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REVIEW

# FUNDAMENTALS OF THE THEORY AND PRACTICE OF POLYMER GEL-PERMEATION CHROMATOGRAPHY AS A METHOD OF CHROMATOGRAPHIC POROSIMETRY

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CONTENTS



## 1. INTRODUCTION

The idea of using chromatography as a porosimetric method has been actively developed by many workers since the 1960s[1–25]. Compared with other methods used for investigating porous structures (mercury porosimetry, electron microscopy, low-angle X-ray scattering, gas and vapour adsorption and desorption methods), chromatographic porosimetry holds a number of advantages and is particularly attractive as a method for studying sorbent structures.

Porosimetry based on gel-permeation chromatography (GPC) is inexpensive and generally accessible, being based on the use of normal chromatographic equipment. There is no need to use high pressure or low temperatures, no call for the

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test specimens to be subjected to special preparation, nor is there any influence on the sorbent structures and properties. Chromatographic porosimetry permits sorbent investigations under conditions identical with, or close to, conditions of practical usage, which is of particular importance for swellable polymeric sorbents. Also, of the above methods, GPC porosimetry is the only one suitable for certifying commercial packed columns for liquid chromatography.

The theoretical fundamentals of polymer GPC have been laid down by Casassa and co-workers[26–28]. The results predicted by Casassa's theory have been corroborated experimentally, have gained recognition and are being widely used by those specializing in the field of polymer analysis. For all that, these ideas do not appear to be generally considered as eventually forming a theoretical basis for a GPC porosimetric method. As a result, some work on chromatographic porosimetry has been found to contain incorrect interpretations of experimental results and, sometimes, incorrect results.

It is for this reason that we consider it necessary once again to discuss the principal conclusions from macromolecular GPC theory, paying particular attention to those questions which have previously been little dwelt upon but which are essential to the understanding of the problems involved in GPC porosimetry, specifically questions of calibration and of the meaning of porous structure characteristics obtainable with this method.

This review surveys current ideas concerning macromolecular GPC using polydisperse sorbents, ideas which are used as a basis for analysing in detail the principal methods employed for interpreting experiments in chromatographic porosimetry. The conditions that are necessary for the GPC porosimetric method to be realized in practice are discussed.

## 2. FUNDAMENTALS OF THE THEORY OF MACROMOLECULAR GPC

The basic quantity measured in chromatography is the retention volume, $V_e$:

$$V_e = V_0 + V_p K \tag{1}$$

where $V_0$ and $V_p$ are the volumes of the mobile and stationary phases, respectively, and $K$ is a distribution coefficient related to the sizes and types of the molecules being chromatographed, the sizes and forms of the sorbent pores and the molecule-to-sorbent interaction conditions.

In gel-permeation (size-exclusion) chromatography realizable in the absence of adsorption interactions, the distribution coefficient depends on the molecule-to-pore size ratio, and for this reason GPC is a suitable method for determining both molecule size and pore size.

The theory of macromolecular GPC was essentially formulated by Casassa and co-workers[26–28]. The theory is based on the calculation of changes in the entropy of a macromolecule as it penetrates from the mobile phase (solution) into a sorbent pore, and makes use of a model of a flexible-chain macromolecule in a thermodynamically ideal solvent, assuming a low polymer concentration in the solution and a quasi-equilibrium nature of the chromatographic process.

*2.1. Universal distribution coefficient versus molecule-to-pore size ratio relationship*

The chief result of the theoretical studies[26–28] was an universal relationship between the distribution coefficient, $K$, and the ratio of the radius of gyration of the macromolecule, $r$, to the pore radius, $R$. For a model of a slit-like pore of width $2R$, this relationship has the following form[26]:

$$K = \frac{8}{\pi^2} \sum_{m=1}^{\infty} m^{-2} \cdot \exp\left[ - \left( \frac{\pi m}{2} \cdot \frac{r}{R} \right)^2 \right] \tag{2}$$

where the summation is performed for odd values of $m$. In the limiting wide-pore and narrow-pore cases, the $K$ *vs.* $r/R$ relationship acquires simpler forms:

$$K \approx 1 - \frac{2}{\sqrt{\pi}} \cdot \frac{r}{R}; \qquad r \ll R \tag{3}$$

$$K \approx \frac{8}{\pi^2} \cdot \exp\left[ - \left( \frac{\pi r}{2R} \right)^2 \right]; \qquad r \gg R \tag{4}$$

Fig. 1 shows the precise relationship of eqn. 2 as a solid line and the asymptotic eqns. 3 and 4 as dashed and dotted lines. It can be seen that the precise $K$ *vs.* $r/R$ function is well approximated by the set of asymptotes for all macromolecule-to-pore size ratios.

It follows from eqn. 4 that with large macromolecules and narrow pores, at $r > R$, the distribution coefficient is not equal to zero. This signifies that a number of large macromolecules penetrate into narrow pores, assuming elongated conformations that are different from the equilibrium conformations of macromolecules in solution (Fig. 2a and b). Penetration of large molecules into narrow pores is a specific feature of polymer chromatography, which Casassa[26] was the first to recognize and which was not accounted for in other GPC theories[29,30].

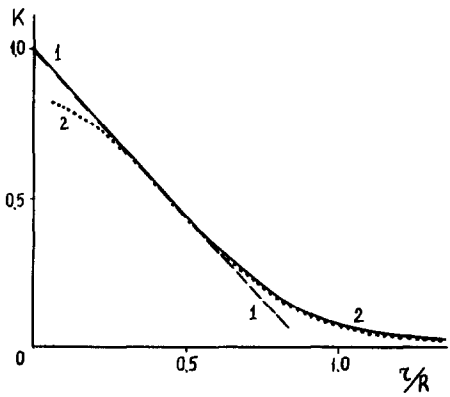


Fig. 1. Distribution coefficient $K$ *versus* the ratio of the radius of gyration, $r$, to the slit-like pore half-width $R$ (based on the theory[26–28]). Solid line, eqn. 2; dashed line (1), eqn. 3; dotted line (2), eqn. 4.

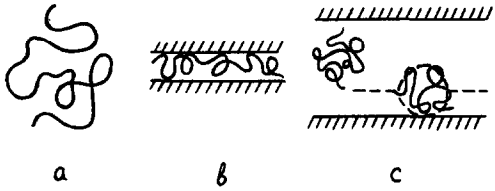


Fig. 2. Typical macromolecular conformations: (a) in a solution; (b) in narrow pores; (c) in wide pores.

The linear dependence of $K$ on $r/R$ in wide pores is of a fairly general nature[28,29]. It reflects a decreasing effective volume available for accommodation of a macromolecule in a pore. In wide pores, macromolecules have approximately the same conformations as in an unrestricted volume (Fig. 2c) and behave in chromatography like spherical solid particles with an equivalent radius. In fact, eqn. 3 is a definition of the effective chromatographic radius of the macromolecule.

*2.2. Chromatographic radius of macromolecules*

It can be seen from comparing eqn. 3 with the expression for the distribution coefficient of a spherical particle of radius $\rho$ in a slit-like pore at $\rho < R$[29],

$$K = 1 - \frac{\rho}{R} \tag{5}$$

that in the process of chromatography in wide pores the polymer chain is like a spherical particle of an equivalent radius

$$S = \rho_{\text{equiv.}} = \frac{2}{\sqrt{\pi}} \cdot r \tag{6}$$

Comparing the results of GPC theory for various forms of molecules, Casassa[28] drew a general conclusion to the effect that the equivalent chromatographic radius of an arbitrary particle is equal to half its mean span (the mean span being the greatest projection of the molecule on to the axis selected, averaged for all possible molecular orientations and conformations).

The effective chromatographic radius for molecules of all types studied is proportional to the radius of gyration, but the coefficients of proportionality have proved to be different both for various forms of rigid particles[29,31], *viz.*, spherical, ellipsoidal and rod-like, and for polymer molecules of various topology, *i.e.*, linear, branched and ring[27,32].

It has thus been shown that the radius of gyration is not strictly a universal chromatographic characteristic for macromolecules. Other characteristic dimensions, *e.g.*, the Stokes radius determinable from the friction coefficient in diffusion and sedimentation processes and the hydrodynamic radius associated with Benoit's "universal calibration" dependence parameter[33], have also been denied the status of universal chromatographic characteristics, which they are not, in fact[28]. However, in a series of molecules of a certain type, any of these dimensions may be used as a characteristic chromatographic radius.

