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Gel Permeation Chromatography

Gel permeation chromatography is used for preparative and analytical fractionation of macromolecules.

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In modern-day chemistry, especially macromolecular chemistry, it is frequently desirable to know the molecular weight, the molecular weight distribution, and the composition distribution of a material. Polymer chemists and biochemists have long sought good methods for making the needed measurements but the ways and means have proved difficult. Over the course of time, however, several methods have been developed which have recently been reviewed (*1*). Many of the methods involve fractionation, are tedious and time-consuming, and yield fractions which are not especially narrow in molecular weight or composition distribution. Johnson and Porter (*2*), for example, state that there has been such difficulty in determining molecular weight distributions that many of the physical properties that depend on molecular weight distribution have been speculated about rather than actually measured.

Recently the drudgery of bulk polymer fractionation for the determination of molecular weight distribution has been overcome. The advent of automated, analytical gel permeation chromatography (GPC) has provided the researcher with a rapid and generally quantitative method for determining the size distribution of polymer molecules. Depending on polymer type and the information needed, GPC experiments are designed to yield information on the molecular weight distribution or composition distribution, or both, from the size separation (*3*). Gel permeation chromatography has reduced the time required for manual separation (formerly possibly weeks) to a few hours' work.

Gel permeation chromatography began as gel filtration chromatography, a technique which is generally attributed to Porath and Flodin (*4*) and which has been extensively reviewed (*1–8*). In general, gel filtration chromatography is a technique for the size separation of macromolecular, natural product materials on water-swellable gels (Sephadex) by means of an aqueous medium. The term gel filtration chromatography is extensively used in the biochemical literature (*9*).

The division between gel permeation chromatography and gel filtration chromatography came with the invention of new gels for organic solvent phase work. The term "gel permeation chromatography" was coined by John C. Moore of the Dow Chemical Company who developed the first gels and published the first major work based on this technique (*10*). The now commonplace, complete separation of synthetic macromolecules, as illustrated by the separation of two polystyrenes shown in Fig. 1 (taken from Moore's paper), was indeed remarkable in 1964.

In the time since Moore's early work a dual literature has developed. In gel filtration chromatography aqueous solvents and hydrophilic gels are used, the experimenter is usually a biochemist and, generally, natural product separations are involved. In contrast, in gel permeation chromatography organic solvents and hydrophobic gels are used, the experimenter is usually a polymer, analytical, or organic chemist, and the product is usually a synthetic macromolecule. Perhaps it is just as well that such a dual literature exists, for most applications in one field are not of significant interest to researchers in the other. All that is really required is that a few scientists keep track of both areas so that theoretical developments and aspects such as universal calibration can be made known to both groups of investigators.

Principle of Gel Permeation Chromatography

Gel permeation chromatography is a form of liquid chromatography which sorts polymer molecules in a gel-packed column according to their size in solution. A size exclusion, or restricted diffusion, principle is used for the size sorting. Figure 2 schematically shows the longitudinal section of a column packed with a typical "gel" material; an electron micrograph of an actual gel is shown in Fig. 3 (*11*). The gel is a rigidly structured porous network and may consist of materials such as polystyrene cross-linked with divinylbenzene (*12*) or porous glass (sold by Waters Associates, Bio-Rad, Du Pont, Corning, and others under various trade names). Solvent is continuously pumped through the gel and column by means of an external pumping system. A solution of the polymer sample is injected onto the top of the column, and the sample is percolated through the column by means of the flowing solvent stream. The size sorting of the polymer molecules takes place in the pores of the gel. As illustrated in Fig. 2, the very large molecules cannot enter many of the pores and thus travel mostly around the gel

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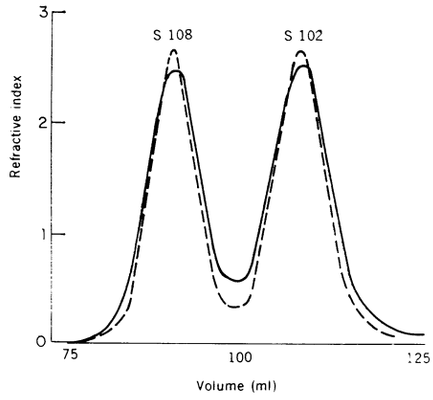


