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Calibration of GPC Columns

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Summary

Calibration in gel permeation chromatography is reviewed with special reference to $M[\eta]$ as the parameter for universal calibration in the case of polymers.

INTRODUCTION

The familiar event in gel permeation chromatography (GPC) is that the largest molecules emerge first from the column and the smallest ones last. Early in the development of GPC it was apparent that the peak elution volume (V) is not a function of molecular weight (M) of the solute species alone, but that molecular structure also plays a role. The task was then to find a formulation for the structural factors with the help of which M can be related to V.

A direct model calculation that starts with a determination of geometry and pore-size distribution of the gel is impractical, if not futile, even if a description could be given entirely in terms of configurational quantities (specific interactions are precluded). The GPC process and the geometry of the gel are in general too complicated for this approach.

The first step in a practical procedure is therefore to calibrate the column with a series of reference solutes usually belonging to a homologous series. In this manner one obtains the familiar plot of log M vs elution volume (cf. Fig. 1), the latter being identified with the volume of column effluent that corresponds to the peak maximum. For this purpose the reference solutes should be monodisperse, or at least exhibit a very narrow molecular-weight distribution (MWD),

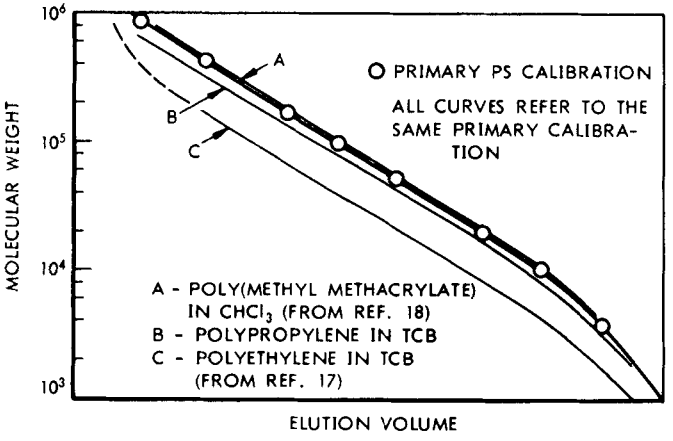

FIG. 1. Calibration curves.

because the position of the peak maximum cannot be directly related to any particular molecular-weight average [in the case of conventional polymers the position of the peak maximum will frequently range between $(M_nM_w)^{1/2}$ and M_w, depending on MWD (1)].

The calibration curve cannot be expected to hold if the material under investigation is structurally different from the reference compounds. This creates problems, particularly in the case of polymers, where it is difficult to obtain sharply fractionated samples. In fact, at the present time polystyrene (PS) standards (Pressure Chemical Company, Pittsburgh, Pa.; Waters Associates, Framingham, Mass; ArRo Laboratories, Joliet, Ill.) are the only readily-available polymeric reference standards for work in nonaqueous solutions.*

It is, therefore, desirable to find a way for transforming a primary calibration curve (as obtained with PS standard, for instance) in such a manner that it can be used with structurally different polymers.

EXTENDED CHAIN LENGTH AND MOLECULAR VOLUME

One of the first attempts to arrive at a useful calibration parameter was to correlate the extended chain length (L) of the solute molecules with the peak elution volume (*4,5*). L can be determined by calcula-

* Balke et al. (*2*) have recently discussed calibration by means of polydisperse standards of known M_w and M_n using a computer search program. A procedure that uses a polymer of very broad but well-defined MWD spanning the molecular-weight range of interest has been described by Cantow et al. (*3*).

tion or from molecular models. This approach held some promise with oligomers, but was not satisfactory with polymers in general, although it represented an improvement over M as a calibration parameter.

The shortcomings of a calibration in terms of L are apparent if one accepts the GPC process to discriminate between molecular species on the basis of effective dimensions in solution. Thus, the concept of molecular volume as a universal parameter is a useful one. This has been shown for small molecules (*6*) provided specific solute–gel interactions (*7, 8*) and solvation effects (*7, 9*) on the elution volume can be discounted (specific effects greatly complicate the problem of universal calibration and will not be considered here).

Complications arise with all nonglobular macromolecules. A configurational effect (*10*) has to be taken into account if the molecules are rodlike. For flexible-coil molecules the concept of molecular volume, in the context of GPC, requires redefinition. The peak elution volume apparently, depends on an "effective" molecular volume operationally defined in terms of hydrodynamic parameters.

