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PRINCIPLES OF GEL CHROMATOGRAPHY

HANS VINK
*Institute of Physical Chemistry, University of Uppsala (Sweden)**
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SUMMARY

The principles of gel chromatography have been examined with regard to general chromatographic theory. It is shown that the elution volume for a solute is solely determined by the partition of a solute between the mobile and stationary phases and that various forms of diffusion only contribute to the width of the peak eluted. It is demonstrated that the treatment based on the diffusion model and the Giddings dynamic theory lead to practically identical results.

INTRODUCTION

Despite the considerable amount of work which has recently been carried out in the field of gel chromatography (other names: gel filtration, gel permeation chromatography and molecular sieve chromatography) some uncertainty concerning the interpretation of the fundamental processes involved still remains. Conflicting views prevail especially about the relative importance of diffusion and partition effects. It is therefore desirable to examine the principles of gel chromatography with regard to the laws of general chromatographic theory and, if possible, draw conclusions about the basic processes involved.

THEORY

Firstly the main results of the theory of linear partition chromatography are summarized. As shown in earlier work[1,2], the basic equations may be written as follows**:

$$v = \frac{\dot{\mu}}{v} = \frac{1}{1 + \frac{\gamma V_2}{V_1}} = w_1 \tag{1}$$

$$\mu_2(t) = \mu_2(0) + 2Dt \tag{2}$$

$$D = D' + D'' = D'_1 w_1 + D_2 w_2 + \frac{v^2 \gamma}{2D_2 V_1 \left(\frac{\gamma}{V_1} + \frac{1}{V_2}\right)^3} \tag{3}$$

* Address: Box 532, 751 21 Uppsala 1, Sweden.
** For symbols, see p. 211.

Eqn. 1 determines the peak mobility (relative peak velocity) in the column under steady state conditions and is valid for peaks of arbitrary form.

Eqn. 2 determines the broadening of the peak in the column under steady state conditions in terms of the variance. This formula is also valid for peaks of arbitrary form. The spreading is characterized by a spreading coefficient D, given by eqn. 3.

These equations are concerned with the situation in the column and are not directly applicable to normal experimental conditions in which the concentration distribution is determined as a function of the efflux volume. However, it may be shown[3] that the moments of the concentration distribution with respect to the efflux volume may be expressed in terms of the corresponding moments in the column. As a good approximation the following equations hold:

$$\mu_V = \theta \frac{L}{\omega} \tag{4}$$

$$\mu_{2V} = \left(\frac{\theta}{\omega}\right)^2 \mu_2 \left(\frac{L}{\omega}\right) \tag{5}$$

where, obviously, L/ω is the time it takes the peak to pass through the column.

Eqns. 1–3 are based on a fairly general model and take into account the various diffusion effects encountered in a chromatography column. This model also takes into account the solute distribution in the gel particles by using a stepwise approach in the treatment of the diffusion process in the particles. From this the continuous model is obtained by passing to the limit of infinitesimal steps. Eqns. 1–3 represent the results for a single step model, which, however, only differ insignificantly from the results obtained for the continuous model. A significant feature of the model is that the geometry of the column filling does not enter the treatment explicitly, but is taken into account by the volume to surface ratios V_1 and V_2. This is not surprising since the mass transfer between the mobile and stationary phases occurs at the surface separating the two phases.

We will next consider in more detail the implications of eqns. 1–5 on the mechanism of gel chromatography.

ELUTION VOLUME

In practical gel chromatography the position of a peak is determined by its elution volume, which is conventionally defined as the position of the maximum of the peak in the elution diagram. The elution volume is related to other column parameters by the formula[4]

$$V_e = V_0 + K_d V_i \tag{6}$$

Alternatively[5]

$$V_e = V_0 + K_{av}(V - V_0) \tag{7}$$

The last definition is more convenient since $V - V_0$, which is the total volume

of the stationary gel phase, is readily determined experimentally. Obviously, for a given solute K_d and K_{av} differ only by a constant factor.