Fig. 1. Separation of two narrow-distribution polystyrenes at two flow rates. The columns measured 0.775 centimeter (inner diameter) by 3.65 meters. The column was packed with a 200- to 325-mesh fraction of a bead polymer made from a mixture of one part commercial 55 percent divinylbenzene and four parts diethylbenzene. The eluent was toluene; flow rate: 26.4 milliliters per hour (solid line); 12.4 milliliters per hour (dashed line). S 108, polystyrene with $\overline{M}_w$ of 267,000; S 102, polystyrene with $\overline{M}_w$ of 82,000. [Reprinted from J. C. Moore (*10*), p. 841, courtesy of John Wiley and Sons, Inc.]

beads and come out of the column first. The very small molecules enter most of the pore volume, have a larger amount of the column volume at their disposal, and take a more tortuous path through the column. These smallest molecules come out last. Molecules intermediate in size exit at intermediate times depending on the general size relationships. Because of the size-sorting process, gel permeation chromatography is used to measure the *size distribution* of polymers.

As the molecules exit from the column, they may be detected by differential refractometry (*13*), ultraviolet photometry (*14*), flame ionization (*15*), infrared absorption (*16*), thermal adsorption (*17*), and other means. Discontinuous detection may also be used. For example, fractions may be collected and the viscosities measured (*18*) or the solvent may be evaporated and the residue weighed. At present, applied gel permeation chromatography is not an absolute technique; that is, calibration is required to yield accurate or quantitative expressions of the size distribution. However, relative information can be obtained without calibration.

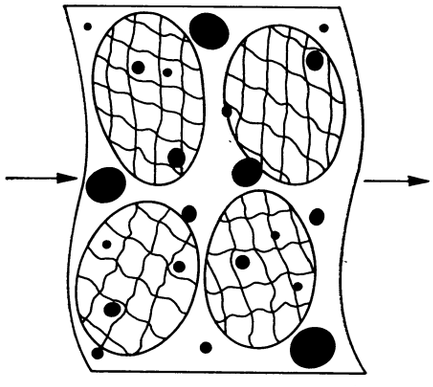


Fig. 2. Longitudinal section of a chromatographic column containing a porous gel matrix and dissolved polymer molecules (schematic).

Calibration can be made with standards by relating the logarithm of the molecular weight, M, or the logarithm of the hydrodynamic volume, log $[\eta] \cdot M$, to the peak retention volume, V_R. Molecular weight data are dependent on polymer type, solvent, and temperature. Type dependency exists because the size of the polymer molecule coil in solution is not the same per unit molecular weight for all polymers. The viscosity of different polymer molecules of the same molecular weight varies widely.

Waters Associates, Inc., introduced the first commercial integrated, automatic gel permeation chromatograph. A flow diagram of the model 200 unit is shown in Fig. 4. The solvent from a reservoir is passed through a filter system into the inlet of a Milton Roy pump. The exit from the pump is damped by a bellows in a surge tank to reduce pump pulsations. The solvent is filtered again and divided into two streams. The reference solvent is passed through a dummy column to provide sufficient back pressure for more evenly controlled flow. It then goes through one side of a differential refractometer detector and into a waste tank. Alternately the reference solvent can be returned to the solvent tank. The other stream passes through the sample columns. Samples are injected onto the columns by means of an automatic four-port injection valve. The exiting polymer solution (containing the sorted molecules) is passed through the other side of the differential refractometer and then through a 5-milliliter siphon, which provides a measure of the flow rate.

To practice gel permeation chromatography it is not necessary to buy a completely integrated unit, although this equipment is best for large-volume, routine industrial type analyses. The laboratory bench-top unit of Rodriguez *et al.* (*19*) shown in Fig. 5 illustrates a rather simple combination of components that can do the job well. Simpler arrangements have been described that provided satisfactory results (*18*).

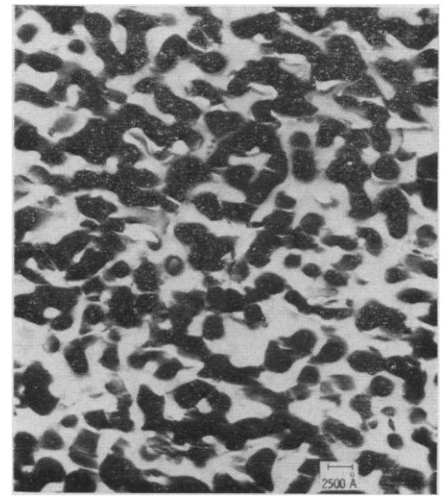


Fig. 3. Electron micrograph of the highly porous glass Bio-Glas 1500. [Reprinted from E. M. Barrall and J. H. Cain (*11*), p. 258, courtesy of John Wiley and Sons, Inc.]

