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A MODEL OF THE MECHANISM OF THE SEPARATION OF MACROMOLECULES IN GEL PERMEATION CHROMATOGRAPHY ON A PACKING WITH NON-HOMOGENEOUS PORES

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SUMMARY

In an attempt to resolve some controversies in published data, a theoretical model of the separation mechanism in gel permeation chromatography has been developed and tested. The model includes the effects of diffusion, both internal and longitudinal, and of steric exclusion; by using the concept of an impermeable boundary inside the particles of the packing, the model takes into account, in an elementary manner, the effect of the complicated geometrical form of pores. The dependences of the elution volume (characterized by the volume partition coefficient, K_{GPC}) and of the height equivalent to a theoretical plate on relevant parameters of the model have been derived. The calculated dependence of the column efficiency on the elution volume is in good agreement with the experimental curves.

INTRODUCTION

In contrast to the chromatography of low-molecular-weight compounds, for which the theory has been developed in great detail and is complete, the theory of the gel permeation chromatography (GPC) of macromolecules is still in an unsatisfactory state, as indicated by a number of unexplained or controversial results. Thus, for instance, nobody has so far offered a satisfactory explanation of the fact that the dependence of the column efficiency on the elution volume sometimes exhibits a minimum, while in other cases it increases monotonically. Further, it follows from the general theory of chromatography^{1,2} that the retention volume of a given compound (if consistently expressed by the first statistical moment of the elution curve^{2,3} and not by the position of its maximum, which, especially in asymmetrical zones, has no physical meaning) is given only by the equilibrium coefficient that characterizes the partitioning of the component between the mobile phase and the carrier. Yau *et al.*⁴ found that the latter statement was fulfilled if porous glass was used as GPC packing, but observed important discrepancies between the equilibrium and dynamic behaviour, particularly of high-molecular-weight polystyrene standards on Styragel columns. This suggests that the differences in the pore type and geometry

between various carriers can affect considerably the course of separation. The same conclusion was also reached by Berek *et al.*⁵ who, in their study of porous silica gels of various origins as GPC packings, demonstrated that there is no simple relationship between the pore size distribution and the shape of the calibration graph. Haller⁶ found experimentally that both for low-molecular-weight and for extremely high-molecular-weight components the equilibrium measurements and the measurements of the sorption kinetics agree with the elution behaviour on columns, but for solutes of medium molecular weight the discrepancy between the results obtained by both methods and the measured retention volumes is so large that it cannot be explained in terms of any of the existing theories.

A model of the mechanism of separation in GPC of polymers suggested in this paper takes into account, in an elementary manner, the fact that the pores in the packing do not have a simple geometrical shape. An explicit dependence of the separation efficiency on the molecular weight of the polymer has been derived and found to be in good agreement with experimental results taken from the literature. The possibilities offered by the model for a qualitative explanation of some experimental discrepancies have been demonstrated.

THEORETICAL

It is very difficult, if not impossible, to include the effect of the complicated geometrical shape of pores in the theory of chromatography in an exact manner. Fig. 1a shows schematically the shape of a real pore; it implies that also for molecules smaller than the mean pore diameter, \bar{d} , the probability of a given molecule penetrating to any depth of the particle will decrease with the effective diameter of the molecule owing to random fluctuations in the cross-section of the real pore. A possibility therefore arises to include the complicated pore geometry in the theoretical analysis by using a simple model concept, which states that, for a given molecule, only a certain

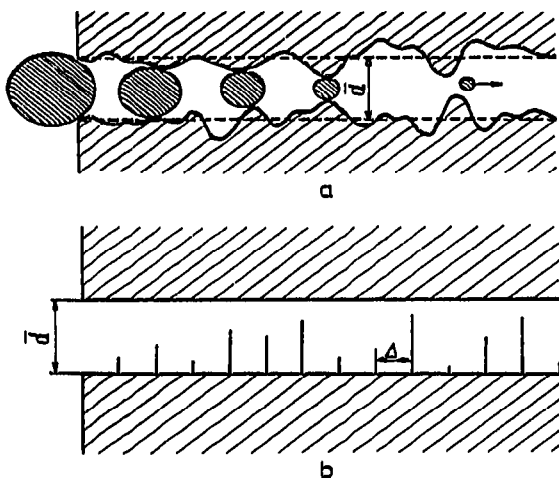


Fig. 1. Penetration of particles of different size into the pore of a complicated geometrical shape. (a) Schematic representation of cross-section of real pore; (b) its idealization.

surface layer of the spherical grain is accessible; the thickness of this layer, ΔR (cf., Fig. 2), is a function of molecular weight.