HYDRODYNAMIC VOLUME AS A UNIVERSAL CALIBRATION PARAMETER

Benoit and co-workers (*11–13*) found that the peak-elution volumes of fractions of a variety of chemically and structurally different polymers* conformed to a single curve if plotted against the product $M[\eta]$, where M is the molecular weight of the respective fraction and $[\eta]$ the intrinsic viscosity. Hence, $M[\eta]$ can be considered as the universal parameter, proportional to R_H^3, R_H being the viscometric hydrodynamic radius of the polymer coil,

$$M[\eta] = 10\pi N_A R_H^3/3 \qquad \text{(cgs)} \tag{1}$$

(N_A designates Avogadro's number). Measurements in other laboratories on other polymer systems have generally confirmed this universality.† An exception are Meyerhoff's data on cellulose nitrate (a rather stiff coil) in tetrahydrofuran (*19*).

* Linear and branched PS in tetrahydrofuran (THF) at 25°C (*11*); PS, poly(vinyl chloride), polybutadiene, poly(phenyl siloxane), PS-poly(methylmethacrylate copolymers in THF, 25°C (*12, 13*).

† PS, polyethylene in trichlorobenzene (TCB), 130°C, PS, polybutadiene in THF, 23°C (*14*). PS, polyisobutylene in TCB (*15*). PS, polyisobutylene in benzene and butanone/isopropanol at 25°C (*16*); PS, polypropylene in TCB at 135°C (*17*); see also Ref. *18*.

Presumably there is nothing unique about $M[\eta]$ and R_H in the GPC process. It is not unlikely that, for instance, the Stokes radius of molecules obtained from diffusion measurements may serve equally well as a universal parameter, as this has indeed been proposed for biopolymers (*20*). (Preference is to be given to R_H because the intrinsic viscosity is much more readily determined than the diffusion coefficient.)

For linear flexible-coil molecules at least, a theoretical justification can be given for the universality of $M[\eta]$ as a calibration parameter:

1. The retardation of a solute molecule in its travel down the column is governed by the probability of the molecule entering into the pores of the gel. The probability of entry into a particular pore is given by the decrease of free energy associated with the volume restriction imposed on the molecule by the dimensions of the pore.
2. The change of free energy is to the largest extent configurational, the heat of mixing (with the solvent) in the deformation is only of minor importance (*21*).
3. It follows from Gaussian statistics that the dimensions of the pore and a single statistical parameter of the polymer coil [rms radius of gyration, or rms end-to-end distance, $(\bar{r}^2)^{1/2}$, for instance] suffice to describe the change of configurational free energy upon deformation (*21*). Here the theoretical work of Casassa (*22*, *22a*) is of special importance. As a result, molecules of the same statistical dimensions should have the same emergence volume under a given set of experimental conditions.
4. Although the argument in Point 3 was restricted to equilibrium conditions, it should also hold, at least approximately, for dynamic processes in which the diffusion coefficient plays a role, since the latter should be equal for linear molecules having the same statistical dimension.
5. Finally, according to the Flory-Fox equation

$$[\eta] = \phi(\bar{r}^2)^{3/2}/M \qquad (\phi = \text{constant}) \tag{2}$$

$M[\eta]$ is proportional to the cube of the statistical parameter.

This line of reasoning cannot be extended to branched molecules. Yet, calculations by Casassa (*22*) have shown that with reference to the theory of Zimm and Kilb (*23*) universal calibration in terms of $M[\eta]$ is still approximately correct for star-shaped molecules (the

calculations were based on an equilibrium model). This has also been indicated by experimental results (*11*, *24*). According to Casassa's treatment, the agreement seems to be fortuitous. Interestingly, GPC studies on the polypeptide benzyl-L-glutamate in dimethylformamide (*25*) show that even this rodlike (helical) molecule conforms to the present scheme of calibration. To which extent this result can be generalized remains to be seen.