Validity of Gel Permeation Chromatography

The result of most GPC experiments is a collection of fractions or an analog tracing of detector response as a function of retention volume, or both. If separate cuts are obtained for distinct chemical species and if separation is complete, one can analytically prove that the fractionation has occurred and that there is a total mass balance in the system. However, when a size distribution of a polymer is needed, complete separation and isolation of all molecular sizes or species are impossible. Furthermore, accurate molecular weight or polydispersity analysis of the fractions is difficult and often limited by small sample size. The question arises: How valid is the gel permeation chromatographic analysis of the size distribution of the polymer? In fact, Does it have validity at all, or is the picture itself some artifact of the gel permeation separation?

Consider a typical chromatogram (Fig. 6) which represents the analog tracing obtained from conventional equipment. Retention volume is measured from the time of sample injection. The position of the curve peak is inverse-

ly proportional to the logarithm of the molecular weight which increases from left to right but the proportionality (equation) is unknown. Figure 6 shows typically what would be obtained for a fractionated homopolymer. The curve for all the polymer molecules lies between the void volume and the water peak. The void volume is that volume at which polymer molecules would exit from the column when no fractionation had occurred, that is, when the molecules travel only around the gel particles. Peaks are often observed for water and air in polymer analyses because water and air have much lower refractive indices than the solvents.

The validity of Fig. 6 for describing a polymer has been shown in various ways. Calibration itself, originally described by Moore (*10*) as a linear relationship between V_R and log M for standard polymers, or by Grubisic *et al.* (*20*) as V_R plotted as a function of the logarithm of the hydrodynamic volume, is a verification of the fractionation process. Figure 7 shows the data of Bly (*21*) which illustrate the dependence of the calibration equation on polymer type in *m*-cresol. On the other hand, Andrews showed [Fig. 8, (*22*)] that the separation of a large number of carbohydrate-free globular proteins in water could be described by one calibration equation. These relationships all show that various molecular sizes do exit from the column at different elution times. Chromatographing the collected fractions and summing or comparing curves is another way in which the technique has been validated [see, for example, the methods of Tung (*23*) and Tung *et al.* (*24*)]. Biochemists have long used gel filtration, the aqueous counterpart of gel permeation chromatography, to separate proteins and bioactive materials. The increased activity of the purified cuts is another confirmation of the process.

I have studied the problem of verifying the validity of gel permeation chromatograms when quantitative calculation of the data is impossible, that is, when no calibration exists because no polymer standards of the polymer type in question are available or the technique does not permit recovery of sufficient amounts of polymer. I have demonstrated that as long as efficient columns are used and the chromatogram falls in the linear region of the semilogarithmic calibration relationship between $\overline{M}_w$ and V_R, then curve *width*

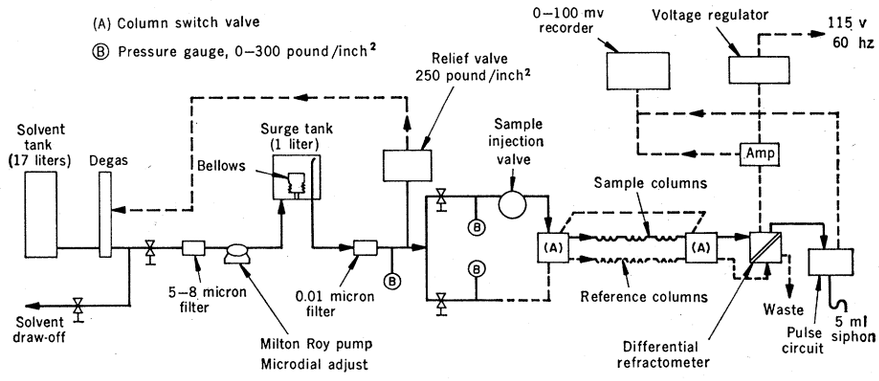


Fig. 4. Flow diagram of the Waters Associates, Inc., model 200 gel permeation chromatograph. [Reprinted from the model 200 service manual, courtesy of Waters Associates, Inc.]