As a consequence, the problem under consideration is divided into two parts: (a) solving the system of partial differential equations that describe the dynamics of elution on a chromatographic column packed with spherical particles with an internal inaccessible core; (b) derivation of a functional dependence of the radius, R , of this core on the molecular weight (size) of particles undergoing separation by using an adequately chosen model of a real pore.

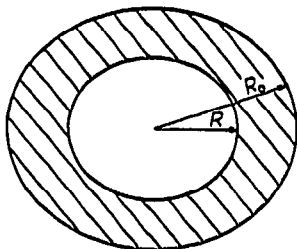


Fig. 2. Spherical particle of the packing (diameter R_0) with the internal impermeable core (diameter R). The hatched area represents the outer layer accessible to molecules of a given size.

Chromatography on a carrier with an inaccessible core

The elution dynamics of a given component on a chromatographic column packed with spherical particles of diameter R_0 is described by a partial differential equation⁷:

$$D_p \cdot \frac{\partial^2 c}{\partial x^2} - w \cdot \frac{\partial c}{\partial x} - \frac{3DH}{R_0} \left[\frac{\partial C}{\partial r} \right]_{r=R_0} - \frac{\partial c}{\partial t} = 0 \quad (1)$$

where D_p (cm²/sec) is the coefficient of longitudinal dispersion, c (g/ml) is the concentration of the respective component in the mobile phase, C is the concentration of the same component in the sorbent, x (cm) is the distance from the front of the column, w (cm/sec) is the linear velocity of the solvent flow, D (cm²/sec) is the diffusion coefficient in the particle, r is the radial coordinate in the grain with the origin in the centre of the sphere, t is time and $H = (1 - \alpha)/\alpha$, where α is the fractional free cross-section of the column. This equation should be solved together with the differential equation

$$D \left[\frac{\partial^2 C}{\partial r^2} + \frac{2}{r} \cdot \frac{\partial C}{\partial r} \right] = \frac{\partial C}{\partial t} \quad (2)$$

which controls the rate of diffusion of the given component in the spherical grain of the packing, and with the boundary conditions

$$t = 0, x \geq 0, c = C = 0 \quad (3a)$$

$$t > 0, r = R_0, C = kc \quad (3b)$$

$$t > 0, r = R, (\partial C / \partial r) = 0 \quad (3c)$$

$$0 \leq t \leq t_0, x = 0, c = c_0 \quad (3d)$$

$$t > t_0, x = 0, c = 0 \quad (3e)$$

According to condition 3b, an equilibrium is assumed to exist on the surface of the sorbent grain between the concentration of the given component in the free cross-section of the column and the concentration inside the sorbent (k is the equilibrium partition coefficient). Conditions 3d and 3e describe the situation at the top of the column at the time of sample injection (for a short time t_0 , a sample with a constant concentration c_0 is injected). A new feature of this model is condition 3c, which expresses zero flux through the spherical surface, $r = R$.

Following the procedure outlined in earlier papers^{2,3}, we shall treat this boundary value problem by using the Laplace–Carson transformation. Let us define the Laplace–Carson transforms of the functions $c(x,t)$ and $C(x,r,t)$ by the relationships

$$S(x,r,p) = \mathcal{L}\{C(x,r,t)\} = p \int_0^\infty C(x,r,t) e^{-pt} dt \quad (4)$$

and

$$s(x,p) = \mathcal{L}\{c(x,t)\} = p \int_0^\infty c(x,t) e^{-pt} dt \quad (5)$$

The solution for a subsidiary equation corresponding to the differential equation 2 can be written as

$$S = r^{-1} [A \sinh(\lambda r) + B \cosh(\lambda r)] \quad (6)$$

where

$$\lambda = (p/D)^{\frac{1}{2}} \quad (6a)$$

and the integration constants A and B calculated from the boundary conditions 3b and 3c have the form

$$A = R_0 k s \cdot \frac{\cosh(\lambda R) - R\lambda \sinh(\lambda R)}{R\lambda \cosh[\lambda(R_0 - R)] + \sinh[\lambda(R_0 - R)]} \quad (7)$$

$$B = R_0 k s \cdot \frac{R\lambda \cosh(\lambda R) - \sinh(\lambda R)}{R\lambda \cosh[\lambda(R_0 - R)] + \sinh[\lambda(R_0 - R)]} \quad (8)$$

