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THEORETICAL STUDY OF CHROMATOGRAPHIC SEPARATIONS PERFORMED WITH CROSS-LINKED ORGANIC POLYMERS

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SUMMARY

A fractionation mechanism in gel permeation chromatography which involves "dissolution" of the solute in the swollen gel is discussed. The solute is considered to be in thermodynamic equilibrium between the swollen gel phase and the mobile phase. Using Flory's theory for gels, an expression for the partition coefficient of the solute is proposed. Variations of this partition coefficient with the structural characteristics of the gel and with the interactions between the molecules present are considered. Predictions are in good agreement with some experimental data.

INTRODUCTION

During the last 15 years, the use of gels as supports for liquid–liquid chromatography has expanded considerably. This technique was first applied for the fractionation of macromolecules[1], but it is now also used for the separation of smaller molecules[2–7], where it appears to be particularly attractive in the preparative field.

In order to explain the fractionation observed, several mechanisms have been proposed and discussed. The hydrodynamic effect[8,9] and the influence of the rate of diffusion[10], which decreases when the molecular size increases, agree with the observed separations. These two mechanisms are in accordance with a dynamic view of the chromatographic process.

A number of experiments (comparison between static and dynamic tests, influence of the flow of the mobile phase, "vacancy chromatography") have indicated that these two effects are not the most important in the observed fractionation[11–13]. Except perhaps for high-molecular-weight solutes, an equilibrium occurs[14–18]:

$$\text{solute in mobile phase} \overset{K_{eq}}{\rightleftharpoons} \text{solute in gel phase}$$
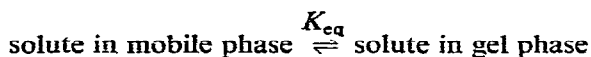
The free energy change involved in the passage of a solute molecule from the mobile phase to the gel phase can be related to the constant K_{eq} of this equilibrium:

$$\Delta F = -kT \log K_{eq}$$

ΔF can be split into several component terms[19], each of which describes one fractionation mechanism. Three terms play a leading part:

$$\Delta F = \Delta F_s + \Delta F_a + \Delta F_d$$

ΔF_s depends upon the entropic variation involved when a macromolecule is transferred from the bulk solution to the vicinity of the gel surface. In other words, if one considers a pore of the gel, the probability that the centre of gravity of a flexible or rigid macromolecule will be near the wall of the pore is less than the probability that it will be far away from it. This steric effect is assumed to be the main effect responsible for fractionation in gel permeation chromatography (GPC). ΔF_a is a function of the adsorption of the solute on the gel surface. This term may be particularly important in the case of rigid supports with a large specific surface area (such as mineral supports). Hence, adsorption of molecules on the silica gel surface and even on polystyrene–divinylbenzene gel has been shown to disturb completely the classical calibration curves[20,21].

In the case of non-rigid gels, swollen by the mobile phase, ΔF_a is probably small, but it is convenient to add a term ΔF_d which arises from the solute "dissolution" in the gel phase. The space accessible to solute molecules is no longer limited by the rigid walls of the pores but these molecules can penetrate into the gel phase as solvent molecules. The gel is, in a way, considered as a classical liquid–liquid chromatographic stationary phase.

We present here the calculation of the partition of the solute between the gel and the mobile phase, according to this process, in terms of the structural characteristics of the system (solvent, solute, gel) and of the interactions between the various molecules. This calculation is developed without taking into account the steric exclusion effect, which, for a macroporous gel, occurs simultaneously with the selective dissolution in the gel.

CALCULATION OF THE RETENTION CHARACTERISTICS

Principle

The retention volume, V, of a solute is given by

$$V = V_0 + KV_s$$

where V_0 is the elution volume of a molecule which does not penetrate into the gel phase; V_s is the volume of the stationary phase that is efficient for the proposed mechanism; when the separation is a partition between the mobile and the gel phases, V_s is the volume of the swollen gel; and K is the partition coefficient of the solute between the mobile and the gel phases:

$$K = \frac{\text{concentration of solute in the gel phase}}{\text{concentration of solute in the mobile phase}}$$

Using Flory's theory of gels[22], K can be expressed in terms of the thermodynamics that characterize the molecules present. (Subscripts 1, 2 and 3 are assigned

to the solvent, solute and gel species, respectively, in the stationary phase; $1'$ and $2'$ are the corresponding subscripts for these species in the mobile phase.)