*2.3. Various pore forms. Universal chromatographic porous medium characteristics*

In addition to the simpler slit-like pore model, Casassa and co-workers[26–28] also considered cylindrical- and spherical-form pore models. They postulated that the expression for the distribution coefficient of a flexible-chain macromolecule, common to all three models, has the following form:

$$K^{(\alpha)} = \sum_{m=1}^{\infty} \frac{2\alpha}{[\beta_m^{(\alpha)}]^2} \cdot \exp\left[ - \left( \beta_m^{(\alpha)} \cdot \frac{r}{R} \right)^2 \right] \tag{7}$$

For slit-like pores, $\alpha = 1$, $\beta_m^{(\alpha=1)} = \pi (m - \frac{1}{2})$; for cylindrical pores, $\alpha = 2$ and $\beta_m^{(\alpha=2)}$ is the $m$th root of the Bessel function $J_0(\beta)$; for spherical pores, $\alpha = 3$, $\beta_m^{(\alpha=3)} = \pi m$. In narrow pores, at $r \gg R$,

$$K^{(\alpha)} \approx \frac{2\alpha}{[\beta_1^{(\alpha)}]^2} \cdot \exp\left[ - \left( \beta_1^{(\alpha)} \cdot \frac{r}{R} \right)^2 \right] \tag{8}$$

In the other limiting case, that of wide pores, at $r \ll R$, it follows from eqn. 7[27] that

$$K^{(\alpha)} \approx 1 - \frac{2\alpha}{\sqrt{\pi}} \cdot \frac{r}{R} \tag{9}$$

Eqn. 9 for the distribution coefficient in wide pores acquires the most universal and model-independent form if a transition is made from the radius of gyration of the macromolecule, $r$, to its effective chromatographic radius $s = \frac{2}{\sqrt{\pi}} \cdot r$ and the parameter $\Sigma = \alpha/R = S_p/V_p$ = the ratio of pore surface $S_p$ to pore volume $V_p$ is introduced in place of pore radius $R$:

$$K \approx 1 - s\Sigma \tag{10}$$

The $K^{(\alpha)}$ *versus* $g = s\Sigma$ relationships calculated from eqn. 7 are shown in Fig. 3. It can be seen that the curves plotted in these coordinates for various pore models are close to one another and at $g < 0.4$ they almost coincide. Hence the pore form is of little effect as regards the laws governing macromolecular GPC. Consequently, chromatographic measurements cannot be used to obtain reliable information about the pore form of a sorbent.

In contrast to the radius $R$, the pore specific surface area, $\Sigma$, is a model-independent characteristic, of significance for pores of any geometry. $\Sigma$ can therefore be considered as a universal sorbent pore characteristic; it can be determined by experiment, without making any assumptions regarding the sorbent pore form. Although the pore form of a real sorbent is generally not known (it may be complicated), knowing the value of $\Sigma$ one can always obtain the effective radius, $R_{equiv.} = \alpha\Sigma^{-1}$, of the equivalent pores of a standard form (*e.g.*, cylindrical or slit-like) and use it as a model characteristic of the porous structure.

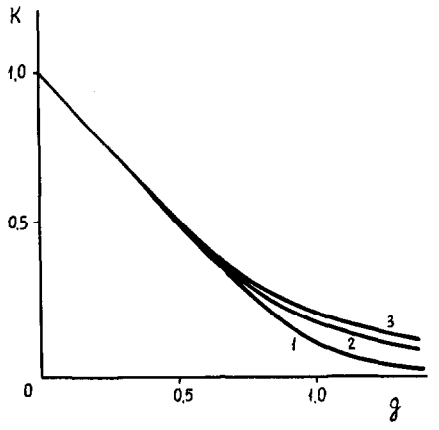


Fig. 3. Distribution coefficient $K$ *versus* $g = s\Sigma$ for (1) slit-like pore model, (2) cylindrical pore model, and (3) spherical pore model. The curves plotted are based on eqn. 7.

### *2.4. Comparison of theory and experiment*

The existence of a universal relationship between distribution coefficient and macromolecule-to-pore size ratio under GPC conditions has been corroborated by many experimental studies and may now be considered as firmly established. Similar relationships have been quoted for polystyrenes[26,34,35], and similar data are also available for water-soluble polymers, *viz.*, dextrans and polyethylene glycols[36,37]. As an example, Fig. 4 shows the $K$ *vs.* $r/R$ relationship plotted in ref. 17 based on data from ref. 38 pertaining to the GPC of dextrans on controlled pore glasses having pore radii varying from 4 to 26 nm. It can be seen that the experimental points related to different pore sizes fit the same curve, fully in agreement with theory.

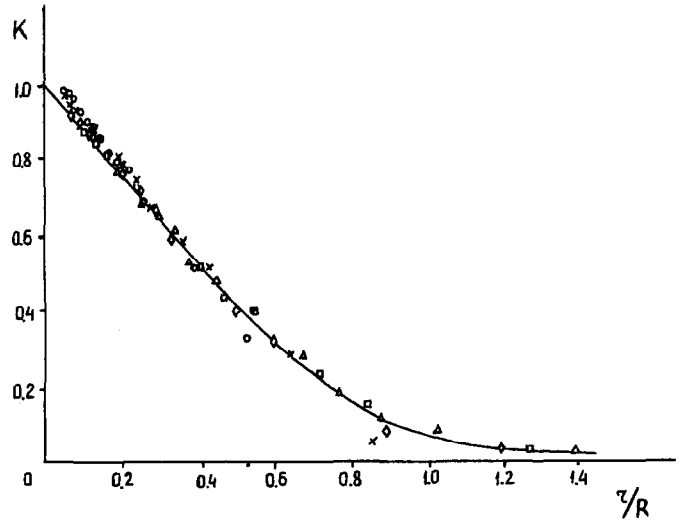


Fig. 4. Distribution coefficient $K$ *versus* $r/R$ for GPC of dextrans on narrow size range porous glasses with pore radii $R$ = 4 (△), 8 (□), 11.5 (◇), 15.5 (×) and 26 (○) nm. Data from ref. 38.

Hence there is complete qualitative agreement between Casassa's theory and experimental data on macromolecular GPC. At the same time, discussions concerning the problem of the quantitative coincidence between theory and experiment have been going on for about 20 years. The reason is that independent methods must be used to measure macromolecule and pore sizes in order to elucidate this problem. However, there are a number of difficulties to be faced. First, there are no independent methods for determining the chromatographic radius of macromolecules, so the radius of gyration, the Stokes radius or some other characteristic molecular dimensions are used for this purpose. Then there also arise problems associated with independent sorbent pore size measurements. Generally, pore sizes are determined using mercury porosimetry[39], electron microscopy[40] or adsorption methods (BET method)[41], and there are limitations for each of these methods. Thus, mercury porosimetry requires the use of high pressures when narrow-pore sorbents are to be investigated (over 1000 atm at $R < 7.5$ nm)[1,5,39]. The nitrogen BET method requires the use of low temperatures (−183°C) and is known to become inaccurate at $R > 20$ nm[5,21]. Additional problems are associated with the fact that different methods provide differently averaged pore sizes. Thus, in mercury porosimetry, the actual quantity measured is the mean radius of curvature of the pore inlet openings[39], whereas the polymer GPC method is also sensitive to inner pore expansions. This is probably the cause of the qualitative variance observed by several workers between mercury porosimetry data and chromatographic data[34]. Difficulties also arise in the interpretation of electron microscopy data when identifying image details with sorbent pores[40].

Discussions on the problem of conformity between GPC data, on the one hand, and theory and data obtainable by other methods, on the other, are of importance for calibrating GPC as a porosimetric method. Two calibration methods are possible, one using GPC theory and the other using other porosimetric methods. The use of GPC theory for calibration purposes would appear preferable, in our opinion, as it makes the method independent of limitations and errors introduced by other porosimetric methods and the pore size measuring accuracy by the GPC method comparable to the test macromolecule measuring accuracy.

