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A COMPARATIVE STUDY OF THE INFLUENCE OF RESTRICTED DIFFUSION UPON THE MOVING RATE OF THE SOLUTE IN VARIOUS FORMS OF CHROMATOGRAPHY

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SUMMARY

The conditions under which the moving rate of a solute in chromatography is influenced by restricted diffusion are investigated.

It appears that this phenomenon does not occur in practice in gas-liquid and liquid-liquid chromatography.

In molecular sieve chromatography, the calculated eluent flow rate for the onset of the phenomenon agrees with the flow rate at which it has been observed.

It is generally believed that in chromatography the ratio of the moving rate of the solute to that of the eluent is equal to the fraction of the solute in the mobile phase at equilibrium. This assumption has been verified repeatedly for gas-liquid chromatography¹⁻⁵.

However, some years ago ACKERS reported that, in molecular sieve chromatography of proteins on Sephadex G-200 and, to a lesser extent G-100, the fraction of the solute in the stationary phase, as calculated from the elution data, is smaller than the equilibrium fraction⁶. He attributed this to restricted diffusion of the solute into the stationary phase.

In this case the possibility exists that due to the prolonged contact of the gel and the protein in the equilibration experiments (24 h) some hydrogen bonds of the former have been broken. This would result in a larger pore size and, consequently, a larger available volume of stationary phase in the gel beads.

Recently, HALLER⁷ observed the same phenomenon in molecular sieve chromatography on porous glass, and so demonstrated conclusively the occurrence of restricted diffusion in molecular sieve chromatography.

In view of these conflicting statements concerning gas-liquid chromatography on the one hand and molecular sieve chromatography on the other, we thought it worthwhile to investigate the conditions under which the moving rate of the solute in chromatography is influenced by restricted diffusion.

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The moving rate of the solute is equal to pu , wherein p is the fraction of the solute in the mobile phase (whether equilibrium is reached or not) and u is the mean flow rate of the eluent. In other words, the solute has a velocity pu , with respect to the stationary phase, and a velocity $(p - 1)u = -qu$, with respect to the mobile phase.

We approximate the Gaussian concentration profile of the solute by a triangular one with a base width equal to four times the standard deviation σ of the Gaussian profile. The maximum residence time of the solute molecules in either phase is then equal to the time required for the solute peak to travel over a distance 4σ with respect to the phase under consideration:

$$\tau_S^{\max} = \frac{4\sigma}{pu} \quad \tau_M^{\max} = \frac{4\sigma}{qu} \quad (1a, b)$$

When the partition equilibrium between the stationary and mobile phases has been established, the solute concentrations in the depths of both phases are equal to the concentrations in the surface layers. So, at equilibrium the mean residence times in the stationary and mobile phases, τ_S and τ_M , are rather long. Obviously, the partition equilibrium can only be reached if:

$$\tau_S^{\max} = \frac{4\sigma}{pu} > \tau_S \text{ and } \tau_M^{\max} = \frac{4\sigma}{qu} > \tau_M \quad (2a, b)$$

whereas equilibrium can no longer be reached and the moving rate of the solute becomes influenced by restricted diffusion if either:

$$\tau_S^{\max} = \frac{4\sigma}{pu} \approx \tau_S \text{ or } \tau_M^{\max} = \frac{4\sigma}{qu} \approx \tau_M \quad (3a, b)$$

When eqn. (3a) applies, diffusion into the stationary phase is restricted, and the retention volume decreases with increasing eluent flow rate. Analogously, in the case of eqn. (3b), the retention volume increases with increasing eluent flow rate.

Anticipating the results derived below, *i.e.*, that restricted diffusion occurs only at large flow rates, we can neglect all terms which are not proportional to u in the expression for the plate height^{8,9}:

$$\frac{\sigma^2}{l} = \frac{B}{u} + C_M u + C_S u + \frac{D}{1 + E/u} \approx C_M u + C_S u = q^2 \tau_M u + pq \tau_S u \quad (4)$$

where l is the column length and B , C_M , C_S , D and E are factors dependent upon the experimental conditions but independent of u .

When eqn. 4 is substituted into eqn. (3a, b) and when it is assumed that $p = q = \frac{1}{2}$, we obtain the following condition for the onset of restricted diffusion:

$$\frac{16l}{u} (\tau_M + \tau_S) \approx \tau_S^2 \text{ or } \frac{16l}{u} (\tau_M + \tau_S) \approx \tau_M^2 \quad (5a, b)$$

The mean residence time τ in a layer of depth d can be calculated as follows. Molecules situated at a distance x from the surface of the layer can leave the layer either by diffusing a distance x towards the surface or by diffusing a distance $d - x$ to the support, being thrown back and diffusing over another distance d towards the

surface, *i.e.*, by diffusing a total distance $2d - x$. The mean distance of diffusion is then equal to d , and the required time is given by:

$$\tau = \frac{\pi d^2}{4D} \approx \frac{d^2}{D} \quad (6)$$

Substitution of this result into eqn. (5a, b) gives:

$$\frac{16l}{u} \left(\frac{d^2_M}{D_M} + \frac{d^2_S}{D_S} \right) \approx \left(\frac{d^2_S}{D_S} \right)^2 \text{ or } \frac{16l}{u} \left(\frac{d^2_M}{D_M} + \frac{d^2_S}{D_S} \right) \approx \left(\frac{d^2_M}{D_M} \right)^2 \quad (7a, b)$$

We shall examine the conditions (5a, b) and (7a, b) for a number of cases below.