According to the hypothesis of a thermodynamic equilibrium, the chemical potentials (μ) of the solvent and the solute in the two phases are assumed to be equal:

$$\mu_1 = \mu_1'; \mu_2 = \mu_2'$$

Pure liquid compounds are chosen as the standard state, their activity (a) being unity. From the above equalities, it follows that

$$a_1 = a_1'; a_2 = a_2'$$

Calculation of solute activities

The free energy change, ΔF, involved in the mixing of the solvent with the gel was calculated by Flory[22]:

$$\Delta F = \Delta F_m + \Delta F_{el}$$

where ΔF_m is the classical free energy of mixing and ΔF_{el} is the elastic free energy due to the expansion of the gel in the solvent. In Flory's theory[22], these two terms are expressed by

$$\Delta F_m = kT (N_1 \log \varphi_1 + \chi_{13} N_1 \varphi_3)$$

$$\Delta F_{el} = kT \cdot \frac{\nu_e}{2} (3 \alpha^2 - 3 - \log \alpha^3)$$

with the hypothesis of a perfect gel, where φ_i is the volume fraction of the species i; N_1 is the number of solvent molecules in the gel phase; χ_{13} is Flory's interaction parameter; and α is the factor expressing the isotropic linear deformation of the gel due to solvent–polymer interactions.

Extension of the previous equations to a system of three components leads to the following expression for the free energy change involved in the mixing of the solute with the swollen gel:

$$\Delta F = kT [N_1 \log \varphi_1 + N_2 \log \varphi_2 + \chi_{13} N_1 \varphi_3 + \chi_{12} N_1 \varphi_2 + \chi_{32} r_3 N_3 \varphi_2 +$$

$$+ \frac{\nu_e}{2} (3 \alpha^2 - 3 - \log \alpha^3)]$$

where N_2 is the number of solute molecules in the stationary phase; N_3 is the number of polymer chains, the statistical reticulation of which gave the gel; χ_{32} represents the interaction intensity between a polymer segment of the gel and a segment of the solute; and ν_e is the total number of cross-linked units which constitute the gel. Other expressions of ΔF for gel–polymer–solvent systems have been proposed[23,24].

In such a system, the so-called "lattice model" of polymer solutions, the three kinds of molecules present are assumed to be constituted by segments, r, of identical volume. Thus, each molecule of solvent, solute and cross-linked polymer is composed of r_1, r_2 and r_3 segments, respectively.

The activity of the solute in the stationary phase is

$$\log a_2 = \frac{1}{kT} \cdot \frac{\partial(\Delta F)}{\partial N_2} = \log \varphi_2 + 1 - \varphi_2 - \frac{r_2}{r_1} \cdot \varphi_1 - \chi_{13} \cdot \frac{r_2}{r_1} \cdot \varphi_1 \varphi_3 +$$

$$+ \chi_{12} \cdot \frac{r_2}{r_1} \cdot \varphi_1 (1 - \varphi_2) + \chi_{32} r_2 \varphi_3 (1 - \varphi_2) + \frac{\nu_e r_2}{N_3 r_3} \left(\varphi_3^{1/3} - \frac{\varphi_3}{2} \right)$$

and the free energy of mixing of the solute in the mobile phase is

$$\Delta F_M' = kT (N'_1 \log \varphi'_1 + N'_2 \log \varphi'_2 + x_{12} N'_1 \varphi'_2)$$

Consequently, its activity in this phase can be written as

$$\log a'_2 = \frac{1}{kT} \cdot \frac{\partial(\Delta F'_m)}{\partial N'_2} = \log \varphi'_2 + 1 - \varphi'_2 - \frac{r_2}{r_1} \cdot \varphi'_1 +$$

$$+ \chi_{12} \cdot \frac{r_2}{r_1} \cdot \varphi'_1 (1 - \varphi'_2)$$

A calculation based upon similar thermodynamical principles was applied to the particular case of polystyrene–cross-linked polystyrene–benzene systems by Hild et al.[25].