## 3. MACROMOLECULAR GPC USING POLYDISPERSE SORBENTS

Studies aimed at ascertaining the principles of macromolecular GPC using inhomogeneous sorbents are generally undertaken with a view to optimizing polymer separation and analysis conditions[34]. At the same time, these studies form an indispensable theoretical and methodological basis for the GPC analysis of sorbent structures. We may briefly consider the main results of theoretical studies[10,17,42] dealing with macromolecular GPC using polydisperse sorbents.

### *3.1. Distribution functions, mean sizes and pore size inhomogeneity characteristics*

Real sorbents are mostly polydisperse, *i.e.*, inhomogeneous in pore size. To describe such sorbents, Gorbunov *et al.*[17] introduced differential functions for the pore volume distribution, $f_v(R)$, and for the pore surface distribution, $f_s(R)$, normalized for the total pore volume, $V_p$, and for the total pore surface area, $S_p$. The

mean sizes $R_v$ and $R_s$ corresponding to these functions are defined by the following relationships:

$$R_s = S_p^{-1} \int_0^\infty R f_s(R)\, dR = V_p \left[ \int_0^\infty R^{-1} f_v(R)\, dR \right]^{-1} = \alpha \cdot \frac{V_p}{S_p} \tag{11}$$

$$R_v = V_p^{-1} \int_0^\infty R f_v(R)\, dR = R_s^{-1} S_p^{-1} \int_0^\infty R^2 f_s(R)\, dR \tag{12}$$

Pore size inhomogeneity (polydispersity) can be characterized by the standard width $\sigma$ or dispersion $\sigma^2$:

$$\sigma^2 = S_p^{-1} \int_0^\infty R^2 f_s(R)\, dR - R_s^2 \tag{13}$$

More convenient than polydispersity characteristics are the dimensionless parameters relative distribution function width,

$$\gamma = \frac{\sigma}{R_s} \tag{14}$$

and polydispersity,

$$U = 1 + \gamma^2 \tag{15}$$

It follows from eqns. 11–15 that $U$ is equal to the ratio of the two different mean pore sizes:

$$U = R_v/R_s \tag{16}$$

and resembles, from the standpoint of meaning, the well known parameter $M_w/M_n$ which is normally used to characterize the molecular weight inhomogeneity of polymers. For a sorbent with all pores identical, $\gamma = 0$ ($U = 1$), and both $\gamma$ and $U$ increase with increasing polydispersity.

*3.2. Theory of polymer GPC using polydisperse sorbents*

So far as we know, the first model that can be regarded as a model of macromolecular GPC using a polydisperse sorbent was considered by Doi[42], who discussed an equilibrium distribution of a flexible-chain macromolecule of radius of gyration $r$ between the solution phase and the stationary phase, with a space randomly filled with impermeable spherical elements of radius $a$ used as a model of the latter. In the free volume, $V_p$, of the stationary phase there were $n$ spherical elements having

a concentration $c = n/V_p$. For this model, Doi obtained an approximate equation for the distribution coefficient:

$$K \approx \exp\left(-8\sqrt{\pi}\, ca^2 r - 4\pi car^2\right) \tag{17}$$

This model is inconvenient, however, for analysing the principles of macromolecular GPC using polydisperse sorbents, as the pore form, pore sizes and pore size distribution are not specified explicitly.

An alternative approach based on the introduction of a model pore size distribution function, $f_v(R)$, was developed[4,10,17]. As the retention volume, $V_e$, is the overall statistical sum of the macromolecule in the mobile and stationary phases, then in calculating $V_e$ for a polydisperse sorbent the summation should be performed for all the elements of the stationary phase, which results in

$$V_e = V_0 + \int_0^\infty f_v(R) K\left(\frac{r}{R}\right) dR \tag{18}$$

where $K(r/R)$ is the distribution coefficient for a monodisperse sorbent having pores of radius $R$. Eqn. 18 can be written in the usual form as

$$V_e = V_0 + V_p \bar{K} \tag{19}$$

in which case the distribution coefficient for a polydisperse sorbent, $\bar{K}$, will be defined by the following equation[10,17]:

$$\bar{K}(r) = V_p^{-1} \int_0^\infty K(r/R) \cdot f_v(R)\, dR \tag{20}$$

A detailed analysis of the theoretical $\bar{K}(r)$ relationships for polydisperse sorbents was given by Gorbunov *et al.*[17]. It was established,in particular, that with polydisperse sorbents also the initial course of the $K(r)$ relationship is described by a simple universal equation

$$\bar{K} \approx 1 - \frac{2\alpha}{\sqrt{\pi}} \cdot \frac{r}{R_s} = 1 - s\Sigma; \qquad r \ll R_s \tag{21}$$

with $\Sigma = S_p/V_p = \alpha/R_s$ now having the meaning of the ratio of the total surface area of all pores to their total volume.

It will be noted that for the random sphere model in the limiting case of $ca^2r \ll 1$, eqn. 17 also leads to eqn. 21, because for this model $\Sigma = S_p/V_p = 4\pi ca^2$ (ref. 42).

Hence the initial slope of the $\bar{K}(r)$ relationship depends only on the value of the sorbent's specific surface area, $\Sigma$ (mean pore radius $R_s$), and is independent of the width and type of the pore size distribution function.

When the macromolecule sizes are comparable to the mean pore radius $R_s$, the distribution coefficient becomes dependent on the width and type of the function $f_v(R)$. Eqns. 20 and 7 enable the $\bar{K}(r)$ relationship to be calculated for sorbents having model distribution functions $f_v(R)$. An example of such a calculation is given in Fig. 5. Monodisperse sorbents are considered with $R$ = (1) 5 nm and (2) 15 nm, in addition to inhomogeneous model sorbents with (3) a unimodal and (4) a bimodal distribution function. Case (3) corresponds to a logarithmically normal distribution of the type

$$f_v(R) = \frac{V_p}{R\sqrt{2\pi \ln U}} \cdot \exp\left\{ -\frac{\left[\ln\left(\frac{R}{R_s\sqrt{U}}\right)\right]^2}{2 \ln U} \right\} \tag{22}$$

with parameters $R_s$ = 15 nm and $U$ = 2. Case (4) is a bimodal distribution with the same values of $R_s$ and $U$ (two maxima at $R_1$ = 8.8 nm and $R_2$ = 51.2 nm). With bimodal distributions with greatly (by an order of magnitude or more) differing pore sizes, two linear segments can be observed in the $\bar{K}(r)$ curve. The initial segment contains the information regarding the mean pore size $R_s$, just as in the general case, whereas the slope and intercept of the second linear segment depend on the large pore size and volume fraction[17].

The analysis carried out previously[17] showed that the type of $\bar{K}$ *versus* $r$ relationship depends chiefly on two parameters, the mean pore radius $R_s$ and the pore

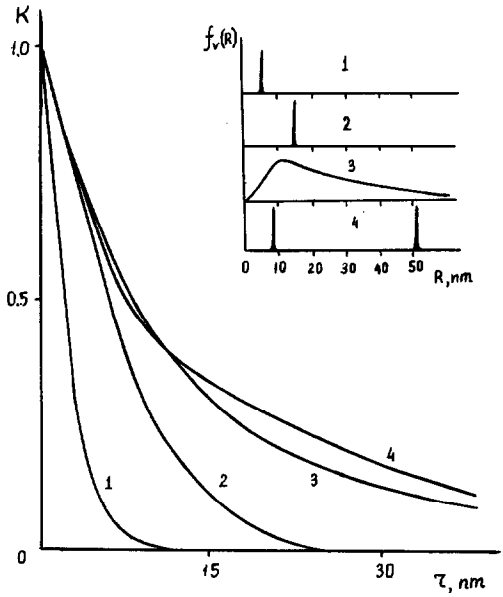


Fig. 5. Theoretical dependences of the distribution coefficient $K$ on the radius of gyration of the macromolecules chromatographed for two monodisperse sorbents (1 and 2) and for inhomogeneous model sorbents having (3) a unimodal and (4) a bimodal pore size distribution. Mean pore radius $R_s$ = (1) 5 and (2–4) 15 nm; polydispersity parameter U = (1 and 2) 1 and (3 and 4) 2. Shown at top are the types of the respective distributions.

size distribution function width. The type of distribution function $f_v(R)$ has little effect on the macromolecular GPC principles.