features are truly representative of the sample even though the true calibration remains unknown (*25*). [The quantity $\overline{M}_w$ is the weight average molecular weight or second moment of the polymer molecular weight distribution. This moment and the first moment, $\overline{M}_n$ (also called the number average molecular weight), as well as higher moments such as $\overline{M}_z$ and $\overline{M}_{z+1}$, are defined in most standard polymer text books.] Thus, comparison of a standard polymer curve to an unknown polymer curve can be made with the assurance that relative variations in curve features really represent the sample. Because calibration for molecular weight is ignored, absolute molecular weights are not obtained by this technique, but the relative curve features and thus the *relative size distributions* are obtained. If the polymer size distribution is the most probable or Gaussian-like, the polydispersity d can be calculated from Eq. 1

$$W_1/d_1 = W_2/d_2 \qquad (1)$$

where W is the width of the curve determined as the distance between the base line intercepts of lines drawn tangent to the points of inflection of the trace, d is $\overline{M}_w/\overline{M}_n$, and subscripts 1 and 2 refer to different polymers, one of which is any standard.

Mechanism

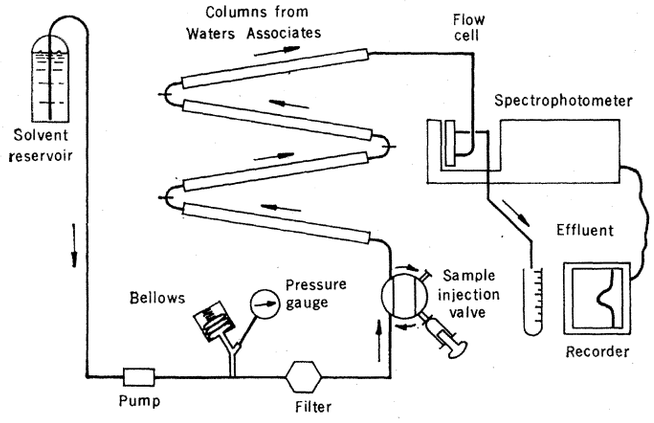
A number of authors have discussed the mechanism of the GPC size-sorting process. It is generally agreed that the process does sort molecules according to their size in solution but it is not generally agreed as to how this size sorting takes place. The phenomenon has been reviewed by Altgelt (*5*), Determann (*6*), Pecsok and Saunders (*7*), and others. Three different mechanisms have evolved: (i) steric exclusion, (ii) restricted diffusion, and (iii) thermodynamic theories. Determann (*6*) has prepared tables of various parameters described in the literature for the elution behavior, mechanism, and calibration in gel chromatography. These are included here as Tables 1 and 2.

The restricted diffusion mechanism for GPC was originally proposed by Ackers (*26*). Additional work has been done by Yau and Malone (*27*), and recently a significant variation has been studied by DiMarzio and Guttman (*28*). Ackers' theory assumes that the time required for a molecule to diffuse in and out of a gel pore is significant relative to the time the molecule spends in the vicinity of that pore. DiMarzio and Guttman show that the separation might be made by flow alone and that no diffusion into pores is required.

The theory of steric exclusion was originally proposed by Flodin (*29*) and has been considered by many others. This theory assumes that the separation process involves differential equilibrium. In contrast to the assumptions made in the restricted diffusion mechanism, according to this mechanism the time required for a molecule to diffuse into, occupy, and come back out of a pore is less than the residence time of the solute around the gel. Under these conditions the process would be neither diffusion-controlled nor sensitive to flow rate, and the distribution coefficient would be described by Eqs. 2 and 3