Calculation of the partition coefficient, K

As the chemical potential of the solute in the two phases has the same value, the equality $a_2 = a'_2$ (or $\log a_2 = \log a'_2$) allows us to calculate the ratio φ_2/φ'_2, i.e., the K constant. In usual chromatographic conditions, the solute can be assumed to be infinitely dilute in the two phases, i.e., φ_2 and φ'_2 are close to zero. Hence, K can be expressed as

$$\log K = \log \frac{\varphi_2}{\varphi'_2} = - \frac{r_2}{r_1} \cdot \varphi_3 - \left(\varphi_3^{1/3} - \frac{\varphi_3}{2} \right) \frac{r_2}{r_3 N_3} \cdot \nu_e +$$

$$+ \chi_{12} \cdot \frac{r_2}{r_1} \cdot \varphi_3 + \chi_{13} \cdot \frac{r_2}{r_1} (1 - \varphi_3) \varphi_3 - \chi_{32} r_2 \varphi_3 \qquad (1)$$

Calculation of the swelling of the gel phase

The condition $\mu_1 = \mu'_1$ must be satisfied for equilibrium between the mobile and the gel phases. As a consequence of having assumed that φ_2 and φ'_2 are close to zero, $\log a'_1$ is also negligible. Accordingly:

$$\log a_1 = \frac{1}{kT} \cdot \frac{\partial(\Delta F)}{\partial N_1} \approx \log (1 - \varphi_3) + \varphi_3 + \chi_{13} \varphi_3^2 + \left(\varphi_3^{1/3} - \frac{\varphi_3}{2} \right) \frac{\nu_e r_1}{r_3 N_3} \approx 0$$

Hence

$$\log (1 - \varphi_3) + \varphi_3 + \chi_{13} \varphi_3^2 = - \left(\varphi_3^{1/3} - \frac{\varphi_3}{2} \right) \frac{\nu_e r_1}{r_3 N_3} \qquad (2)$$

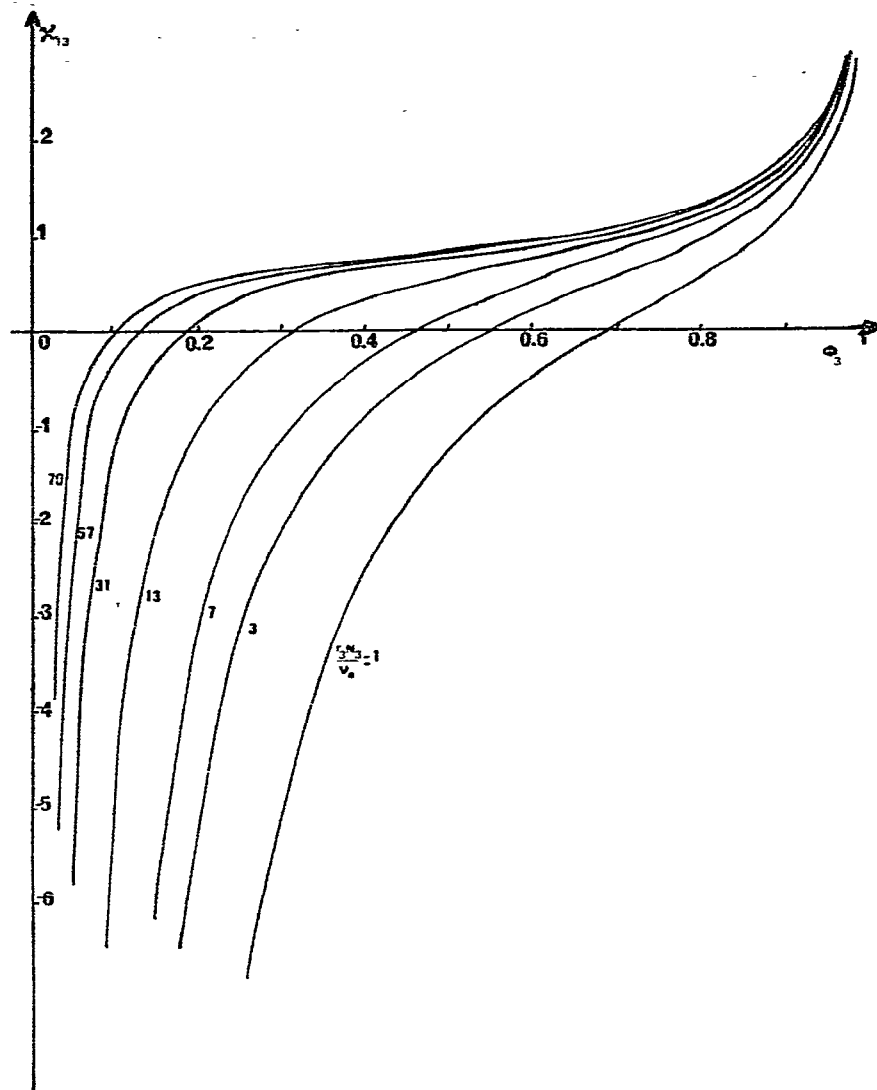In Fig. 1, χ_{13} is plotted against φ_3 for each value of the ratio $r_3 N_3/\nu_e$.

