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EVALUATING DISPERSION IN GEL PERMEATION CHROMATOGRAPHY

I. THEORETICAL ANALYSIS*

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SUMMARY

Dispersion (peak spreading, zone broadening) in gel permeation chromatography (GPC) results from a variety of mechanisms, largely independent and additive in their effects. Among the more important of these, in addition to instrumental contributions, is expected to be eddy diffusion within the chromatographic columns. An experimental approach to the study of dispersion in GPC is proposed, based on existing theories and concepts from gas chromatography and liquid–liquid dispersion in packed beds.

INTRODUCTION

Ever since the early realization that virtually all polymer systems contain a distribution of molecular weights, the study of the details of such a distribution has been a problem of major concern. It was not until the development of gel permeation chromatography (GPC) by MOORE[1] that a practical solution to this problem evolved.

With reference to the usual definition of chromatography[2], GPC may be defined as a chromatographic method in which a liquid phase (polymer solution) percolates through a stationary bed (porous gel), the separation depending on the ability of the solute molecules to penetrate into the gel. The amount of internal pore volume accessible to a given molecular species depends on its size, which in simple cases correlates with its molecular weight. Since higher-molecular-size species can permeate less of the internal pore structure of the gel, they are eluted first. (With MOORE[3], we prefer the terminology of gel permeation to gel filtration[4] or molecular-sieve chromatography[5].)

For a given set of experimental conditions, the volume at which a single solute species is eluted from the column depends on the extent to which it permeates the gel, each molecular size having a characteristic elution volume or (at constant flow rate) elution time. The relations between elution volume, molecular size, and polymer chain

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structure are now rather well understood[6], and we will not discuss this aspect of the subject.

For a variety of reasons, however, not all molecules of a single species are eluted at the same time, resulting in a spread of elution or retention times about a mean. This zone broadening or peak spreading is, in our opinion, the major factor imposing an ultimate limit on the accuracy with which molecular-size distributions can be determined by GPC. Much attention has been given to the empirical correction of chromatograms for dispersion[7–10] but to date no comprehensive theory for this recently-developed technique has appeared. In addition, very little work has been published on liquid-system dispersion in packed beds at low Reynolds numbers. It is the purpose of this paper to develop a method for studying dispersion in GPC columns, with the eventual hope of arriving at a satisfactory theory to explain both the dispersion and the permeation processes.

THEORY

The separation process in a chromatographic column can be considered to occur in a series of hypothetical steps, within each of which equilibrium is achieved between solute concentrations in the mobile and stationary phases. In analogy with distillation theory, each step is termed a plate and is considered to correspond to a specified height of the column. Although the actual separation does not occur in this manner, the concept of height equivalent to a theoretical plate (HETP) is very useful for characterizing column efficiency[11]. The number N of theoretical plates is related to the average retention time T_r and its standard deviation σ by $N = (T_r/\sigma)^2$, and the HETP is simply L/N, where L is the column height. For a Gaussian peak, the width of the chromatogram measured at one-half its height is $2.36\,\sigma$.

We assume that gel permeation chromatography is based on a two-phase model, in which the stationary phase consists of the liquid within the pores of the gel, and the mobile phase is the liquid contained in the interstitial volume, *i.e.*, everywhere outside the gel. We now write a diffusion equation describing each phase.

The diffusion equation for the solute concentration in the mobile phase may be written:

$$\partial C_l/\partial t + U(\partial C_l/\partial x) = D(\partial^2 C_l/\partial x^2) + (k/F_l)\,(C_s - C_l/K) \qquad (1)$$

where C is concentration, t is time, F is volume fraction, and x is distance along the column. The subscripts l and s denote the mobile and stationary phases, U is the interstitial velocity (flow rate), D the effective longitudinal-dispersion coefficient, k the mass-transfer coefficient, and K the distribution coefficient.

Excluding the last term on the right-hand side, this equation is conventional and describes all aspects of the process except for permeation. Its use requires the assumption of plug flow.