*3.3. Experimental data on macromolecular GPC using polydisperse sorbents*

Inhomogeneously porous sorbents are used in chromatographic practice for optimizing methods used for analysing polymer molecular weights and molecular weight distributions (MWDs). It is known, for instance, that the use of a set of columns containing sorbents of varying pore sizes or mixtures of such sorbents permits the working range of molecular weights in the GPC analysis of polymers to be expanded. Yau *et al.*[34] and Vilenchik *et al.*[43] described specially selected sorbent mixtures ensuring linearity of the calibration graph (coordinates $K$ *versus* ln $M$) over a wide range of molecular weights, the latter group making use of the theoretical eqn. 21 for designing such sorbents. Experimental data have also been published on molecular GPC based on the use of biporous sorbents[10,15,18,21,34]. Most of these studies, however, were not aimed at comparing experimental data and GPC theory based on the use of polydisperse sorbents.

The sensitivity of the chromatographic method to pore size polydispersity was demonstrated experimentally[17]. Based on four sorbents of narrow size range (modified porous glasses and silica gel) with pore sizes $R$ = 2.3 (I), 7.2 (II), 11.7 (III) and 31.3 nm (IV), two model specimens were prepared: a two-component mixture of sorbents II and III (61:39, v/v), and a four-component mixture of sorbents I–IV (10:22:31:37, v/v). The compositions of these mixtures were specially selected so that while having identical mean pore radii, the two- and four-component sorbents would have different polydispersities. In accordance with theory, one would expect for such sorbents $\bar{K}(r)$ relationships similar to curves 2 and 3 in Fig. 5, *i.e.*, coinciding initially but diverging at large values of $r$. The $\bar{K}(r)$ relationships obtained experimentally for the two- and four-component sorbents (Fig. 6) are in fact as would be expected, which is evidence that the theoretical views are correct.

Careful measurements of the distribution coefficients of dextrans using as the sorbent a mixture of CPG-10 porous glasses with various pore sizes were performed by

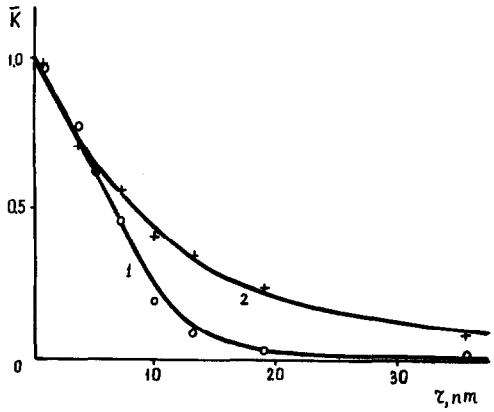


Fig. 6. Experimental $\bar{K}(r)$ relationships for dextran molecules on mixed sorbents. 1, Two-component sorbent; 2, four-component sorbent. Data from ref. 17.

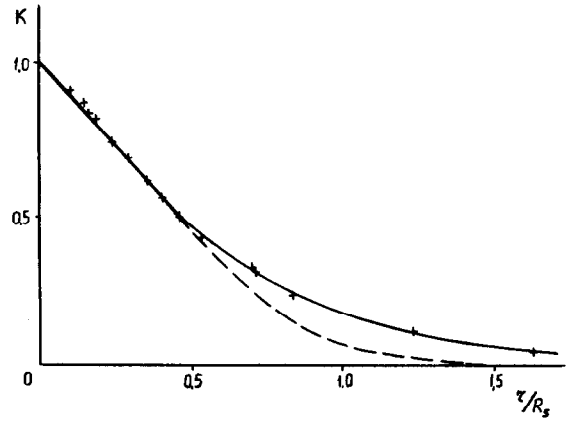


Fig. 7. Distribution coefficient $K$ *versus* $r/R_s$ for chromatography of dextrans on mixed sorbent CPG-10. Experimental data from ref. 44; dashed line as per theory, ref. 26; solid line as per theory, ref. 17, at $U = 1.22$.

Basedow *et al.*[44]. All the glasses used for preparing the mixed sorbent had a high degree of pore size homogeneity. The data on the GPC of dextrans on these monodisperse glasses[38] fitted the same common curve in the coordinates of $K$ *versus* $r/R$, coinciding with the theoretical relationship (eqn. 2) for monodisperse sorbents (Fig. 4). The results for the mixed sorbent[44] are shown in the same coordinates in Fig. 7. The experimental points for high-molecular-weight dextrans can be seen to deviate noticeably from the $K(r/R)$ relationship for monodisperse sorbents, shown by the dashed line. The solid line in Fig. 7, passing through the experimental points, was calculated from eqns. 20, 22 and 2 of the polydisperse sorbent GPC theory and corresponds to a value of the polydispersity parameter $U = 1.22$.

Hence the available experimental data are in good agreement with the theory[17] of macromolecular GPC based on the use of polydisperse sorbents.

## 4. CHROMATOGRAPHIC POROSIMETRY OF SORBENTS

The establishment of the basic principles of macromolecular GPC using inhomogeneously porous sorbents permits the solution of a practically important inverse problem, *viz.*, finding the porous structure characteristics of sorbents based on macromolecular GPC data.

Various methods have been proposed, and have gained acceptance in practical work, for the chromatographic determination of pore sizes, polydispersities and pore size distribution functions. However, there have been no detailed discussions until now of these methods, based on common theoretical grounds, which makes it difficult to assess and compare experimental results obtained by different workers.

We shall analyse the principal approaches used to interpret experiments in GPC porosimetry and discuss the conditions required for the correct practical realization of the method.

### *4.1. Determination of sorbent pore volume, surface area and size*

Pore volume is an important sorbent characteristic as it defines the maximum

range of $V_e$ values in which the size separation of macromolecules is possible. The pore volume is required for calculating the distribution coefficients $K$ in eqn. 1. Normally, the pore volume, $V_p$, is determined by measuring the retention volume difference, $V_t - V_0$, for small molecules capable of penetrating into every pore and large particles that are known *a priori* to be incapable of entering the pores. Sorbent porosity can be conveniently characterized as the pore volume fraction, $x = V_p/(V_0 + V_p)$.

The GPC method likewise readily allows the sorbent pore surface area, $S_p$, to be assessed. By combining eqns. 1 and 21 we obtain

$$V_e \approx V_0 + V_p - \frac{2}{\sqrt{\pi}} \cdot rS_p \tag{23}$$

Eqn. 23 is valid only when the size of the molecule is smaller than the pore size, as for eqn. 21. According to eqn. 23, the value of $S_p$ can be determined from the initial slope of the curve of $V_e$ *versus* $r$.

Hence $S_p$ and $V_p$ can easily be measured, the specific surface area $\Sigma = S_p/V_p$ determined and the mean pore size $R_s \approx \Sigma^{-1}$ assessed. However, this method will not be accurate for polydisperse sorbents as the linear region of the $\bar{K}(r)$ curve will decrease with increasing polydispersity, leading to large errors in the determination of the initial slope.

Other simple methods are also known for assessing pore sizes from GPC data, based on the empirically determined principles of macromolecule retention.

*4.2. Empirical methods for pore size assessment*

One such method is associated with determining the "critical" size of macromolecules which are still capable of penetrating into pores[45]. This method is based on a linear relationship between $\ln(1 - K)$ and $\ln M$, discovered empirically by Haller *et al.*[38,46]. The "critical" radius of a macromolecule, $r_0$, can be determined from this relationship by extrapolation to $K \to 0$.