By differentiating eqn. 6 and substituting into a subsidiary differential equation corresponding to eqn. 1, we obtain a second-order differential equation with constant coefficients:

$$\frac{d^2 s}{dx^2} - \frac{w}{D_p} \cdot \frac{ds}{dx} - \frac{p}{D_p} [1 + g(p)] s = 0 \quad (9)$$

the solution of which (with respect to conditions 3d and 3c) can be written as

$$s(x,p) = c_0 (1 - e^{-p't_0}) \exp(-\eta x) \quad (10)$$

where

$$\eta = - \frac{w}{2D_p} + \left\{ \left(\frac{w}{2D_p} \right)^2 + \frac{p}{D_p} [1 + g(p)] \right\}^{\frac{1}{2}} \quad (11)$$

In eqns. 9 and 11, the term $g(p)$ is given by

$$g(p) = \frac{3Hk}{R_0^2\lambda} \cdot \frac{\Delta R \cosh(\lambda\Delta R) + (R_0R\lambda - \lambda^{-1}) \sinh(\lambda\Delta R)}{R\lambda \cosh(\lambda\Delta R) + \sinh(\lambda\Delta R)} \quad (12)$$

where

$$\Delta R = R_0 - R \quad (13)$$

The position of the chromatographic band on the time axis is characterized by the first statistical moment about zero of the elution curve $c(x,t)$, *i.e.*, by the quantity

$$\mu'_1 = (1/c_0t_0) \int_0^\infty t c(x,t) dt \quad (14)$$

The width of the band is characterized by its second central moment μ_2 (variance):

$$\mu_2 = \mu'_2 - \mu_1'^2 \quad (15)$$

where

$$\mu'_2 = (1/c_0t_0) \int_0^\infty t^2 c(x,t) dt \quad (16)$$

[The factor $(c_0t_0)^{-1}$ normalizes the elution curve so as to make $\mu'_0 = \int_0^\infty c(x,t) dt = 1$.] As shown earlier², the moments μ'_n can be calculated directly from the Laplace-Carson transform of the elution curve according to the equation

$$\mu'_n = (-1)^n (c_0t_0)^{-1} \lim_{p \rightarrow 0} \frac{d^n}{dp^n} \left[\frac{s(x,p)}{p} \right] \quad (17)$$

It is easy to demonstrate, by applying operator 17 to eqn. 10 (and taking into account eqns. 11 and 12) that at the column outlet

$$\mu'_1 = \frac{l}{w} [1 + \lim_{p \rightarrow 0} g(p)] + \frac{t_0}{2} \quad (18)$$

and

$$\mu_2 = \frac{2l}{w} \left\{ D_p \cdot \frac{[1 + \lim_{p \rightarrow 0} g(p)]^2}{w^2} - \lim_{p \rightarrow 0} \frac{dg(p)}{dp} \right\} + \frac{t_0^2}{12} \quad (19)$$

where l is the length of the chromatographic column.

By substituting for the respective derivatives and limits, we obtain

$$\mu'_1 = \frac{l}{w} [1 + Hk(1 - \varrho^3)] \quad (20)$$

$$\mu_2 = \frac{2l}{w} \left\{ D_p \cdot \frac{[1 + Hk(1 - \varrho^3)]^2}{w^2} + \frac{R_0^2}{15D} \cdot Hk(1 - \varrho^3)[1 - G(\varrho)] \right\} + \frac{t_0^2}{12} \quad (21)$$

where the new dimensionless parameter ϱ ($0 \leq \varrho \leq 1$) is defined by

$$\varrho = R/R_0 \quad (22)$$

and characterizes the maximum depth of penetration of the given particles into the grain of the packing. For the function G ,

$$G(\varrho) = \varrho^3 \cdot \frac{4 + 4\varrho - 5\varrho^2}{1 + \varrho + \varrho^2} \quad (23)$$