$$K = \frac{V_R - V_0}{V_1} \qquad (2)$$

$$V_R = V_0 + (K V_1) \qquad (3)$$



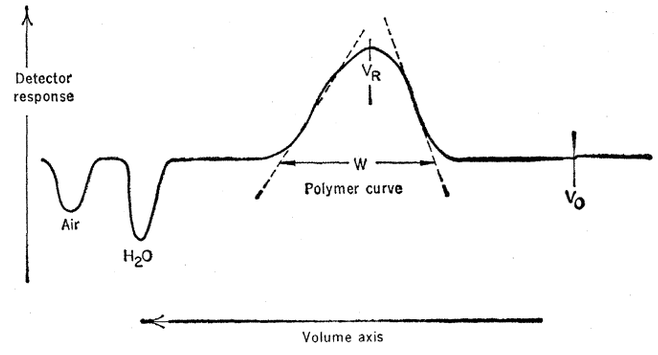
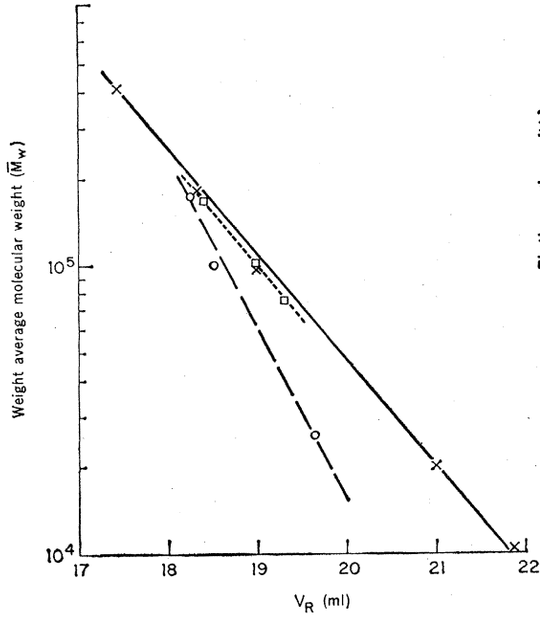


Fig. 5 (left). Bench-top gel permeation chromatographic assembly. [Reprinted from F. Rodriguez, R. A. Kulakowski, O. K. Clark (*19*), p. 122, courtesy of the American Chemical Society] Fig. 6 (right). Typical gel permeation chromatogram for a homopolymer.

where V_R is the retention volume at the peak position maxima, V_0 is the interstitial volume of the column, and V_i is the total volume in the pores. Several workers, using different models for the gel network, have tried to solve theoretically for K. Laurent and Killander (*30*), for example, considered the gel to be a long network of randomly distributed rigid rods. The spaces between these rods represented the pores of the gel. Experimental data validate, fairly well, the calculations for this model in terms of the volume quantities. Porath (*31*), on the other hand, has viewed the network as a cone-like volume, and Squire (*32*), using cylindrical openings and crevices, expanded on Porath's work but considered only spherical solutes.

In the thermodynamic theories experimental gel permeation chromatograms are compared to theoretical curves. For example, DeVries *et al.* (*33*) calculated the elution curve for rigid-sphere polymer molecules in terms of the size distribution of the pores of the gel, the packing efficiency of the gel, the molecular weight distribution of the sample, and size exclusion. Experimental results obtained by DeVries *et al.* are in qualitative agreement with the theoretical curves. Cantow *et al.* (*34*) have shown that the sizes of gel pores determined by mercury porosimetry and by nitrogen desorption isotherms correspond with excluded polymer coil sizes. These authors did not get a 1-to-1 correspondence of gel size to coil size, but the difference was a constant value. Casassa (*35*) described the separation that would be obtained on a uniform gel pore size but with varying macromolecular chain conformation. He assumed that solute molecules inside the cavities are in equilibrium with those outside. He further assumed that the conformation of the molecules could be described by random-flight statistics. Casassa then computed concentration ratios inside the gel and outside, and plotted these as a function of the radius of the molecule and the hollow cavity. He obtained qualitative agreement between calculated values and the experimental values obtained by Moore and Arrington (*36*), an indication that separation is made on a size basis in line with the conformational degrees of freedom of the polymer molecule.

Casassa was testing Moore and Ar-



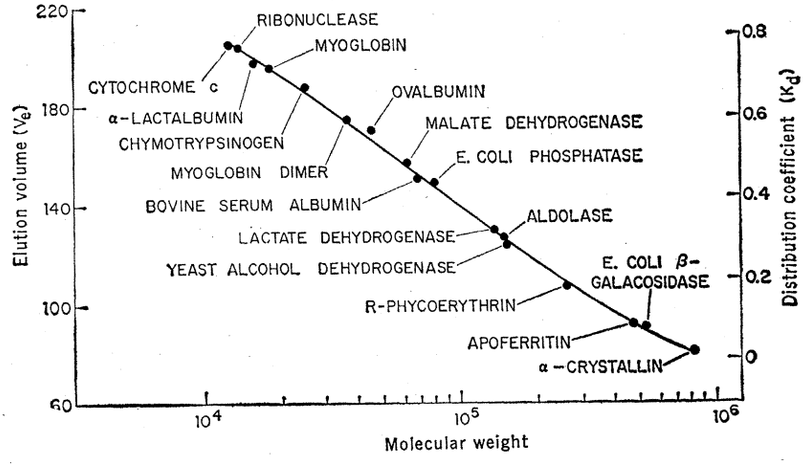


Fig. 7 (left). Relationship between molecular weight and elution volume for various polymers in *m*-cresol. Polycaprolactam (circles); poly(methyl methacrylate) (squares); polystyrene (x's). [Redrawn from D. D. Bly (*21*), p. 2088 (figure 2)] Fig. 8 (right). Relationship between molecular weight and elution volume for certain proteins in water. [Reprinted from P. Andrews (*22*), p. 113, courtesy of the British Council Medical Department]