Let us consider this method from the standpoint of the theory of macromolecular chromatography. It follows from eqn. 9 that under conditions where macromolecule sizes are substantially smaller than pore sizes, a linear relationship between $\ln(1 - K)$ and $\ln M$ must indeed occur while the "critical" radius $r_0$ extrapolated to $K \to 0$ must be related to $\Sigma = S_p/V_p$ by

$$r_0 \approx \frac{\sqrt{\pi}}{2} \cdot \Sigma^{-1} \tag{24}$$

However, with comparable macromolecule and pore sizes, theory predicts a different functional $K(r)$ relationship (eqn. 8) which fails to give a straight line in the coordinates of $\ln(1 - K)$ *versus* $\ln M$. Consequently, within the region of small $K$ values, the method of size extrapolation to $K \to 0$, suggested by Basedow *et al.*[45], becomes incorrect. A rigorous theoretical relationship (eqn. 7) in the coordinates of $\ln(1 - K)$ *versus* $\ln(r\Sigma)$ is illustrated in Fig. 8. Fig. 8 also illustrates dependences calculated for polydisperse model sorbents. Polydispersity can be seen to lead to expanding non-linear regions in the curves and hence to incorrect, *i.e.*, too high, pore

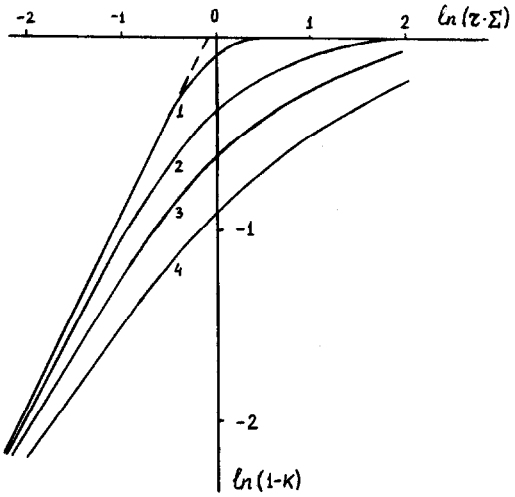


Fig. 8. Theoretical ln ($r\Sigma$) dependence of ln (1 − $K$) for a monodisperse sorbent of $U$ = (1) 1 and for polydisperse sorbents of $U$ = (2) 2, (3) 5 and (4) 20.

size values obtained by the use of the above procedure. It will be noted that any procedures for determining a "critical" size are based on the concepts of rigid non-deformable test particles and are therefore inapplicable where flexible-chain molecules are used as chromatographic standards.

Another technique currently in use is the "median" method of pore size determination, which consists in determining experimentally the macromolecular radius $r_{1/2}$ corresponding to a distribution coefficient $K = 1/2$. This dimension is identified with the mean pore radius, various correction factors being introduced, as a rule[21,23].

It follows, in fact, from macromolecular GPC theory that $r_{1/2} \approx R$. Using the approximate eqn. 3 and assuming $K = 0.5$, we obtain

$$r_{1/2} = \frac{\sqrt{\pi}}{4} \cdot \Sigma^{-1} \tag{25}$$

whence, considering the connection between $\Sigma$ and the radii of equivalent pores of regular geometry, a transition can be made to equivalent model pore sizes. To give more precise relationships based on the use of the rigorous eqn. 7:

$$r_{1/2} = 0.444R_{\text{equiv. slit}} = 0.251R_{\text{equiv. cyl.}} = 0.175R_{\text{equiv. sphere}} \tag{26}$$

Thus, an uncorrected "median" method will give too low pore size values, yet, by using eqn. 26, this method can be employed to obtain correct results for sorbents with identical pores.

Let us now consider how the value of the "median radius" $r_{1/2}$ is affected by pore size inhomogeneity. Specifying a logarithmically normal pore size distribution function (eqn. 22), we calculated the dependence of $\bar{K}$ on $r\Sigma$ for various values of the

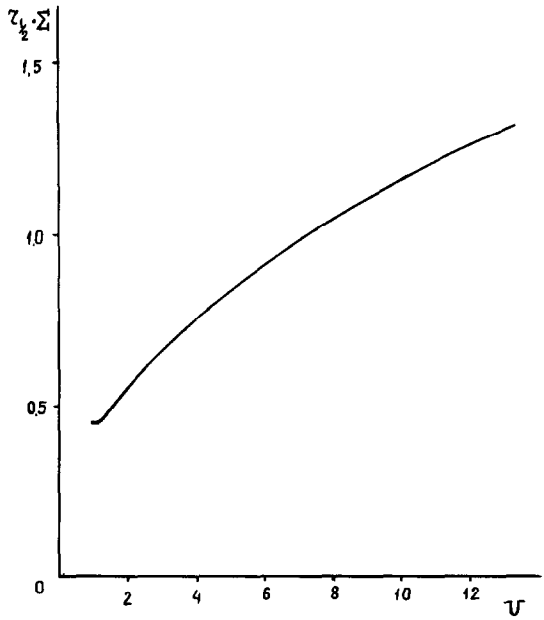


Fig. 9. $r_{1/2}\Sigma$ *versus* sorbent pore polydispersity parameter $U$.

polydispersity parameter $U$, and used these data to plot $r_{1/2} \cdot \Sigma$ *versus* $U$ (Fig. 9). It can be seen that in the general case of polydisperse sorbents there is no definite relationship between $r_{1/2}$ and $\Sigma$ (pore sizes), which means that the median method produces distorted results in this instance.

To summarize, all of the simple methods discussed above produce inaccurate results for sorbents that are inhomogeneous in pore size. More complicated methods are required in this instance, accounting not only for mean sizes, but also for pore size distribution.

### *4.3. Methods for calculating pore size distribution functions*

Halász and Martin[2,5] suggested a method for the chromatographic determination of pore size distribution functions, which has gained wide acceptance owing to its simplicity. This method was based on the assumption that $K = 1$ for all macromolecules of a size smaller than the pore size and $K = 0$ if the molecule size exceeds the pore size. Identified with the sorbent pore diameter was the "exclusion value" of the molecule diameter $\varphi$, which was selected by the trial-and-error method such that the mean pore sizes obtained in the process would agree with the results obtained by the "classical" methods[5]. As a consequence of the assumption made[2,5], the ln $\varphi$ dependence of $-dK/d(\ln \varphi)$ was interpreted as a differential pore size distribution function.

Unfortunately, the method described above and all of the results obtained therewith are not correct, as first noted by Knox and Scott[16]. The assumption that $K = 1$ for all macromolecules capable of penetrating into the pores is in contradiction with GPC theories[26–29]; also, it does not agree with the available experimental results. Owing to the incorrect conceptions of the GPC mechanism, which Halász and Martin

used as the basis for their method, this method will give pore size distribution functions that are far too wide, with gross errors for narrow size range and monodisperse sorbents. This may be demonstrated by using the example of a monodisperse model sorbent with identical slit-like pores of width $2R_0$. The theoretical $K(r)$ relationship for this sorbent is obtainable from eqn. 7 at $\alpha = 1$. Considering this relationship as ideal for an experimental $K(r)$ function, free from any measuring errors, a differentiation procedure may be applied, such as proposed by Halász and Martin[2,5]. Fig. 10 shows the results of such differentiation (curve 2) compared with the specified pore size distribution in the form of a delta function, $f(R) = \delta(R - R_0)$ (curve 1). It can be seen that curve 2 is shifted towards lower $R$ values relative to the initial distribution function, and is considerably broader. Calculation of the moments of function 2 gives a mean pore size $\bar{R} = 0.48\ R_0$, and a relative width for this function $\gamma = \sigma_R/\bar{R} \approx 0.67$ ($U = 1.44$), while the true distribution is characterized by $\bar{R} = R_0$ and $\gamma = 0$ ($U = 1$). Of course, by choosing a correction factor for the relationship between $r$ and $\varphi$, as recommended[5], curve 2 can be shifted parallel to the right until its maximum coincides with the value of $R = R_0$. Such a correction will not improve the assessment of relative width $\gamma$ and polydispersity $U$, however.

Curve 3 in Fig. 10 was plotted in a similar manner, using eqn. 7 at $\alpha = 2$. This curve illustrates the use of the method[5] with a monodisperse sorbent with cylindrical pores. Curve 3 is characterized by the parameters $\bar{R} = 0.29\ R_0$ and $\gamma = 0.72$ ($U = 1.52$). Hence the method advanced by Halász and Martin produces unsatisfactory results for cylindrical pore sorbents also.