Let us now pass from the variable t to the more common independent variable V (elution volume):

$$V = a_w F t \quad (24)$$

where F (cm²) is the total cross-section of the (empty) column. Let us restrict ourselves to a case when the injection time, t_0 , is negligibly short, i.e., $t_0 \rightarrow 0$. If ν_n are the moments of the elution curve with respect to the variable V , then⁸ (considering eqn. 24)

$$\nu_n = (a_w F)^n \mu_n \quad (25)$$

As $a_w F = V_0$ (V_0 being the void volume of the column) and $(1 - a)F$ is equal to the total volume of the packing, V_p , we obtain for the retention volume, V_R (defined as the first statistical moment of the elution curve with respect to the independent variable V), a relationship in the usual form:

$$\nu_1 \equiv V_R = V_0 + K_{GPC} V_p \quad (26)$$

For the coefficient K_{GPC} , the theory yields an explicit relationship:

$$K_{GPC} = k(1 - \varrho^3) \quad (27)$$

The separation efficiency can be characterized by the height equivalent to a theoretical plate (HETP), which is defined in terms of the moments of the elution curve by

$$\text{HETP} = l \cdot \frac{\nu_2}{\nu_1^2} \quad (28)$$

By combining eqns. 21 and 25–28, the following expression is derived for HETP:

$$\text{HETP} = \frac{2D_p}{w} + w \cdot \frac{2R_0^2}{15D} \cdot \frac{Hk(1 - \varrho^3)}{[1 + Hk(1 - \varrho^3)]^2} \cdot [1 - G(\varrho)] \quad (29)$$

Dependence of ϱ on molecular weight

In order to compare the above relationships with experimental results obtained by studying the behaviour of macromolecular solutes on GPC columns, it is necessary to derive the dependence of the thickness of the accessible surface layer (characterized by the dimensionless parameter ϱ) on the molecular weight of the solute. For this purpose, it is necessary to choose a suitable model of the real pore geometry. In this paper, relationships are derived for the simplest case of a conical pore⁹ and for the model of a pore with a randomly fluctuating cross-section.

Conical pore

We shall assume that the molecules in solution are approximately spherical and their size can be characterized by the effective hydrodynamic radius, R_h . According to Porath's model of a conical pore⁹, we also assume that ρ and R_h are proportional, that is, $\rho = R/R_0 = KR_h$. The effective hydrodynamic volume of a polymer coil is proportional to the product $[\eta]M$; after substitution for the intrinsic viscosity $[\eta]$ from the Mark-Houwink equation, we have $R_h = K'M^{1+\alpha_\eta}$ (K and K' being proportionality constants); by combining the two latter expressions, we obtain the required dependence between ρ and M in the form

$$\rho = K_\rho M^{(1+\alpha_\eta)/3} \quad (30)$$

where K_ρ is an as yet unknown coefficient of proportionality and α_η is the Mark-Houwink exponent.

Pore model with a randomly fluctuating cross-section

In order to obtain a more realistic model, let us start from a schematic view of a real pore according to Fig. 1a. Let us assume that a pore with a diameter \bar{d} contains partitions placed at regular intervals (*cf.*, Fig. 1b), and that the length of these partitions is a random variable with a rectangular probability density. It is easy to see that the probability p_1 that a particle with a diameter $d_p = 2R_h$ will pass through a single partition is given by

$$p_1 = 1 - d_p/\bar{d}$$

while the probability that the particle will pass through at least n partitions from the pore orifice to the depth $n\Delta$ is proportional to $(1 - d_p/\bar{d})^n$. It seems reasonable to assume that Δ is very small compared with the radius of the grain, R_0 ; we can then describe the position of the particle of a given size in the grain by means of a continuous dimensionless random variable, σ , for which $\sigma = 0$ if the particle is on the grain surface and $\sigma = 1$ if the particle is in the centre of the sphere. By analogy with the preceding illustrative reasoning, we can write for the probability density of this random variable the equation