rington's proposition (*36*) that the "entire range of probable domain dimensions of flexible coil macromolecules" is likely to be important in the mechanism of GPC. To this end Carmichael (*37*) has also approached the problem but by means of a stochastic model. He calculated for a monodisperse sample that the end-to-end distance of a polymer coil and the pore size distribution of the gel should correlate with the retention volume. Carmichael's calculated data are in semiquantitative agreement with the data of Moore and Arrington and with the data of Cantow *et al.*, thus giving weight to the stochastic model concept. (Most of the scientists mentioned in the last three paragraphs are presently active in the field, and it is expected that they will publish additional work.)

Chang (*38*), Yau *et al.* (*39*), and Grubisic-Gallot and Benoit (*40*) systematically investigated certain components in the mechanism of separation in GPC. Their experiments showed that the solute molecule is excluded from a part of the inner space of the gel under static conditions and that the excluded volume increases with the molecular size of the solute. Further, they showed that, although diffusion may play a part in the sorting process, it is relatively unimportant under most conditions. Yau (*41*) has also proposed a coupled theory for both the size exclusion and restricted diffusion mechanisms. Even though Yau's coupling theory is in good agreement with his experimental data, he concludes that this theory should be considered as additional evidence but not as proof for attributing the GPC separation mechanism solely to exclusion and diffusion effects. Despite the complexities and the lack of uniform agreement about the mechanism, I have found it expedient to consider that the mechanism of GPC is exclusively size exclusion and to regard other factors such as flow, restricted diffusion, adsorption, shear strain, viscosity drag, and so on as biases operating on that simple mechanism.

Calculations

The first and often the only data obtained in GPC are the analog tracings of detector response as a function of retention volume. This "picture" is the gel permeation chromatogram. To eliminate personal bias from the pictorial evaluation of gel chromatograms, I have attempted to establish foolproof comparative techniques. These were discussed above and are illustrated by Eq. 1. Because further quantitative computation often is not possible (polymer standards of the polymer type in question do not exist for many research polymers), it is of considerable value just to be able to interpret pictures of GPC but the interpretation must be accurate.

Semiquantitative computation requires calibration with polymer standards and involves calculation of classical molecular weight parameters, $\overline{M}_w$, $\overline{M}_n$, $\overline{M}_z$, $\overline{M}_{z+1}$, and the like. The computation has been discussed by Cazes (*8*).

Truly quantitative calculations of

Table 1. Parameters for the description of the elution behavior in gel chromatography. [Reprinted from H. Determann (*6*), p. 68, courtesy of Springer-Verlag] Original symbols have been retained; references are numbered according to the listing in this article.

Parameter	Calculation	Remarks	Reference
V_e	Direct measurement	Only the results obtained on the same gel bed can be compared	
V_e/V_t	Division of the elution volume (V_e) by the total volume of the gel bed (V_t)	Independent of the geometry of the column; sensitive to differences in packing density; greatest accuracy for small V_e (large molecules)	(*54*)
V_e/V_0	Division of the elution volume (V_e) by the elution volume of an excluded substance (V_0)	Independent of the geometry of the column; sensitive to differences in packing density; greatest accuracy for small V_e (large molecules)	(*55*)
K_d	$K_d = (V_e - V_0)V_i$ V_i (volume inside of the gel grains); dependent on the amount of dry gel and its solvent regain upon swelling (S_r)	Independent of the geometry and packing density of the column; uncertainty in the determination of V_i; greatest accuracy for large V_e (small molecules)	(*56*)
K_{av}	$K_{av} = \frac{V_e - V_0}{V_t - V_0}$	Independent of the geometry and packing density of the column; all columns are easily measured; greatest accuracy for large V_e (small molecules)	(*30*)

Table 2. Relations between the elution behavior of macromolecules and their molecular weights. [Reprinted from H. Determann (*6*), p. 108, courtesy of Springer-Verlag] Original symbols have been retained; references are numbered according to the listing in this article.