Another method for calculating pore size distribution functions was suggested by Knox and Scott[16] for cylindrically shaped pores and further developed by

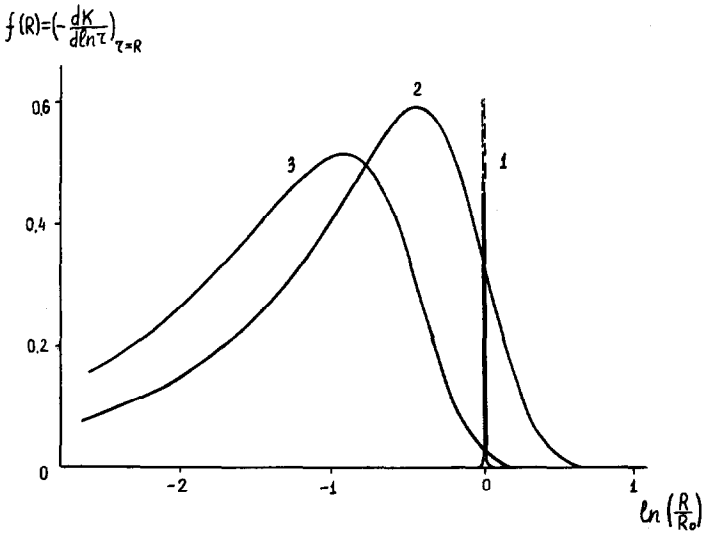


Fig. 10. $\left(-\frac{dK}{d \ln r}\right)_{r=R}$ versus $\ln (R/R_0)$ as calculated from eqn. 7 for monodisperse model sorbents with slit-like (curve 2) and cylindrical (curve 3) pores. Curve 1 represents the true pore size distribution.

Nikolov[24] for pores of other geometries. Some studies[16,20,24,25] were based on the equations of GPC theory for rigid spherical particles[29]:

$$K^{(\alpha)}\left(\frac{\rho}{R}\right) = \begin{cases} \left(1 - \frac{\rho}{R}\right)^{\alpha}; & \rho < R \\ 0; & \rho > R \end{cases} \tag{27}$$

where $\alpha = 1$ for slit-like pores, $\alpha = 2$ for cylindrical pores and $\alpha = 3$ for spherical pores.

By substitution of eqn. 27 into eqn. 20 and subsequent differentiation, equations were obtained[16,24] for determining pore size distribution functions for pores of different shapes. These equations can be conveniently written in the following general form:

$$f^{(\alpha)}(R) = (-1)^{(\alpha+1)} \cdot \frac{R^{\alpha}}{\alpha!}\left[\frac{d^{(\alpha+1)}K}{d\rho^{(\alpha+1)}}\right]_{\rho=R} \tag{28}$$

In our opinion, this method of calculating pore size distribution should be correct if non-deformable spherical particles are used as standards, such as a series of proteins of approximately spherical form and known size.

However, in the original works[16,24,25] flexible-chain macromolecules, polystyrenes, were used. At the same time, the theory based on a rigid solid particle model is not fully usable for describing the principles of flexible-chain macromolecular GPC (particularly where the molecule and pore sizes are comparable), as it does not account for changes in the conformational entropy of macromolecules as they enter the pores. It is for this reason that the method advanced by Knox and Scott, which uses macromolecules as test particles, will produce distorted results.

Fig. 11 demonstrates the use of eqn. 28 for monodisperse model sorbents of slit-like (curve 2) and cylindrical (curve 3) forms. In calculating these functions as "experimental" $K(r)$ relationships, use was made, as previously, of eqn. 7 from Casassa's theory of macromolecular GPC[26].

Comparing Figs. 10 and 11, one can see that, in general, the Knox and Scott method is better than that of Halász and Martin. The functions in Fig. 11 give near-correct mean pore sizes ($\bar{R} = 0.96R_0$ for slit-like pores and $\bar{R} = 0.86R_0$ for cylindrical pores). Consequently, the Knox and Scott method needs almost no correction factors. However, the function width obtainable by this method is still found to be excessive, *e.g.*, $\gamma = \sigma_R/\bar{R} \approx 0.28$ instead of $\gamma = 0$, for slit-like pores. In addition, as Fig. 11 shows, the use of eqn. 28, with $\alpha = 2$ (for the cylindrical pore model), leads to an artefact, giving negative function values in the smaller $R$ region.

In our opinion, a more accurate method for calculating pore size distribution functions is that proposed by Vilenchik and co-workers[10,14]; this method consists in solving directly the integral eqn. 20 having the experimentally determined function $K(r/R)$ for its kernel.

One difficulty should be noted, however, as a matter of principle, this difficulty

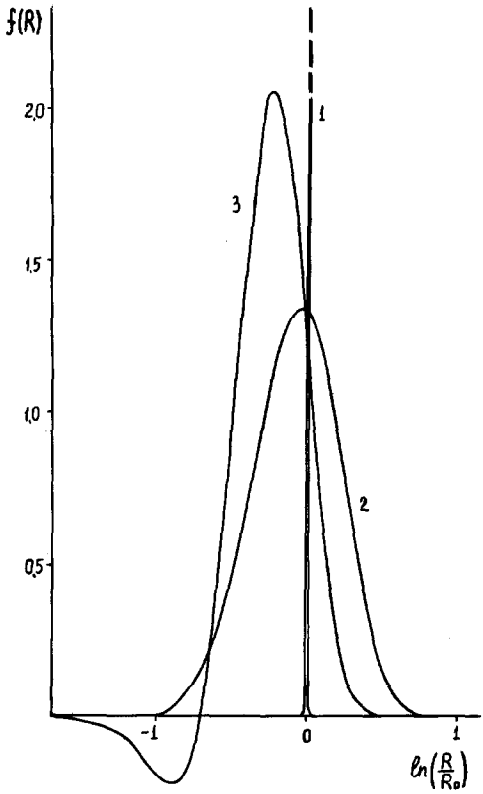


Fig. 11. "Pore size distributions" as calculated from eqns. 28 and 7 for monodisperse model sorbents with slit-like (curve 2) and cylindrical (curve 3) pores. Curve 1 represents the true pore size distribution.

arising in all instances where a pore size distribution function is derived from GPC data: eqn. 20 is a first-kind Fredholm equation, and the problem of using this equation to find an unknown $f_v(R)$ function is classed among "ill-posed" mathematical problems. For all practical purposes, it implies that minor errors in the initial data will have a considerable effect on the calculation results. In fact, the Knox and Scott method[16], whose only difference from the method advanced by Vilenchik *et al.*[10] is in the $K(r/R)$ function as the kernel type, also comes down to solving eqn. 20 numerically. In this method there are great uncertainties arising in calculating high-order derivatives of the experimental $\bar{K}(r)$ function.

In this connection, the question had arisen as to how reliably a distribution function and its moments could be derived from experimental GPC data. In other words, it was thought necessary to establish how sensitive the GPC porosimetric method was to the mean pore size, and also to the distribution function width and type.

*4.4. Analysing the sensitivity of GPC porosimetry to various porous structure characteristics*

The possibility of reliably determining various characteristics of porous structures by using the GPC method was treated theoretically by Gorbunov *et al.*[17].

The principal conclusions are evident from Fig. 5. Comparing curves 1 and 2–4 in Fig. 5 shows that the mean pore size has a pronounced effect on the type of $\bar{K}(r)$ relationship and, as a result, can be reliably determined from GPC experiments. It can also be seen that curves 3 and 4 for inhomogeneous model sorbents deviate from curve 2 at large $r$ values. Analysis shows that the difference increases with increasing polydispersity parameters $\gamma$ and $U$. Consequently, chromatographic measurements can also be used for quantitatively assessing the distribution function width and the polydispersity characteristics $\gamma$ and $U$ related thereto.

Now let us discuss the question of the sensitivity of the chromatographic method to the type of distribution function. Consider curves 3 and 4 in Fig. 5. They are close to each other although calculated for distributions that differ greatly in type. This signifies that the chromatographic method is only slightly sensitive to the type of pore size distribution function. Therefore, attempts at calculating in detail the type of distribution function from chromatographic data appear to us to be of little promise at present. Evidently, it is only in certain special instances, namely those of bimodal distributions with greatly differing (by an order of magnitude or more) pore sizes, that a reliable conclusion can be drawn from the type of $\bar{K}(r)$ relationship regarding the distribution function form.

We are hopeful, nevertheless, that in the future, when the accuracy and reproducibility of chromatographic measurements have been improved as a result of improvements in chromatographic equipment, chromatography will also be capable of providing information about details of porous structures.

For the present, however, we suggest that chromatographic porosimetry be considered as a method for determining the basic porous structure characteristics of sorbents, *viz.*, the mean pore size $R_s$ (or specific surface area $\Sigma$) and the polydispersity parameter $\gamma$ or $U$ related to the pore size distribution function width. The knowledge of these characteristics has been shown to be sufficient for predicting GPC principles.