Fig. 1. Swelling of the gel (φ_3) *versus* χ_{13} for various values of the ratio $r_3 N_3/v_c$.

According to eqns. 1 and 2, K can be calculated when the degree of cross-linking of the gel, the values of the interaction parameters χ_{ij} and the solute size parameter r_2/r_1 are known.

DISCUSSION

Validity of the proposed model

Our calculations are based upon the lattice theory of polymer solutions, developed by Flory[26] and Huggins[27]. This theory assumes that the interactions be-

tween the species present do not permit the formation of complexes that could not be easily dissociated by thermal agitation at room temperature; hence, in practice, rigorous tests to check our calculations would require non-polar gels, solutes and solvents. In addition, according to the assumption of infinite dilution of the solute, the mass of solute injected must be sufficiently small to have no influence on the elution characteristics of the system.

The gel is assumed to be gaussian and perfect so that calculations are simple. However, a factor expressing the correction for gel imperfections resulting from chain ends can be introduced by replacing v_e with $v_e(1 - 2 M_e/M)$, where M is the primary molecular weight (before polymer cross-linking) and M_e the molecular weight per cross-linked unit[22]. In fact, this correction neglects the variation of the configurational entropy provided by chain ends, and this variation may become important for low cross-linked gels. It is assumed that χ_{13} values are independent of φ_3, which is not always true[28].

The steric exclusion effect and the "dissolution" effect occur simultaneously, in another way, in the case of a macroporous gel and lead to a double equilibrium:

solute in the mobile phase $\rightleftharpoons$ solute in the pores $\rightleftharpoons$ solute in the gel phase
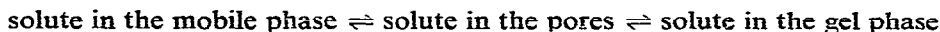
Consequently, the elution volume can take the form

$$V = V_0 + K_1 V_p + K_1 K_2 V_g$$

where K_1 and K_2 characterize fractionation by the steric exclusion effect and the "dissolution" effect, respectively, and V_p and V_g are the pore volume and the volume of the swollen gel, respectively.

Influence of the structure of the gel

In conventional liquid–liquid partition chromatography, the difference between the sizes of the solutes may be sufficient to explain a separation[29]. The same is true in the case of gels: if there is no enthalpic effect ($\chi_{ij} = 0$), K varies from 0 to 1 when the molecular weight decreases from ∞ to 0. Fig. 2, in which the logarithm of the macromolecular weight of the solute, M_2, is plotted *versus* K, illustrates these predictions.

The ratio $r = r_3 N_3/v_e$ may be considered to be close to the average degree of polymerization, $\overline{DP}$, of macromolecular cross-linked units. Accordingly, the system is the more efficient at high molecular weights as the gel is less cross-linked (see Fig. 2). On the other hand, K is always zero for entirely cross-linked gels (rigid gels), *i.e.*, there is no partition phenomenon.