Instead of the Flory-Fox equation—Eq. (2)—one may use the expression of Ptitsyn and Eizner (*26*). The universal calibration parameter then becomes $M[\eta]/f(\epsilon)$, where $f(\epsilon) = 1 - 2.63\epsilon + 2.89\epsilon^2$, and $\epsilon = (2a - 1)/3$, a being the exponent in the Mark-Houwink equation. Some arguments may be advanced in favor of including $f(\epsilon)$, but no clear distinction can be made on the basis of presently available GPC data (*17*).*

Substitution for $[\eta]$ by means of the Mark-Houwink equation, $[\eta] = KM^a$, immediately leads to an equation (*17*, *27*) which transforms a primary calibration curve (obtained with polymer 1) for use with some other polymer (subscript 2)

$$\log M_2 = \frac{1}{1 + a_2} \log \frac{K_1 f(\epsilon_2)}{K_2 f(\epsilon_2)} + \frac{1 + a_1}{1 + a_2} \log M_1 \qquad (3)$$

K and a are the parameters of the respective Mark-Houwink equations. In certain cases these may be found in the literature, but they can usually be determined even if fractionated polymer samples are not available (*17*). (The transformation of the primary calibration curve may have to be carried out by segments if one set of Mark-Houwink parameters is insufficient for the whole range of molecular weights under consideration.)

An application of Eq. (3) may be illustrated by the example of polypropylene in trichlorobenzene at 135°C, PS serving as the primary calibration standard (*17*). The Mark-Houwink equations were determined as $[\eta] = 1.37 \times 10^{-4} M^{0.75}$ (dl/g) for polypropylene, and $[\eta] = 1.21 \times 10^{-4} M^{0.707}$ for polystyrene. From this, one calculates $\log M_{PP} = 0.0496 + 0.975 \log M_{PS}$. The displacement of the calibration curve for this example, and a few others, is shown in Fig. 1.

If $a_1 = a_2$, the calibration curves, $\log M$ vs V, are parallel. Therefore, if equality of the Mark-Houwink exponents can be anticipated for a particular pair of polymers in a given solvent, M_w/M_n, M_z/M_w,

* In the case of Meyerhoff's data on PS and cellulose nitrate ($a_1 = 0.74$, $a_2 = 1$) omission of $f(\epsilon)$ reduces the disagreement between results (*19*).

etc., for polymer 2, can be calculated from the chromatogram with reference to the primary calibration curve without a need for transformation (*28*). Furthermore, it is evident that the factor $f(\epsilon)$ can only be important if a_1 differs significantly from a_2.

M_1 and M_2 in Eq. (3) do not represent any particular averages of molecular weight. Thus, M_2 refers to the molecular weight of monodisperse samples provided M_1 and the Mark-Houwink parameters are valid for monodisperse polymer, as they should be.

DISCUSSION

It seems that the accuracy of calibration in terms of $M[\eta]$—or similarly, by Eq. (3)—is most likely impaired by unreliable Mark-Houwink parameters. Here a critical selection from published data is imperative. (Mark-Houwink equations based on number-average molecular weight should not be used because of differences between M_n and viscosity-average molecular weight, unless the sharpness of the polymer fractions has been documented.) More and better data for many polymer–solvent systems are needed.

One may expect universal calibration in terms of $M[\eta]$ to become unreliable in a molecular-weight range sufficiently low for substantial deviations from Gaussian coil statistics. But it should be kept in mind that the absolute magnitude of these deviations do not matter here; only the deviations of one polymer with respect to another are reflected in the calibration, which is definitely a mitigating factor.

Recently, Dawkins (*18*) has expressed some dissent, and he suggested that universal calibration should be based on the unperturbed dimensions of the polymer coil rather than on the dimensions of the expanded coil as implied in the $M[\eta]$ calibration. He correctly points out that present experimental data do not refute this hypothesis since practically all measurements which compare elution volumes of different polymers have been made in solvents where coil expansion was approximately the same for the polymers under investigation. For evidence Dawkins replotted the data of other investigators and added his own results on PS, poly(methyl methacrylate) and poly(dimethyl siloxane) in chloroform (*18*). Significantly, Meyerhoff's nonconformist cellulose nitrate (*19*) also falls on the same plot, in support of Dawkin's hypothesis. An explanation for the significance of the unperturbed dimensions is offered in terms of an interaction between the solute and polymer chains of the gel in the interior of the pores.

It should be possible to settle this argument by measurements under

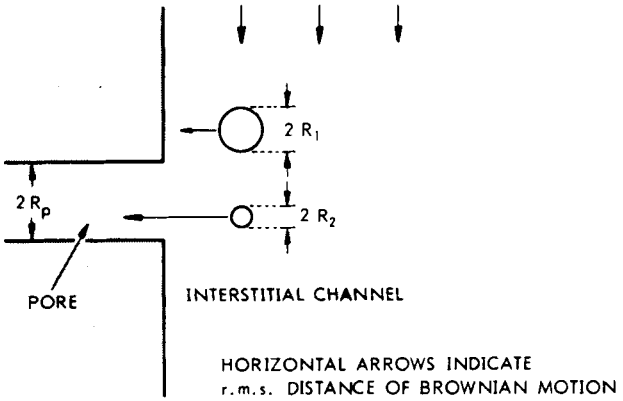

FIG. 2. Schematic representation of capture of molecules by pore.

conditions such that the coil expansions of the respective polymers differ significantly from each other. Experiments of this kind should also reveal whether calibration in terms of $M[\eta]$ or $M[\eta]/f(\epsilon)$ gives more consistent results.