Eqn. 7 provides an operational definition of the partition coefficient. It is therefore of great interest to compare eqn. 7 with eqn. 4, in which the equilibrium partition coefficient γ is used and which has been derived under non-static conditions. Rearranging eqn. 4 we get

$$\mu_V = \frac{L\theta}{\omega} = \frac{L\sigma V_1}{v} = L\sigma(V_1 + \gamma V_2) = V_0 + \gamma(V - V_0) \tag{8}$$

Thus, if μ_V is identified with the elution volume V_e (which is strictly valid only for symmetrical peaks) we find

$$\gamma = K_{av} \tag{9}$$

Eqns. 4 and 7 are based on a fundamental principle in chromatography, namely that only the solute present in the mobile phase contributes to the migration of the peak in the column. A mathematically rigorous form of this statement is given by eqn. 1. In the case of eqns. 4 and 7 some approximations are involved, which are necessary because of the distortion of the peak which occurs when it leaves the column, an effect which is not at present amenable to a rigorous mathematical treatment (*cf.* ref. 3).

Although there is substantial experimental evidence in support of eqn. 1 (the elution volume is in general independent of the elution rate) one may enquire under what conditions the solute distribution between the mobile and stationary phases depends on dynamic variables, such as the velocity v.

It should first be noted that the validity of eqn. 1 is a consequence of the linearity of the general diffusion equation

$$\frac{\partial c}{\partial t} = D_2 \frac{\partial^2 c}{\partial x^2} \tag{10}$$

which governs the diffusion in the stationary phase. If D_2 is concentration dependent the linearity of eqn. 10 is lost and eqn. 1 would no longer hold. This is of course also the case when γ depends on the concentration.

We can also ask whether γ is affected by the velocity gradient existing in the vicinity of the gel particles. Since it is normally assumed that the liquid in the immediate vicinity of the particle surface forms a stationary film, the problem reduces to the existence of an uneven partition of solute between the liquid which is flowing past and the film. This effect has been treated in detail in some recent articles[6,7]. However, in view of the low flow rates encountered in gel chromatography this effect appears to be negligible.

There is the further possibility that the partition volume V_2 depends on the velocity. This is the case if the thickness of the stationary liquid film depends on the velocity[8]. However, in general the film constitutes only a small fraction of the partition volume V_2 and therefore this effect is also expected to be rather small.

We may thus conclude that the elution volume of a peak is solely determined by solute partition between the mobile and stationary phases. This is normally inde-

pendent of the elution rate and is determined by the partition coefficient γ and the partition volume V_2 (V_1 can be expressed in terms of V_2). To treat the partition effect we need to dispose of only one of these parameters. However, as we shall see later, both parameters are needed when intraparticle diffusion is considered.

PEAK BROADENING

The broadening of a concentration peak in linear partition chromatography may be expressed in terms of the change of its variance with time and it then assumes the remarkably simple form of eqn. 2. This equation has the form of a diffusion equation and the generalized diffusion coefficient, or spreading coefficient, D, consists of two terms representing the longitudinal diffusion and chromatographic dispersion, respectively.

Longitudinal diffusion

The longitudinal diffusion in the mobile phase is due to the Brownian diffusion and diffusion arising from irregular flow (eddy diffusion). Considering these effects to be additive we may write

$$D'_1 = D_1 + kv \tag{11}$$

where the dependence of eddy diffusion on velocity has been taken into account explicitly. This dependence on velocity is obvious, since the eddy diffusion vanishes when v tends to zero. In the first order approximation the effect is proportional to velocity[9,10]. From eqn. 3 it follows that the contribution of longitudinal diffusion in the mobile phase is proportional to the mass fraction of solute in the mobile phase.