(1) Gas-liquid chromatography

In the first place we shall estimate the relative magnitudes of d^2_S/D_S and d^2_M/D_M , to judge whether eqns. (5a) and (7a) or (5b) and (7b) apply.

From PERRETT AND PURNELL'S¹⁰ data on C_S for the elution of heptane and benzene from a 20% octadecane column, their data on D_S for these substances and eqns. (4) and (6), it can be concluded that $d_S \approx 5 \cdot 10^{-4}$ cm. As the support for the stationary phase consists of porous particles, d_M is about equal to half the particle diameter d_p , *i.e.*, about $5 \cdot 10^{-3}$ cm.

As $D_M/D_S \approx 10^4$, it follows that:

$$\frac{\tau_S}{\tau_M} = \frac{d^2_S D_M}{d^2_M D_S} \approx 10^2 \quad (8)$$

Therefore eqn. (5a) applies, and can be simplified to:

$$\frac{16l}{u} \approx \tau_S \quad (9a)$$

Substituting $l = 100$ cm and the value of τ_S for benzene derived from PERRETT AND PURNELL'S data ($2 \cdot 10^{-2}$ sec), we obtain $u \approx 8 \cdot 10^4$ cm/sec which is a far larger velocity than can be realized in practice.

(2) Liquid-liquid chromatography

Here $D_M/D_S \approx 1$, so that $\tau_S/\tau_M \approx 10^{-2}$ *. Therefore, in this case eqn. (5b) applies, and can be simplified to:

$$\frac{16l}{u} \approx \tau_M \quad (9b)$$

From recent data^{11,12} it can be concluded that $C_M + C_S = q^2\tau_M + pq\tau_S \approx 0.25\tau_M$ is about equal to 0.5 sec. Substituting this result, and $l = 100$ cm, we obtain $u \approx 8 \cdot 10^2$ cm/sec. Again, this velocity cannot be realized in practice.

(3) Molecular sieve chromatography

In the special case of molecular sieve chromatography on porous glass, deep, tortuous pores are filled with the stationary phase. The average depth will be of the order of the particles' diameter.

* The expectation that $C_S \ll C_M$ is in accord with recent investigations on peak broadening in liquid-liquid chromatography, using paper or a thin layer of cellulose powder as the support for the stationary phase. In both cases C_S appeared to be negligibly small^{13,14}.

As the particles have a smooth surface, d_M is smaller than in the case of gas-liquid or liquid-liquid chromatography, say about $0.1 d_p$.

As diffusion in narrow pores is sterically and frictionally hindered, $D_M/D_S > 1$. Therefore, $\tau_S/\tau_M > 10^2$ and eqn. (7a) applies, and can be simplified to:

$$\frac{16l}{u} \approx \frac{d^2_S}{D_S} \quad (10)$$

In HALLER'S¹⁵ experiments, $l = 50$ cm and $d_S = d_p \approx 0.03$ cm.

The diffusion coefficient of a spherical molecule in free solution is equal to:

$$D = \frac{kT}{6\pi\eta a} \quad (11)$$

where:

k = Boltzmann's constant

T = absolute temperature

η = dynamic viscosity of the solvent

a = radius of the diffusing molecule

However, in narrow pores the diffusion coefficient D^p is smaller as a result of steric hindrance and increased hydrodynamic frictional resistance to motion. According to RENKIN¹⁶:

$$\frac{D^p}{D} = \left(1 - \frac{a}{r}\right)^2 \left\{1 - 2.104 \frac{a}{r} + 2.09 \left(\frac{a}{r}\right)^3 - 0.95 \left(\frac{a}{r}\right)^5\right\} \quad (12)$$

where r is the pore radius. The first factor on the right accounts for steric hindrance, the second for increased frictional resistance.

In HALLER'S experiments, $\eta = 1$ cP, $a = 130$ Å and $r = 310$ Å. Substituting these values, we find from eqns. (10), (11) and (12) $u \approx 0.01$ cm/sec. If, instead of $p = q = \frac{1}{2}$, $p = \frac{2}{3}$ and $q = \frac{1}{3}$ are used, as in HALLER'S work, an even smaller value for u is found: $u \approx 0.005$ cm/sec. These values are of the same order of magnitude as the flow rates at which HALLER observed the influence of restricted diffusion on the moving rate of the solute (0.001–0.05 cm/sec).

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