$$h(\sigma)d\sigma = A\kappa^\sigma d\sigma \quad (31)$$

where $h(\sigma)d\sigma$ gives the probability that the particle in the grain can penetrate as far as a point between the coordinates σ and $\sigma + d\sigma$, and

$$\kappa = 1 - d_p/\bar{d} \quad (32)$$

The normalization constant A can be calculated from the condition

$$\int_0^1 h(\sigma) d\sigma = 1$$

as

$$A = \ln \kappa / (\kappa - 1) \quad (33)$$

Now, we have to decide the connection between the random variable σ (or, more exactly, between the parameter \varkappa of its probability distribution) and the quantity ϱ , which characterizes the position of the assumed impermeable boundary inside the particle of the packing. Bearing in mind that the model concepts used idealize the actual state to a great extent, the simplest possible solution can be chosen. Let us therefore calculate the mean value, $\bar{\sigma}$:

$$\bar{\sigma} = \int_0^1 \sigma h(\sigma) d\sigma = \frac{\varkappa (\ln \varkappa - 1) + 1}{(\varkappa - 1) \ln \varkappa} \quad (34)$$

It can easily be demonstrated that for $\varkappa \rightarrow 0$ (i.e., for $d_p \rightarrow d$), $\lim \bar{\sigma} = 0$, and for the other limiting case $\lim_{\varkappa \rightarrow 1} \bar{\sigma} = 1/2$. In order to satisfy the requirement $0 \leq \varrho \leq 1$ and with respect to the physical meaning of ϱ , we put

$$\varrho = 1 - 2\bar{\sigma} \quad (35)$$

DISCUSSION

As pointed out by Casassa¹⁰, all GPC theories give for the retention volume a relationship analogous to eqn. 26 but differ in the explicit form that they predict for K_{GPC} as a function of relevant parameters of the model. The present theory gives K_{GPC} as a product of two factors. The first factor, k , is the equilibrium coefficient that characterizes the partitioning of the given component between the mobile phase and the grain of the packing (according to the principle of steric exclusion) (cf., eqn. 3b). This coefficient has been analysed in detail by Casassa and co-workers¹⁰⁻¹² and need not be discussed here. The second factor, $(1 - \varrho^3)$, takes into account in an elementary way the complicated geometry of the porous packing.

The structure of the porous grain is, of course, much more complex than is implied by the scheme in Fig. 1a: a large number of interconnected cavities of a complicated shape are actually involved, and one may expect that in many instances the diffusing molecules could find another, although perhaps very complicated, route in order to avoid the obstacle and to penetrate deeper into the grain. In fact, the model under consideration includes the assumption that during a comparatively short contact time between the grain and the travelling chromatographic zone, the non-uniform cross-section of the pore can effectively play the role of an impermeable boundary. (It should be remembered that the characteristic diffusion time is proportional to the square of the characteristic distance.) In static sorption experiments, the contact time between the grain and the surrounding solution is so long that all cavities inside the grain may become operative, not excluding the least accessible ones. From this viewpoint, the factor $1 - \varrho^3$ in principle has the character of a contribution of restricted diffusion, so that eqn. 26 may be regarded as a theoretical justification of the formal decomposition of K_{GPC} into the contributions of steric exclusion and restricted diffusion suggested by Yau¹³ in an effort to bridge the gap between the results of measurements of the static partition coefficients and of retention volumes on different GPC packings⁴.

In this paper, the dependence of ϱ on the solvent flow-rate is not explicitly taken into account (although such an assumption would not be at variance with the

procedure used for deriving eqns. 26 and 29). However, it may be said qualitatively that $\varrho(w)$ would be an increasing function, which would be in agreement with the trend of the dependence of the retention volume on the flow-rate found experimentally by Yau *et al.*⁴ and also by Haller⁶.