Longitudinal diffusion in the stationary phase is represented by the term D_2w_2 in eqn. 3. This term is only significant if the column packing consists of particles having large dimensions in the axial direction of the column. If these dimensions are small the particles are surrounded by a solution of uniform concentration and this term should be omitted.

Chromatographic dispersion

The last term in eqn. 3, the chromatographic dispersion coefficient D'', represents the dispersion due to the finite rate of mass transfer between the mobile and stationary phases. Obviously this term vanishes when equilibration is instantaneous ($D_2 = \infty$). To bring out the physical significance of the various factors appearing in D'', we may rearrange the expression in eqn. 3 and get

$$D'' = \frac{v^2\gamma V_2^2 w_1^2(1 - w_1)}{2D_2} \tag{12}$$

We find that in this case geometrical factors are important, since D'' is directly dependent on the partition volume V_2, and thus on the dimensions of the particles used to fill the column. If the column packing consists of particles having a simple geometrical form, V_2 is directly computable. For instance, if the column packing consists of spherical particles with the radius r, we have

$$V_2 = \frac{\frac{4}{3}\pi r^3}{4\pi r^2} = \frac{1}{3} r \tag{13}$$

and eqn. 12 becomes

$$D'' = \frac{v^2 \gamma r^2 w_1^2 (1 - w_1)}{18 D_2} \tag{14}$$

In the treatment of partition effects in connection with the elution volume we found that it was sufficient to use only one of the parameters γ and V_2 to characterize the partition process. When diffusion effects are considered it is essential that the correct value of the partition volume V_2 is used. This also permits the consideration of those cases when the gel particles are non-uniform. For instance, when only the surface layer of a gel particle is accessible to the solution, the equilibration time is very much shorter than it would be for a uniform particle. This can be taken into account in eqn. 12 by assigning to V_2 a value comprising only the surface layer.

PEAK FORM AND END EFFECTS

The chromatographic theory used here is based on the moments of the concentration distribution in a peak and is not concerned with the form of these distributions. However, some information about the form of the concentration distribution may be obtained from the general form of eqn. 2. Since the spreading of a peak is governed by the law of diffusion, we can conclude that an initially sharp distribution (δ-function distribution) will give rise to a Gaussian peak. Similarly a peak which is initially Gaussian will remain so during the time it resides in the column.

In practice non-Gaussian peak forms are often encountered. This may be due to adsorption or other effects which make the process non-linear. However, even in the absence of non-linear effects non-Gaussian peaks may occur, and are then due to "end effects" occurring at the column ends. At the loading end the effect naturally depends on the loading conditions, and the magnitude of the effect can be reduced by using a proper loading procedure. Under normal operational conditions the most important factors which are responsible for non-Gaussian peaks are the finite width of the loading zone and the initial departure of the process from steady state conditions. The latter effect is probably also responsible for the initial skewness of the peaks.

At the effluent end, the peak is distorted when it leaves the column. A peak which is Gaussian within the column thus becomes non-Gaussian in the elution diagram. However, this effect is probably quite small, since the variance of the peak only undergoes a slight change when the peak leaves the column[3]. It is also possible that the "end effects" at the two ends of the column counteract each other, which results in nearly Gaussian curves in the elution diagram. The "end effects" are in general difficult to study analytically and it seems that numerical methods[11] are better suited for this purpose.

COLUMN EFFICIENCY

Perhaps the most direct way of indicating the efficiency of a chromatographic

column is to express the peak width relative to its position. In the elution diagram this would be given by the standard deviation of the peak divided by the elution volume. However, in order to avoid the root sign, we may use the square of this quantity instead, *i.e.*, the variance divided by the square of the elution volume. This is a dimensionless quantity and for an efficient column it should be as small as possible. Calling it the reduced dispersion and denoting it by S, we have

$$S = \frac{\mu_{2V}}{V_e^2} \tag{15}$$

Using eqns. 4 and 5 and rearranging with the help of eqns. 1–3 we get

$$S = \frac{2D_1}{Lv} + \frac{2D_2(1 - w_1)}{Lvw_1} + \frac{2k}{L} + \frac{vV_2^2 w_1(1 - w_1)}{LD_2} + \frac{\mu_{2V}^0}{V_e^2} \tag{16}$$

