in diameter. The void fraction, α, is taken as 0.4. We presume equilibrium chromatography in this example.

We first considered the particles to be porous and nonabsorbing, having 2×10^6 conical pores reaching to the center of the particle and having an external radius of 2.38×10^{-6} cm. Calculations were based on eqs. (2) and (3). For D, the elution volume is 25.1 ml; for C, 32.5 ml; for B, 38.4 ml; and for A, 42 ml. This is a typical elution diagram for molecular sieve chromatography. Elution volume decreases with molecular size. (The number of pores is an important parameter. If there be only 10^5 such conical pores, the separation will be insignificant. Also, no separation of significance is obtained if the pore radius is made too small or large. Decreasing it to 0.50×10^{-6} cm or increasing it to 5.0×10^{-6} cm causes the polymer mixture to elute a pulse and not to separate.)

Turning to impermeable carbonaceous particles with adsorbent properties equivalent to those found for carbon black by Binford and Gessler,[14] an analytical representation of the linear region of their equilibrium data is:

$$\lambda_i = 1.72 \times 10^{-12} M_i^{1.67} \frac{\text{moles ads/cm}^2 \text{ surface}}{\text{moles diss/cm}^3 \text{ soln}}. \quad (7)$$

The elution volumes under the same flow conditions were found from eqs. (2) and (4) to be for A, 27.2 ml; for B, 78.7 ml; for C, 175.8 ml; and for D, 309 ml. This shows the effect of increasing extent of adsorption with molecular weight. The large molecules are retarded. The extent of retardation of polymer molecules in the column would increase if the particle size were decreased.

For porous adsorbents with the same structure as our permeating gel and the same internal and external adsorbent properties as our impermeable particles, the following ordering was obtained from eqs. (2) through (5): for D, 181 ml; for A, 980 ml; for B, 16,000 ml; and for C, 22,700 ml. The reversal clearly shows the interaction of exclusion and adsorption in separating macromolecules.

Let us now consider two types of macromolecules moving through a selectively porous medium. Let them again have the dimensions of A, B, C, and D, but now A and C are a species that are adsorbed but B and D are nonadsorbing. The elution volumes will be D, 25.1 ml; B, 38.4 ml; A, 980 ml; and C, 22,700 ml. We thus both separate the two species and fractionate them. (One must be wary about the extent of dispersion in such a column, especially that due to adsorption-desorption equilibrium. This might be calculated from the expression for σ_t^2 given in the footnote [eqs. (F-1) through (F-4)]. Such calculations have been made by Kingry.[7])

DISCUSSION AND CONCLUSIONS

The question arises as to whether separations of this type have been observed in chromatographic experiments. Mark and Saito,[15] who in 1936

were the first to report the chromatographic fractionation of polymers, passed solutions of cellulose acetate through a porous carbon (blood charcoal) adsorbent. Mark and Saito found that the high molecular weight polymer eluted first from the column, followed by the intermediate and low molecular weight fractions. This was unexpected, as among small molecules high molecular weight compounds generally adsorb to a greater extent than do low molecular weight compounds. Baum and Broda[16] set out to resolve this question by studying the extent of adsorption of poly-(acetyl glucoses) of varying chain length on aluminum oxide and charcoal. They observed that adsorption increased from monomer to dimer to low molecular weight polymer but then decreased as the polymer molecular weight increased. In succeeding years, other researchers constructed columns with different adsorbent materials and gels to separate polymer mixtures. In some cases, large molecules eluted first,[17-23] and in others, small molecules did.[24-26] Pharmacia researchers have found selective variation of elution volume of macromolecules in dextran gel columns when they varied the pH.[27,28] The porous adsorbent effect described in this paper would seem to be the only rational explanation of such experimental results. Experiments aimed toward chromatographic separation mixtures of macromolecules of varying composition with porous adsorbents are in progress in our laboratory.

In summary, a rate theory of chromatographic fractionation of polymers with porous adsorbents has been developed. Expressions for elution time and dispersion were obtained. A model of particle structure consisting of spheres with conical pores was developed and integrated into the results of the rate theory. Sample calculations and comparison with the literature indicate that this may be a powerful separation technique.

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