All of the curves obtained have an inflexion point, the coordinates of which can be calculated by differentiating eqn. 1 with respect to $\log r_2$:

$$(\text{For all } \chi_{ij}) \cdot \frac{dK}{d \log r_2} = A e^u \cdot e^{Ae^u}$$

where

$$u = \log r_2$$

$$A = -\frac{\varphi_3}{r_1} - \left(\varphi_3^{1/3} - \frac{\varphi_3}{2}\right) \frac{v_e}{r_3 N_3} + \chi_{12} \cdot \frac{\varphi_3}{r_1} + \frac{\chi_{13}}{r_1} \cdot \varphi_3 (1 - \varphi_3) - \chi_{32} \varphi_3$$
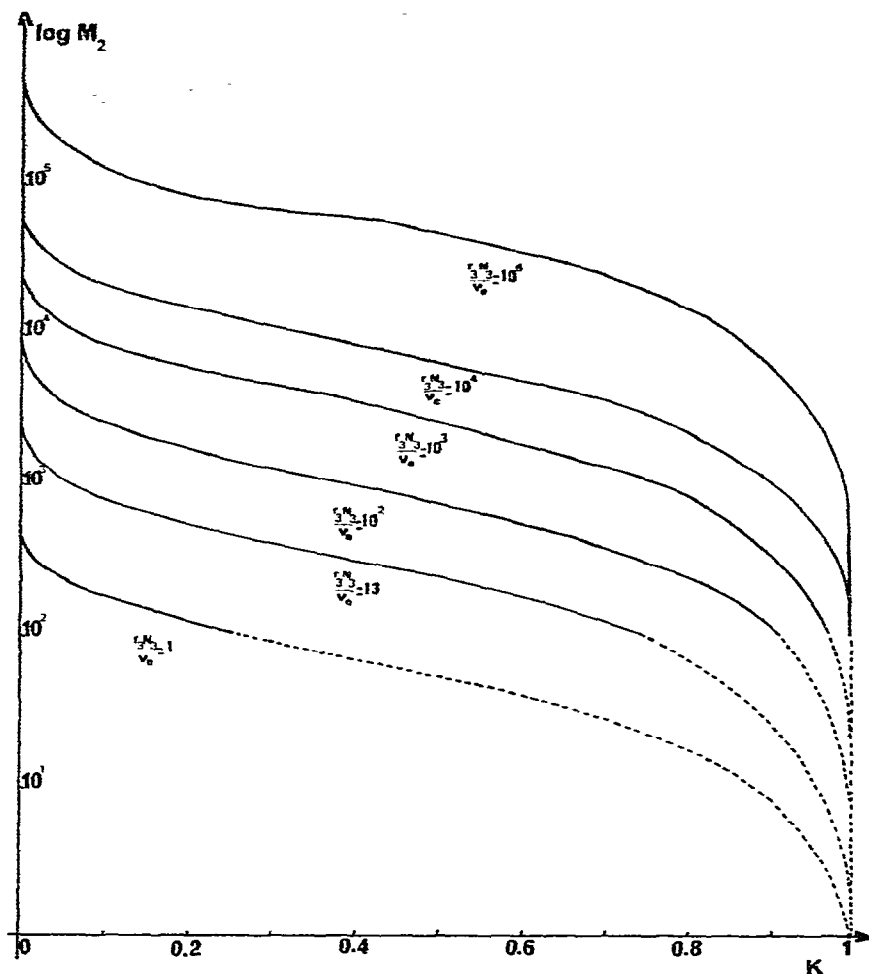
Fig. 2. Log M_2 *versus* K (partition coefficient) for various values of the ratio $r_3 N_3/v_e$. Curves were plotted with $M_2 = 10^2 r_2$ and $\chi_{13} = \chi_{12} = \chi_{32} = 0$.

Consequently, whatever the degree of cross-linking of the gel (*i.e.*, the r value), the abscissa of the inflexion point is $K = 1/e$ and the slope of the inflexion tangent is d log $r_2/dK = -e$ or d log $M_2/dK = -1$.