Eqn. 16 reflects the requirements for an efficient column operation immediately. Thus, an efficient column should be long and have a fine-grained filling (small V_2). The requirements for the elution rate are conflicting. For optimal conditions the sum of the terms representing longitudinal Brownian diffusion should be equal to the term representing the chromatographic dispersion. Omitting the longitudinal diffusion in the stationary phase we get for the optimal velocity

$$v = \frac{1}{V_2}\sqrt{\frac{2D_1D_2}{w_1(1 - w_1)}} \tag{17}$$

The efficiency also depends on the solute distribution between the mobile and stationary phases, the term representing the chromatographic dispersion having a flat maximum when the solute is equally distributed between the two phases. Finally, the efficiency depends on the initial peak width, represented by μ_{2V}^0. This term depends on the loading conditions and thus characterizes the efficiency of the overall chromatographic process, rather than the efficiency of the column.

The reduced dispersion defined by eqn. 15 also constitutes the basis of the theoretical plate concept, which is often used to express the efficiency of chromatographic columns. The plate height in a column, H, is given by

$$H = L\left(\frac{w}{4V_e}\right)^2 \tag{18}$$

Since the width w of the peak is usually taken to be four times the standard deviation, we have

$$H = LS \tag{19}$$

In the present study we prefer not to use the theoretical plate concept, since its use in chromatography is somewhat misleading. Its physical significance does not extend beyond that of a relative peak width and it does not represent a genuine analogy to the similar concept in the theory of distillation.

Finally, we shall compare the present theory with the dynamic theory of GID-

DINGS AND MALLIK[12]. Translating their expression for the plate height into our notations we get

$$H = LS = \frac{2D_1}{v} + \frac{2D_2(1 - w_1)}{vw_1} + \frac{1.2vV_2^2w_1(1 - w_1)}{D_2} + \sum_t \frac{1}{1/A_t + 1/C_{mt}v} \qquad (20)$$

We have assumed that the gel particles here are spherical, hence $d_p = 6V_2$, where d_p is the particle diameter in ref. 12.

A comparison with eqn. 16 shows that, apart from the term for the initial peak width, which is absent in eqn. 20, the results are almost identical. In the case of the term representing the chromatographic dispersion, there is a small difference in the numerical constant, and in the term representing the eddy dispersion some additional terms are included in eqn. 20. This close agreement between the two theories, which are quite different in approach, is of course very satisfactory and gives us confidence in the results obtained.

SYMBOLS

v = translational velocity of mobile phase
γ = partition coefficient
t = time
w_1, w_2 = mass fractions of solute in the mobile and stationary phase, respectively
D = spreading coefficient
D' = longitudinal diffusion coefficient
D'' = chromatographic dispersion coefficient
D_1, D_2 = diffusion coefficients in mobile and gel phase, respectively
k = eddy diffusion coefficient
V_1, V_2 = volumes per unit of interphase area of mobile and stationary phase, respectively
σ = interphase area per unit length of the column
L = length of the column
$\theta = v\sigma V_1$ = rate of solvent flow through the column
μ, μ_2 = the mean and the variance of the concentration distribution in a peak in the column, respectively
$\dot{\mu}, \dot{\mu}_2$ = time derivatives of μ and μ_2
ν = peak mobility
$\omega = v\nu$ = velocity of the peak
μ_V, μ_{2V} = the mean and the variance with respect to the efflux volume
V_e = elution volume
V_0 = void volume in the column
V_t = volume of the internal solvent in the gel
V = total volume of the column
S = reduced dispersion
H = height of one theoretical plate.

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