The theoretical discussions of the behavior of molecules in the GPC process have emphasized the equilibrium aspects between the moving phase (interstitial liquid) and the pores of the gel which represent the stationary phase. A few studies dealing with the dynamic behavior have been reported (*29–31*). Yet the following simple considerations suggest that arguments purely on the grounds of equilibrium effects are insufficient to describe exclusion from pores, the central phenomenon in our model of GPC.

Figure 2 represents schematically a wide interstitial channel and a slotlike pore of width $2R_P$. Consider then the progress of two solid spherical molecules with radii (smaller than R_P) in the channel (for the sake of simplicity we may assume uniform flow velocity, v, throughout the channel). Then the probability of entry of a molecule into the pore will first of all be determined by the flow-by time at the entrance to the pore,

$$t = 2(R_p - R)/v \tag{4}$$

R being the molecular radius. Obviously, t is greater for the smaller molecule. Moreover, in this one-dimensional model, entry into the pore can only be brought about by lateral Brownian motion. Therefore, the probability of entry will further depend on the diffusion coefficient

which again favors the smaller molecule, since $D \sim 1/R$. It follows that compact molecules, small enough to enter all pores of the gel, will still exhibit elution volumes depending on their sizes (*32*). In view of conventional flow velocities, gel dimensions, and diffusion coefficients of solute molecules, it appears that this dynamic exclusion effect should be quite significant. In the case of flexible coils, instead of compact molecules, one must further take into account configurational effects—as they apply to the equilibrium model (*22*)—in order to assess the overall probability of entry into a pore.

According to the present model, large molecules are only likely to be captured by a pore if they travel close to the surface of the gel, that is, at distances of the order of 100 Å or less).* This condition becomes less stringent for smaller molecules which diffuse more rapidly, but it is doubtful whether equilibrium between the moving and the stationary phase is ever approached under the conditions of a conventional GPC experiment.

At first it is somewhat surprising that flow rate seems to have only a rather insignificant effect on the peak elution volume (*32*, *33*), unless the molecular weight of the solute is very high (*34*). But as Casassa and Tagami (*22a*) have pointed out, the equilibrium model is still applicable if nonequilibrium exists in the column. The only requirement is that a given molecule undergoes a large number of transfers between the moving and the stationary phase in the course of its passage down the column. Under this condition then, the elution volume that corresponds to the peak maximum should be virtually independent of flow rate.

It has also been suggested that dynamic effects which have to do with the flow pattern of solvent in the interstitial channels may play some role in the chromatographic separation (the assumption of uniform velocities in the channels is very unlikely to apply). One aspect of separation of molecules by laminar flow in the channels has been discussed by DiMarzio and Guttman (*35*).

A theory that takes equilibrium and dynamic effects into account has yet to be formulated. Nevertheless, it appears that the concept of universal calibration, as discussed before, remains valid at least in

* If we assume a pore width of $2R_p = 200$ Å, the molecular diameter $2R = 100$ Å, and a flow velocity of 0.1 cm/sec, one calculates by means of Eq. (4) $t = 10^{-5}$ sec; for a diffusion coefficient of 10^{-7} cm^2/sec one then finds the rms distance of diffusion, corresponding to this time interval, as 141 Å (assuming diffusion in one dimension).

the case of linear flexible molecules, since molecules of the same statistical dimensions should exhibit the same dynamic and equilibrium behavior in the chromatographic process. Similarly, secondary effects, such as peak broadening, skewing, and concentration dependence of elution volume, should be approximately the same for all molecules of the same statistical dimensions.

CONCLUSION

In the absence of specific interactions, calibration in terms of $M[\eta]$ can be considered as universal with reasonable confidence if the polymer molecules are linear and randomly coiled. In the case of long-chain branching somewhat greater reservation is in order. The evidence that this calibration scheme applies to rodlike macromolecules in general is, at the present time, insufficient.

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