Influence of the nature of the solvent

The role of the solvent depends not only upon the size of its molecules but also mainly upon its interactions with the solute and the gel (χ_{12} and χ_{13}). When we consider a sequence of solvents, all of which swell the gel alike (*i.e.*, the gel–solvent interactions are constant) but which have various affinities for the solute, the elution characteristics may be appreciably altered, as shown in Fig. 3. In this figure, curves for the logarithm of the molecular weight, M_2, as a function of K were plotted with the values $\chi_{13} = \chi_{32} = 0$ and $v_e/r_3N_3 = 1/13$ for $\chi_{12} = -1$, 0 or $+0.5$.
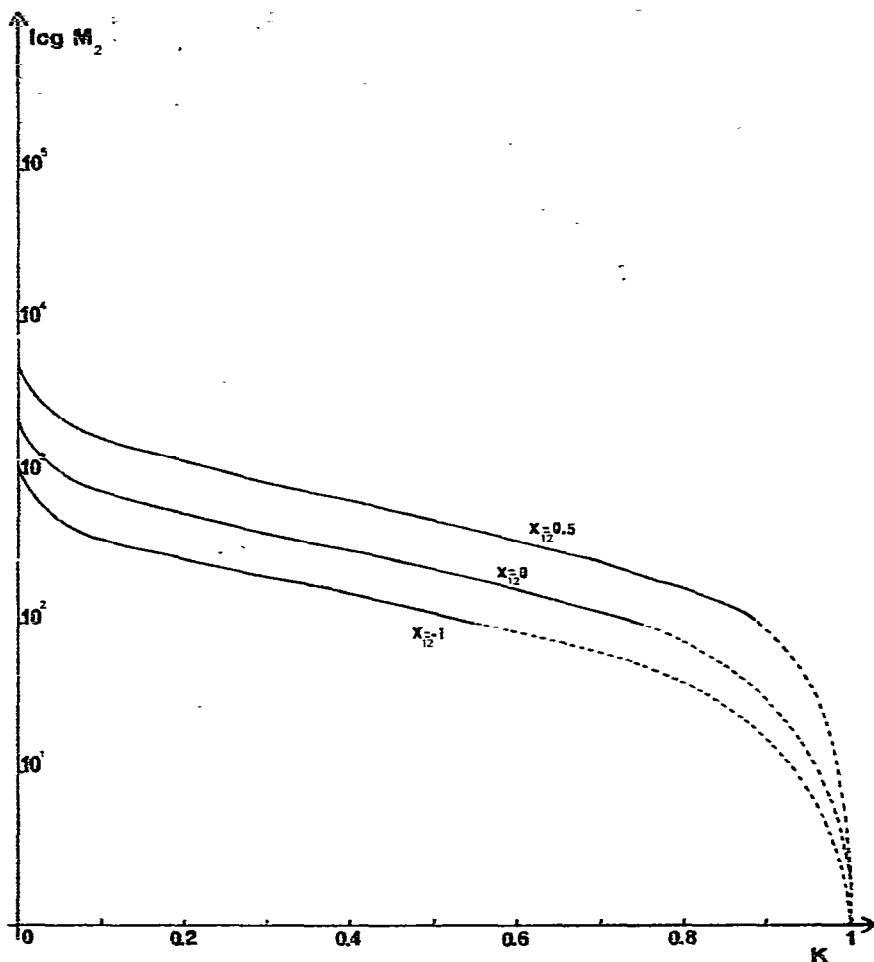
Fig. 3. Log M_2 versus K (partition coefficient) for various values of χ_{12}. Curves were plotted with $M_2 = 10^2\, r_2$, $r_3\, N_3/\nu_e = 13$ and $\chi_{13} = \chi_{32} = 0$.

Influence of gel–solute interactions

Fig. 4 shows, for a given solvent–gel system, the evolution of the elution characteristics with the gel–solute interactions. The values $\chi_{12} = \chi_{13} = 0$ and $r = r_3 N_3/\nu_e = 13$ were taken as an example.

As the affinity of the solute towards the gel increases (*i.e.*, for decreasing values of χ_{32}), the curves become warped and, particularly when the solute–gel interactions are very strong, the solutes are eluted in order of increasing molecular weight.