*4.5. Determination of the mean sizes and polydispersities of sorbent pores*

To calculate the mean pore size and polydispersity, a method was proposed[17] based on approximating the experimental $K^{(i)}(r_i)$ relationship obtained by using a series of polymer homologues of known radii $r_i$, by means of the theoretical eqn. 20. The kernel substituted here into eqn. 20 is the $K(r/R)$ function of the form of eqn. 7, while the desired distribution $f_v(R)$ is specified by a logarithmically normal law (eqn. 22) with varying parameters $R_s$ and $U$. The problem of selecting a distribution function $f_v(R)$ model is not a matter of principle, as the GPC porosimetric method has a low sensitivity to the pore size distribution function form, as shown previously.

A computer is used for calculations by the least-squares method, using a BASIC program specially developed for the purpose. A set of varied values of parameters ($R_s$, $U$) is searched to find those which minimize the function

$$T(R_s, U) = \sum_i (K_i^{exp} - K_i)^2 \tag{29}$$

where $K_i$ are the distribution coefficients for the $i$th experimental point, calculated from eqns. 20, 7 and 22.

TABLE 1

MIXED-SORBENT POROUS STRUCTURE CHARACTERISTICS CALCULATED AND MEASURED BY THE GPC POROSIMETRIC METHOD

| *Sorbent* | *Mean pore radius, $R_s$ (nm)* | | *Polydispersity parameter, U* | |
|---|---|---|---|---|
| | *Calculated* | *Experimental* | *Calculated* | *Experimental* |
| Two-component mixture | 8.5 | 7.8 | 1.06 | 1.04 |
| Four-component mixture | 8.5 | 7.7 | 2.2 | 2.1 |

The solid lines in Fig. 6 show the results of the best approximation by eqns. 20, 7 and 22 of the experimental data for the example discussed above of mixed two- and four-component sorbents. Table 1 gives quantitative data on the pore sizes and degrees of inhomogeneity of the mixed sorbents. The values of $R_s$ and $U$ in Table 1 were obtained in two ways: (a) calculated from eqns. 11, 12 and 15 on the basis of the model mixture preparation method; and (b) determined by the GPC porosimetric method under discussion, using the experimental chromatographic data shown in Fig. 6. Comparison of the theoretical and experimental values in Table 1 shows that the GPC method provides correct quantitative estimates of the polydispersities and pore sizes of inhomogeneous sorbents.

We are using GPC porosimetry as the basic method for studying sorbent characteristics. To give an example, Table 2 gives data relating to the porous structure characteristics of several sorbents, *viz.*, Ultragel AcA (LKB), TSK-gel Toyopearl HW (Toyo Soda), hydrophobic Octyl-Sepharose CL-4B and a series of hydrophobic ion-exchange sorbents, SOLOZA K, with variable amounts of hydrophobic component[37].

TABLE 2

CHROMATOGRAPHIC POROSIMETRIC RESULTS FOR SOME SORBENTS INTENDED FOR USE IN GPC AND HYDROPHOBIC INTERACTION CHROMATOGRAPHY

| *Sorbent* | $x^*$ | $\Sigma^{**}$ | $R_s^{***}$ | $U$ |
|---|---|---|---|---|
| Toyopearl HW-65 F | 0.33 | 83 | 24 | 1.6 |
| Toyopearl HW-60 F | 0.42 | 100 | 20 | 1.1 |
| Toyopearl HW-55 F | 0.49 | 160 | 12.5 | 1.9 |
| Octyl-Sepharose CL-4B | 0.53 | 55 | 36 | 1.1 |
| Ultragel AcA-54 | 0.56 | 83 | 24 | 1.06 |
| Ultragel AcA-34 | 0.60 | 49 | 41 | 1.07 |
| Ultragel AcA-22 | 0.65 | 38 | 52 | 1.1 |
| SOLOZA K-0 | 0.43 | 87 | 23 | 1.1 |
| SOLOZA K-10 | 0.42 | 95 | 21 | 1.0 |
| SOLOZA K-20 | 0.47 | 100 | 20 | 1.3 |
| SOLOZA K-30 | 0.46 | 77 | 26 | 2.0 |
| SOLOZA K-40 | 0.49 | 59 | 34 | 2.1 |

* $x = V_p/(V_0 + V_p)$ = sorbent pore volume fraction.
** $\Sigma$ = Specific pore surface area ($m^2$/ml of pore volume).
*** $R_s = 2\Sigma^{-1}$ = mean radius of equivalent cylindrical pores (nm).

*4.6. Mean size and polydispersity parameter errors*

It is difficult to determine the overall error in absolute pore size values determined by the GPC porosimetric method. Apart from the chromatographic measurements as such, the overall error is contributed to by the errors involved in the determination of test macromolecule sizes, and also by the inaccuracies that may be due to the real-life systems and their theoretical models not being completely compatible with each other. The above types of error may be regarded as systematic errors involved in the method.

When carrying out comparative sorbent studies in which the important point is to establish the equivalence of, or difference between, the structures and chromatographic characteristics of the sorbents, it is convenient to make use of accidental error estimates based on the scatter of experimental points. In the case of two parameters being determined simultaneously by the non-linear least-squares method, the perception of the scattering errors will define the confidence region corresponding to the reliability level specified, normally 90 or 95%[47]. (Using confidence intervals for each of the parameters taken separately is allowable only when the parameter estimates are not correlated, and thus when the parameters are obtained by independent methods.)

In the proposed method, the parameters to be determined simultaneously are the mean pore size $R_s$ and the polydispersity $U$, the confidence region in this case being a set of $\{R_s, U\}$ values, including the point corresponding to the best estimate. The values of $R_s^*$ and $U^*$ corresponding to the confidence region boundary are determined from the following equation[47]:

$$T(R_s^*, U^*) = T_{\min}\left[1 + \frac{2}{N-2} \cdot F_{95}(2, N-2)\right] \tag{30}$$

where $N$ is the number of experimental points, $T_{\min}$ is the minimal value of function $T$ (eqn. 29) and $F_{95}(2, N-2)$ is the Fischer number for a probability of 95% and 2 and $N-2$ degrees of freedom[47].

As an example, Fig. 12 illustrates 95% confidence regions calculated for the two- and four-component mixed sorbents, for which the data can be seen in Fig. 6 and Table 1. The central points of these confidence regions correspond to the most probable values of $R_s$ and $U$ for the sorbents. Generally, confidence regions will have an asymmetric form. For inhomogeneous sorbents these regions are usually more elongated along the $U$ axis. This signifies that the GPC porosimetric method determines polydispersity to a lower accuracy than the mean pore size.

To reduce the scattering errors, it may be convenient to increase the number of experimental points or re-measure the distribution coefficients (two to four times for each polymer standard involved).

*4.7. Conditions for realizing the GPC porosimetry method*

Suitable systems to use for GPC porosimetry are polystyrenes in organic solvents[5,10,16] and dextrans and polyethylene glycols as aqueous solutions[15,17,34]. The polymer homologue series used should preferably have a wide range of molecular weights (molecular sizes), and narrow molecular weight distributions are desirable for all polymer standards.

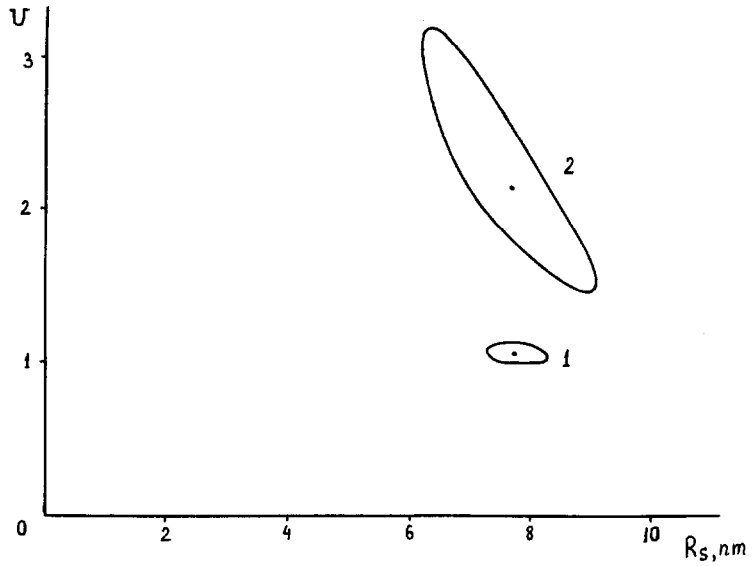


Fig. 12. 95% confidence regions obtained for (1) two- and (2) four-component sorbents.