With respect to the definition of K_{GPC} (eqn. 27) eqn. 29 can be rearranged to give

$$\text{HETP} = \frac{2D_p}{w} + w \cdot \frac{2}{15} \cdot \frac{R_0^2}{D} \cdot \frac{HK_{\text{GPC}}}{[1 + HK_{\text{GPC}}]^2} \cdot [1 - G(\varrho)] \quad (36)$$

A comparison of the second term of this equation with the relationship describing the effect of mass transfer in the stationary phase for the case of a spherical, fully accessible grain¹ leads to the conclusion that condition 3c, reflecting the existence of an impermeable boundary inside the grain, will be reflected only in the correction term $1 - G(\varrho)$. Examination of eqn. 23 shows that the function $1 - G(\varrho)$ is S-shaped and decreases monotonically from unity for $\varrho = 0$ to zero for $\varrho = 1$.

In order to find the extent to which eqn. 29 adequately describes the experimentally determined dependences of the efficiency of GPC columns on the retention volume, the results of Tung and Runyon¹⁴ and Smit *et al.*¹⁵ were considered. In both instances, the effect of the polydispersity of polystyrene standards was eliminated by using the exact reverse-flow technique suggested by Tung *et al.*¹⁶. However, the dependence of the column efficiency on V_R according to Tung and Runyon¹⁴ exhibits a clear extreme in the region of high molecular weights (low elution volumes), while a similar dependence given by Smit *et al.*¹⁵ is monotonic; both types of behaviour have been described elsewhere^{16,17}.

The following assumptions and approximations were introduced in the calculations of HETP according to eqn. 29. (a) It was assumed that the molecular-weight dependence of the diffusion coefficient, D , in the packing has the same form as a similar dependence in solution, *i.e.*, $D = K_D M^{-\alpha_D}$. In calculations for experimental data taken from Smit *et al.*¹⁵, who used toluene as solvent, the results of Mukherjea and Rempp¹⁸ were taken for K_D and α_D , and the decrease in the diffusion coefficient in gel as a result of the role played by the obstruction factor¹⁹ was respected by reducing K_D by 50% (*cf.*, ref. 20). The constants of a similar dependence for polystyrene in tetrahydrofuran (the solvent used by Tung and Runyon¹⁴) have not been published so far; therefore, α_D for this case was calculated by means of the expression $\alpha_D = (1 - \alpha_\eta)/3$ from the Mark-Houwink exponent α_η published²¹ for this system, and K_D was chosen so as to make the diffusion coefficients in both solvents coincide at an arbitrarily chosen value $M = 10^5$. (b) In the model of a conical pore, the coefficient K_ϱ in eqn. 30 was determined so that the exclusion limit, M_{11m} , was first calculated by linear extrapolation of the calibration curve to the void volume V_0 , and K_ϱ was determined from eqn. 30 assuming that $\varrho = 1$ for $M = M_{11m}$. A similar procedure was employed for the determination of the mean diameter, \bar{d} , needed for calculations in the model of a pore with a fluctuating cross-section.

A comparison of experimental data¹⁴ with the course calculated from eqn. 29 for both pore models is given in Fig. 3. It is obvious that both models correctly describe qualitatively the course of the experimental dependence with an extreme in the region of low elution volumes, although the position of this extreme on the K_{GPC}

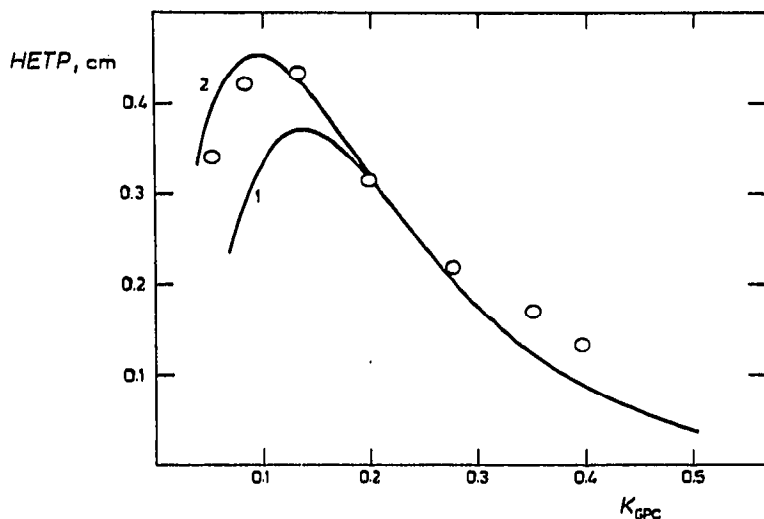


Fig. 3. Comparison of the experimental and calculated dependence of HETP on the elution volume characterized by the coefficient K_{GPC} . Points: data from Tung and Runyon¹⁴ (the highest exclusion limit of gel 10^6 \AA). 1, Calculated for conical pore model; 2, calculated for pore model with fluctuating cross-section.