The contribution of permeation, described by the last term on the right in eqn. (1), is given by the product of a mass-transfer coefficient k and a driving force equal to the difference in solute concentration across the boundary for permeation. To evaluate this concentration difference, we assume that the solute in the stationary phase (concentration C_s) is in equilibrium with that in the mobile phase at the entrance to the pore. The solute in the mobile phase at this point is assumed to have a

concentration differing from its bulk concentration in the mobile phase, C_l, by a factor $1/K$, where K is the distribution coefficient for permeation. This implies a linear isotherm for the permeation process.

Similarly, the diffusion equation for the solute concentration in the stationary phase may be written:

$$\partial C_s/\partial t = (k/F_s)\,(C_l/K - C_s) \tag{2}$$

By making these equations dimensionless, solving them simultaneously by Laplace-transform techniques, and introducing the boundary conditions ($C(0,t) = C_0(t)$, a unit injection impulse at $t = 0$, and $C(\infty,t) = 0$, it is possible to obtain VAN DEEMTER's equation[11]:

$$\text{HETP} = 2D/U + 2F_sK'U/(1 + K')^2k \tag{3}$$

where $K' = F_s/KF_l$. This approach does not restrict VAN DEEMTER's equation to Gaussian distributions, since no mention was made of the form of the resulting chromatogram. Neither is there any restriction on the nature of the injection impulse, provided that its variance is subtracted from that of the final chromatogram.

Taking the first moment of the distribution of retention times gives the average retention time T_r, while the second moment gives its variance. T_r is related to the distribution coefficient by the equation $T_r = 1 + K'$. This relation is in a dimensionless form such that with no permeation, $T_r = 1$.

Experimentally, care must the taken that the retention time and variance of the sample-injection step are negligible or are subtracted from those observed, to obtain the contribution of the column alone.

The longitudinal-dispersion coefficient is assumed[12] to be the sum of contributions from each of the dispersion mechanisms operating in the column:

$$D = \phi D_l + \lambda U d_p + hR^2U^2/D_r \tag{4}$$

where ϕ is a tortuosity factor, D_l the mobile-phase diffusivity, λ an eddy-diffusion factor, d_p the effective particle diameter, h a velocity-profile constant, R the column radius, and D_r is a radial-diffusivity coefficient. Following DORWEILER[13], we assume[14] that D_r varies in the same way as does the velocity profile across the column, and can be approximated by the sum $\phi D_l + \lambda U d_p$.

On the right-hand side of eqn. (4), the first term represents the tortuosity-corrected liquid diffusivity, or "molecular diffusivity". The second term is the "eddy diffusivity", taking into account the particle diameter and the characteristics of the column packing. The third term accounts for velocity-profile effects and radial dispersion. If, as is usual in liquid systems, the molecular diffusivity is negligibly small compared to the eddy diffusivity, the dispersion coefficient becomes a linear function of flow rate. This was confirmed by LEVENSPIEL AND BISCHOFF[15], who showed that for axial dispersion in liquid systems with fixed beds, D/Ud_p was approximately constant for Reynolds numbers from less than 1.0 to 100. VAN DEEMTER did not include a velocity-profile term in his equation for D.

By utilizing high-molecular-size solutes that do not permeate the gel, it is possible to characterize dispersion in the interstitial regions of the column. Since there is no mass transfer, eqn. (3) reduces to HETP $= 2D/U$, and D is readily studied as a function of operating and column-packing variables. Knowing D, F_s and F_l, it is

possible to evaluate the distribution and mass-transfer coefficients for homologous low-molecular-size solutes which permeate the gel. We believe that studies of this sort offer the possibility of obtaining insight into the mechanisms of the GPC separation.

DISCUSSION

Alternate approaches

In a recent theory for zone broadening in GPC based on well-known concepts in gas chromatography, GIDDINGS AND MALLIK[16] assumed that the individual dispersion mechanisms act independently and contribute additively to the total dispersion observed. An eddy-diffusivity term was coupled with one describing the mobile-phase resistance to mass transfer. LEPAGE *et al.*[17] recently applied this theory to the case of permeating solutes with porous silica beads.