Molecular type	Solvent	Relation (derived)*	Reference
Polysaccharides	Water	$V_e/V_t = k \cdot \log M$ (E)	(*54*)
Paraffins	Toluene	$V_e - V_0 = k \cdot \log M$ (E)	(*57*)
Polysaccharides	Water	$K_d^{1/3} = k_1 - k_2 \cdot M^{1/2}$ (T)	(*31*)
Proteins	Water	$K_d^{1/3} = k_1 - k_2 \cdot M^{1/2}$ (E)	(*58*)
Proteins	Water	$V_e V_0 = k \cdot \log M$ (E)	(*55*)
Oligonucleotides	Water	$\ln K_d = k \cdot \log M$ (E)	(*59*)
Proteins	Water	$V_e = k \cdot \log M$ (E)	(*60*)
Oligostyrenes	Chloroform	$K_d^{1/3} = k_1 - k_2 \cdot M^{1/2}$ (E)	(*61*)
Proteins	Water	$K_d = f(M^{1/3}/k)$ (T)	(*26*)
Proteins	Water	$(V_e/V_0)^{1/3} = k_1 - k_2 \cdot M^{1/3}$ (T)	(*32*)
Polyethylenes Polyethers	Different lipophilic solvents	$V_e = k \cdot \log$ chain length (E)	(*62*)
Cellulose nitrate	Tetrahydrofuran	$V_e = k_1 - k_2 \cdot \log (M^{1/2} \cdot R)$	(*63*)
Polystyrenes	Tetrahydrofuran	$V_e = k_1 - k_2 \cdot \log (M^{1/2} \cdot [\eta]^{1/3})$ (E,T)	(*63*)
Polymethacrylate	Tetrahydrofuran	$V_e = k_1 - k_2 \cdot \log (M^{1/2} \cdot [\eta]^{1/3})$ (E,T)	(*63*)
Oligopeptides	Phenol/glacial acetic acid/water	$K_d^{1/3} = k_1 - k_2 \cdot M^{1/2}$ (E)	(*64*)
Hydrocarbons	Cyclohexane	$k_1 - k_2 \cdot \log V_e = M$ (E)	(*65*)

* Symbols k, k_1, and k_2 are constants which have a different value for each formula; (E) refers to the empirical derivation of the formula, and (T), to its derivation from theoretical assumptions on the mechanism of gel chromatography. Other symbols are explained in Table 1.

molecular weight parameters and of the integral and differential molecular weight distributions require calibration and *correction* for curve-broadening operatives which distort the chromatograms. The latter include longitudinal diffusion, channeling in the column, viscosity drag, mixing in the column end fittings and connector tubing, mixing in the detector cell, and overloading due to high concentration. Various authors have evaluated the magnitude of these broadening operatives (*42–44*), and several computer programs have been written to make the necessary corrections (*23, 42, 44, 45*). Most of the computer programs are only partly successful. Usually fairly accurate data for polydispersity are obtained for broad molecular weight distributions with any of the programs, although the absolute values of $\overline{M}_w$ and $\overline{M}_n$ may themselves be rather inaccurate. Most of the programs exhibit mathematical cycling in the computational part of the program. It appears that probably the best quantitative calculations in the future will come from a calibration curve which compensates for all the broadening functions and which is then followed by a straightforward computational program. The procedures described, of course, evaluate the size distribution of a polymer. If two materials, such as proteins, are completely separated, there really is no need to describe the size distribution in terms of a monodisperse material. The picture itself or the collected fractions are the data desired. The calibration curve shown in Fig. 8 is a case in point.

Performance

It is useful, both theoretically and pragmatically, to know the efficiency and performance of GPC columns. The terminology and equations used in gel permeation chromatography are the same as those used in other chromatographic techniques. Efficiency is normally expressed in terms of the height equivalent to a theoretical plate, HETP, or a number of theoretical plates, N (or n), whereas performance is measured in terms of resolution. Special ways of expressing resolution R have been described. For example, Bly has preferred to consider conventional resolution equations (*46*) normalized for the peculiarities of gel permeation chromatography. The difference in elution volumes is normalized for the molecular weights of the samples being used, and the widths of the curves are normalized for the molecular weight distribution of the samples expressed in terms of polydispersity. In this way the conventional Eq. 4 is transformed into Eq. 5:

$$R = \frac{V_2 - V_1}{W_1 + W_2} \quad (4)$$

$$R_s = \frac{V_2 - V_1}{\left(\frac{W_1}{d_1} + \frac{W_2}{d_2}\right) \cdot \left(\log \frac{\overline{M}_{w_2}}{\overline{M}_{w_1}}\right)} \quad (5)$$

The specific resolution, R_s, is an absolute measure of the performance of the columns independent of and not biased by the samples chosen, although it is dependent on the polymer generics. In contrast to the case in gas chromatography or other forms of liquid chromatography, once R_s is known, it can be used a priori to predict the percentage of fractionation between two materials of the same type (*46*).