The requirements that experiments in GPC porosimetry should meet have been discussed[5,17,22]. For porous structure characteristics to be estimated reliably in such experiments, the following basic conditions should be satisfied: (i) no adsorption interactions between polymer and porous material: (ii) chromatography to be carried out in a quasi-equilibrium mode; and (iii) no intermolecular interactions in the solution or in the stationary phase. Provisions to satisfy these conditions include selection of a suitable polymer-solvent system, low polymer concentration in the sample and a sufficiently low elution rate. The criteria that will signify the correct selection of the conditions are the chromatographic results being independent of temperature, solvent composition, concentration and flow-rate, with the conditions selected varying only slightly, and also no double or asymmetric peaks present in the chromatograms.

The condition that is most difficult to satisfy but of extreme importance at the same time is the first one, *i.e.*, that of guaranteeing a macromolecular GPC mode free from adsorption effects. Adsorption effects, coupled with a size-exclusion mechanism, are frequently encountered in polymer chromatography[4,22,35,48–55], and they may seriously distort chromatographic porosimetric results. One must be able to recognize such events and prevent them by selecting suitable polymer-solvent systems, temperatures, pH values and other experimental conditions.

The conformational properties of macromolecules adsorbed within pores and the adsorption effects in polymer chromatography have been investigated theoretically[56–64]. Recently, a general theory of macromolecular chromatography has been developed[65–67] that agrees well with experiment, holds good no matter what adsorption interactions may be present and incorporates Casassa's theory of GPC as a specific case.

This theory[65] implies that several different modes of polymer chromatography may be realized depending on the energy of interaction between the polymer and the

sorbent. Strong interactions lead to an adsorption chromatographic mode that is characterized by a different order of chromatography of macromolecules than in GPC. A "critical" chromatographic mode is also possible when $K$ is independent of molecular weight. Generally, such modes are easily recognizable in experiments.

At the same time, for weak adsorption interactions the theory predicts a "subcritical" GPC-like mode, in which the $K(r)$ relationship is similar to its GPC counterpart in quality, but differs from it quantitatively. Specifically, in place of eqn. 4 the general theory[65] yields for the GPC-like mode the following expression:

$$K \approx \frac{8}{\pi^2} \cdot \exp\left\{-\left[\frac{\pi r}{2\,(R + |H|)}\right]^2\right\} \tag{31}$$

where $|H|$ is a length-dimensioned parameter which depends on the adsorption interaction energy and is referred to as the "correlation length of adsorption". Corresponding to GPC conditions $|H| = 0$; as adsorption effects come into play, $|H|$ varies on a small scale initially, but then starts to increase sharply.

It follows from eqns. 4 and 31 that the apparent pore radius $\tilde{R}$ measured under such conditions will be greater than the true radius $R$ by the value of $|H|$:

$$\tilde{R} = R + |H| \tag{32}$$

The theoretical dependence of $\tilde{R}$ on the adsorption energy for a macromolecule in a cylindrical pore was calculated by Gorbunov *et al.*[61]. Experimentally, variation of adsorption interaction energy (and $|H|$) is usually achieved by varying the mixed solvent composition or the temperature. Nefyodov and co-workers[35,68] gave experimental data on the chromatography of polystyrenes with a molecular weight range between 200 and 400 000. Silica gel KSK was used as the sorbent. The parameters varied were temperature and the composition of the mixed carbon tetrachloride–

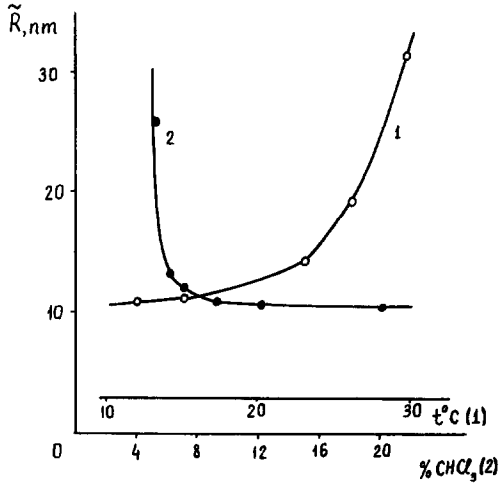


Fig. 13. Apparent pore size $\tilde{R}$ *versus* (1) temperature and (2) mixed solvent composition for GPC-like chromatographic modes. Data from refs. 35, 67 and 68.

chloroform solvent. All of the characteristic chromatographic modes were observable, including the GPC-like mode. Using GLC-like mode data[35,67,68], we have calculated apparent pore sizes in relation to temperature and mixed solvent composition (Fig. 13). The GPC mode is seen to be attained as the temperature drops to 12°C or as the chloroform content in the mixed solvent rises to 20%, both of these methods of eliminating adsorption leading, within the limits of error, to an identical pore radius $R$ of 11 nm. Similar results were obtained in a study[37] of the chromatography of polyethylene glycols on SOLOZA hydrophobic ion-exchange sorbents in aqueous buffer solutions. It was shown[37] that the GPC mode may be achieved in various ways, by temperature or pH variations or by adding agents to reduce adsorption interactions (Triton X-100[37]).

Hence the GPC porosimetric method can be used to analyse not only the structures of neutral porous materials, but also those of sorbents for use in adsorption (hydrophobic, ion-exchange, bioaffinity) chromatography.

## 5. CONCLUSIONS

The macromolecular GPC method permits direct sorbent pore surface and volume measurements. The pore surface-to-volume ratio is an universal model-independent sorbent pore characteristic which is meaningful for practical sorbents with irregularly shaped and variously sized pores. Together with this characteristic, use can be made of the more readily visualizable such as those of equivalent model pore sizes.

The known simple methods of pore size determination (the "critical molecular weight" and "median" methods) require the use of correction factors. No correction is needed for the method using the initial slope of the $K(r)$ plot for pore size assessment. All of these methods, however, become inaccurate with polydisperse sorbents.

The pore size distribution function calculation method proposed by Halász and Martin is essentially incorrect. The mean pore sizes as determined by this method can be corrected, but the function width is far too large. The method advanced by Knox and Scott does not require mean size corrections, but will give too high estimates for polydispersity as it fails to account for the specific behaviour of polymeric molecules in the process of chromatography. A more accurate method is that advanced by Vilenchik and co-workers. It will be noted, however, that in most instances the type of pore size distribution function cannot be determined reliably from GPC porosimetric data, nor can chromatographic data be used to determine the pore form.

It has been shown that reliable measurements are possible for the mean pore size and for the polydispersity parameter that characterizes the pore size distribution function width. An algorithm has been developed for determining these parameters from data obtainable by GPC porosimetry based on the theory of macromolecular GPC using inhomogeneous sorbents.

Conditions have been discussed to permit the proper realization of chromatographic porosimetry. It has been shown that adsorption effects may lead to incorrect, *i.e.*, too high, pore size estimates and, for this reason, precautions must be taken to guard against such non-exclusion effects while undertaking the practical realization of the GPC porosimetric method.

The theoretical fundamentals of the GPC porosimetric method have by now

been well developed, and the possibilities offered by this method, as also its application potential, are recognized. We believe that, if the necessary conditions are duly observed and the resulting data are correctly interpreted, chromatographic porosimetry will become one of the best methods for investigating the porous structures of sorbents.

## 6. ACKNOWLEDGEMENTS

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## 7. SUMMARY

Considered in this work are the basic results from the theory of, and experiments in, macromolecular GPC using porous sorbents, which are essential for understanding the problems involved in GPC porosimetry. Current views on macromolecular GPC based on the use of inhomogeneous sorbents are presented. The known methods of interpreting experiments on GPC porosimetry are analysed on the basis of common theoretical grounds. The necessary conditions for proper realization of the GPC porosimetric method are discussed.

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