axis is better represented by the model with a fluctuating cross-section (curve 2). A similar comparison for experimental data from Smit *et al.*¹⁵ (where the first column in the series was designated 10^7 \AA) is shown in Fig. 4. In this case, both pore models exhibit a virtually indistinguishable course (curve 2), which again gives a qualitatively correct description of the monotonic dependence.

With respect to the fact that no attempt has been made to adjust the

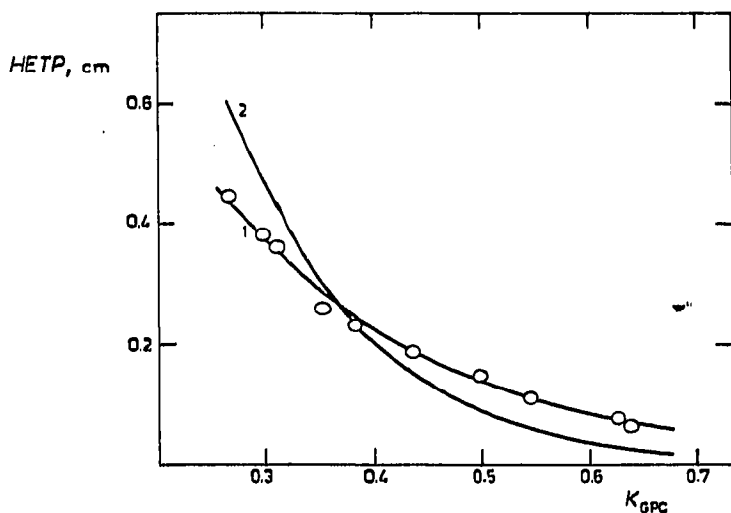


Fig. 4. Comparison of the experimental and calculated dependence of HETP on K_{GPC} . 1, Data from Smit *et al.*¹⁵ (gel with the highest exclusion limit of 10^7 \AA); 2, course calculated according to the present theory (both pore models give virtually identical results).

numerical values of the individual parameters so that the calculated courses would coincide with the experimental data, and also bearing in mind the rather idealized models used in the derivation as well as the number of approximations necessary, the coincidence between the experimental and calculated curves can be regarded as satisfactory. In fact, the very good agreement between the absolute HETP values calculated for the pore model with a randomly fluctuating cross-section with the experimental data of Tung and Runyon¹⁴ is probably fortuitous, especially as in the derivation of eqns. 27 and 29 by the above method, that is, by solving the respective systems of differential equations, it was not possible to take into account the zone broadening due to effects in the mobile phase, and in accordance with Hermans²² it was necessary to assume a piston flow of the solvent through the free cross-section of the column. Conflicting evidence exists in the literature on GPC concerning the relative importance of mobile phase effects and effects due to permeation on the total zone width: Kelley and Billmeyer²³ and Yau *et al.*¹⁷ assigned great importance to the non-equilibrium mobile phase effects, while the data of Ouano and Biesenberger²⁴ and Hendrickson²⁵ indicate that the piston-flow approximation is valid under the normal operating conditions of GPC columns. In any event, non-equilibrium in the mobile phase definitely contributes to the width of the chromatographic zone; it is not difficult, however, to modify eqn. 29 by using a term that adequately describes this contribution, *e.g.*, in the sense of Giddings' coupling theory²⁶. One may say that the present theory of separation of macromolecules by GPC, although it contains several rather rough approximations, can correctly describe qualitatively some interesting experimental data that previously could not be satisfactorily explained by existing theoretical approaches.

It should be mentioned in conclusion that eqn. 29 may be of interest not only in connection with the model of the separation mechanism on GPC columns discussed here, but also as an exact expression of the contribution of mass transfer in the stationary phase in chromatographic columns packed with the so-called pellicular carriers (*e.g.*, of the Corasil type).

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