Applications

Applications of gel permeation chromatography and gel filtration chromatography to macromolecular problems are extensive. The great advantage of these techniques is that they permit one to perform on a micro scale ($<$ 50 milligrams of sample) that kind of fractionation which was previously impossible or was possible only on a macro scale. In addition, the time required to complete a fractionation or to analyze the molecular weight distribution is very much shorter than that previously required for the bulk techniques.

Single crystals, for example, have been analyzed for their molecular weight distribution (*47*) and the effect of degradation on small samples was obtained (*48*). Harmon (*49*) used GPC to study the results of the milling of natural and synthetic rubbers. Law (*50*) has studied in detail solid propellant binders for rocket engines, and Duerksen and Hamielec (*51*) have used GPC data to analyze polymer reactors and kinetic parameters. Many other applications have been cited by Cazes and Gaskill (*52*), and several review articles are in preparation.

Very small amounts of protein materials or other large natural product macromolecules can be separated with ease. In chapter 5 of his book Determann (*6*) reviews many applications in the gel filtration area, including the isolation of enzymes, the separation of various hormones, preparative-scale separations of serum proteins and plasma proteins, the sorting of nucleic acids, the sorting of oligonucleotides, and separations of various viruses and carbohydrates. The separation of carbohydrates has been twice reviewed by Granath (*6*, p. 158).

Waters Associates, Inc., has sponsored a series of very useful technical seminars on gel permeation chromatography, and the reprints from these seminars contain extensive listings of applications.

Future

The futures of gel permeation chromatography and gel filtration chromatography appear to be bright. There will be considerable advancement in high-pressure chromatography (which means high speed) and in large, preparative-scale chromatography. Considerable improvement in resolution and performance ability through the recycle technique is furthering application to small molecules (*53*). For this purpose GPC has the advantage of full automation and column stability. The columns do not bleed a liquid phase as is the case in partition chromatography. With enhancement in resolution and greater speed of analysis, more on-line monitoring for products and processes will come. Monitoring will probably even yield to control by coupling GPC and process to a computer.

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Biochemical Differentiation during Amphibian Metamorphosis

Thyroxine affects liver cytology, transcription, translation, and mitochondrial enzyme level.

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Anuran metamorphosis represents a postembryonic period of extensive morphological, cytological, and biochemical changes by which the tadpole, adapted to an aquatic life, is transformed into a frog adapted to a terrestrial life. This animal system thus provides an unusual opportunity for study of a number of aspects of differentiation and comparative and developmental biochemistry (*1*, *2*). Several aspects of metamorphosis in vertebrates and invertebrates have been reviewed recently (*3*).

While metamorphosis has traditionally been viewed from the standpoint of changes in gross morphology and physiology, biochemical interests in the underlying molecular changes have resulted during the past decade in a considerable study of biochemical changes involved in differentiation and development as aspects of metamorphosis (*2–4*).

This review is confined mainly to results from the author's laboratory, particularly the biochemical changes observed in the liver of the amphibian *Rana catesbeiana* during metamorphosis. Stages of development of *Rana catesbeiana* tadpoles are shown in Fig. 1.

Tadpole liver has been reported to undergo no cell division during metamorphosis (*5*). From a biochemical standpoint this consideration is of prime importance in that the biochemical changes occurring in essentially a fixed population of cells can be studied without concern with the additional biochemical factors associated with cell division and mixed populations of new and old cells (*2*).

Relation of Metamorphosis to Ammonotelism and Ureotelism

Certain species of tadpole excrete ammonia predominantly during their premetamorphic stages, but begin to excrete an increasing amount of urea after onset of metamorphosis (*6*). With the development of suitable assay procedures (*7*) for the different enzymes involved in urea biosynthesis (Fig. 2) from bicarbonate and ammonia (*8*), it became possible to determine the activities of these enzymes at different stages of natural

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