Another experimental approach was taken by HENDRICKSON[18], who postulated that peak spreading obeys an equation of the form

$$\overline{W}_b^2 = \overline{W}_m^2 + \overline{W}_a^2 + \overline{W}_i^2 + \overline{W}_d^2 + \overline{W}_s^2 \qquad (5)$$

where $\overline{W}_b$ is the observed width (at the base) of the chromatogram, and the other terms represent, in order, the contributions to that width from the molecular-size distribution of the test sample, the apparatus, spreading in the interstitial volume within the column, diffusional spreading due to holdup of molecules within the pores of the gel, and sorption. HENDRICKSON attempted to evaluate the last four contributions, and thus obtained $\overline{W}_m$ by difference. TUNG *et al.*[8] utilized a reverse-flow scheme to isolate the contribution of longitudinal mixing to peak broadening. This approach required the assumption that the chromatogram was Gaussian.

It has been suggested[19] that a capacitive effect, produced by stagnant-flow areas in a packed column, may occur in liquid systems. This case was analysed by TURNER[20], who pictured the fluid as occupying a channel with distributed pockets of stagnant liquid. The transport of solute into or out of the "dead volume" was assumed to be controlled only by molecular diffusion. This approach was extended[21] by dividing a packed bed into a turbulent region with complete mixing and a stagnant volume with incomplete mixing controlled by molecular diffusion. It is conceivable that such stagnant volumes (exclusive of the pores in the gel) could exist in GPC columns in the form of regions where the local fluid flow rate was very small compared to the bulk flow rate. They would be less important in the GPC of macromolecules as compared to small molecules because of their widely-different diffusivities. While it would be difficult to prove the presence or absence of such regions, it is important to recognize that they could exist.

Advantages of the present approach

The residence-time distribution for any packed-bed system is closely related to chromatographic efficiency. For example, GIDDINGS AND MALLIK[16] draw an analogy between the causes of peak broadening in GPC and in gas chromatography. One might expect, however, that because the retention mechanisms differ, neither GIDDINGS' nor VAN DEEMTER's equations would describe GPC adequately. The real value in these approaches may lie in studying why they do *not* describe the GPC process accurately.

Perhaps more directly applicable are the models proposed to describe the spread of residence times caused by dispersion in packed beds: the diffusion model and the cascade-of-mixers model[22]. Our approach is based on a diffusion model embodying plug flow and expressing results in terms of an effective dispersion coefficient which includes velocity-profile effects as well as diffusivity.

We feel that the method we have outlined can provide a fruitful approach to a better understanding of GPC. The success of this approach depends on one's ability to obtain accurate dispersion data, and this in turn rests on the need to minimize and correct for extra-column contributions from the apparatus and from the finite width of the sample-injection pulse. We have made a detailed study of these factors, the results of which, together with preliminary dispersion data, will be presented in Part II of this series[23]. We and others[24] have observed significant non-Gaussian behavior (tailing) in these extra-column contributions, which can seriously limit the accuracy and column-efficiency of the GPC apparatus.

While HETP is widely used to describe zone broadening in packed-bed processes, its use has a disadvantage in that the HETP of a given column may change significantly with the nature of the solute and with other operating variables, such as flow rate. Thus, SMITH AND KOLLMANSBERGER[25] showed that HETP decreased linearly with decreasing flow rate for permeating solutes, except for a downward concavity at very low flow rates, as predicted by GIDDINGS' proposal[16]. The theory presented here similarly predicts the downswing, followed by a sharp increase at extremely-low flow rates where molecular diffusion becomes important.

In addition to the extra-column contributions mentioned, a thorough study of dispersion in GPC will have to deal with such complexities as velocity-profile effects, molecular diffusion, eddy diffusion, stagnant-flow areas, non-Fickian diffusion, non-linear permeation isotherms, viscosity effects with macromolecular solutes, sorption, particle-size effects, and column-packing variables, to mention only a few. Each of these will have to be evaluated in an as nearly independent fashion as possible prior to the development of a detailed understanding of the mechanism of the GPC process.

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