Importance of the "dissolution" partition phenomenon to the GPC of polymers

The curves in Figs. 2 and 3 appear similar to the molecular weight *versus* elution volume calibration curves obtained experimentally with gels classically used for GPC of polymers, *i.e.* cross-linked dextrans or acrylamide, styrene–divinylbenzene copolymers and porous silica. However, for high-molecular-weight solutes, a partition
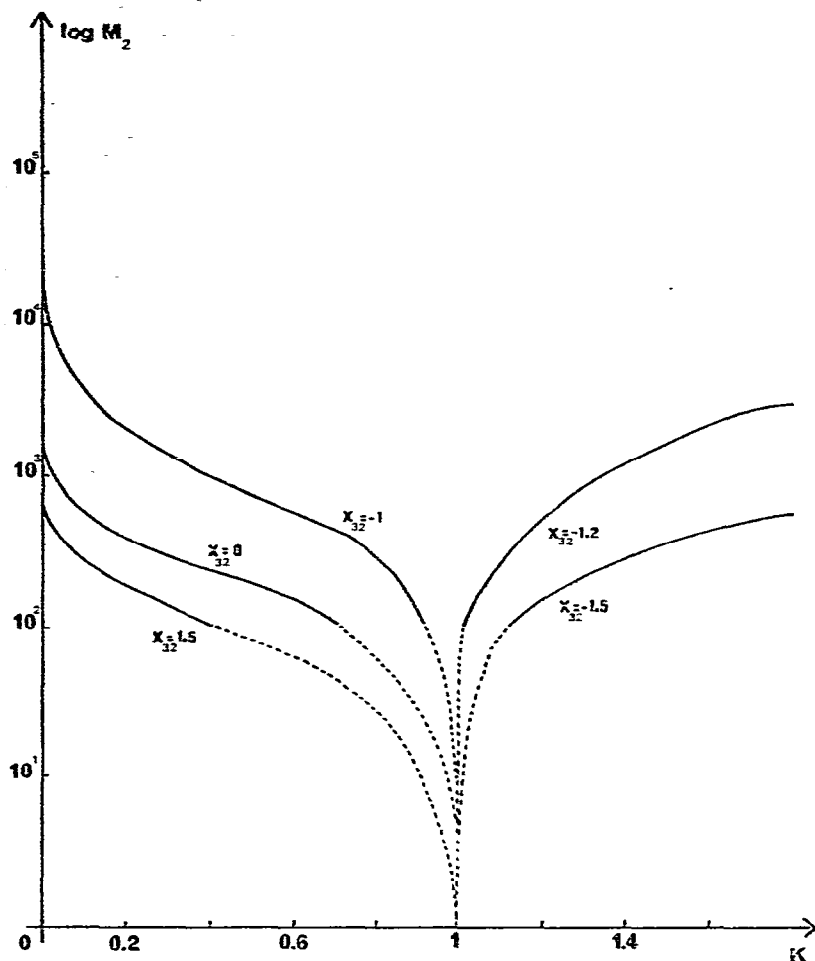
Fig. 4. Log M_2 *versus* K (partition coefficient) for various values of χ_{32}. Curves were plotted with $M_2 = 10^2 r_2$, $r_3 N_3/v_e = 13$ and $\chi_{12} = \chi_{13} = 0$.

separation requires very low cross-linked gels. This is not the case for organic gels available commercially as supports for GPC. But, if the partition fractionation mechanism is not the most important in the case of high-molecular-weight species, it certainly plays a leading role for compounds with molecular weights of less than a few thousands.

Contrary to the steric exclusion effect, the partition phenomenon allows both the influence of the gel–solute interactions and the role of the solvent and of the structure of the gel for small molecules to be explained quantitatively.

Experimental verification

Firstly, the incidence of r_3 is well illustrated by the experiments of Weiss *et al.*[30], who studied chromatographic systems with anionically prepared polystyrene

gels of various but well known structures. They obtained calibration curves which are close to those in Fig. 2.

By another means for a given solvent–gel system, eqn. 1 takes the form

$$\log K = \left[A + \left(\frac{\chi_{12}}{r_1} - \chi_{32} \right) \varphi_3 \right] r_2$$

with $0 < A < -1$. Consequently, when the affinity of the solute towards the solvent is weak ($\chi_{12} > 0$) or when its affinity towards the gel is strong ($\chi_{32} < 0$), the proportionality constant between $\log K$ and r_2 may be positive, so that solutes are eluted in order of increasing molecular weight (if χ_{12} and χ_{32} do not depend upon molecular weight). This case is considered in Fig. 4 and such systems do exist. A typical example of such behaviour was described by Marsden[31], who studied the elution of hydroxylated compounds on a cross-linked dextran gel ($\Delta V_s = 9$) in water. They noticed that polyhydroxy compounds of general formula $HO-CH_2-(CHOH)_n-CH_2-OH$, whose affinity for water is strong, are eluted in order of decreasing molecular weight (i.e., $K < 1$). On the other hand, for a sequence of n-alcohols whose solubility in water decreases with increasing molecular size, the elution volume increases with increasing molecular weight of the solute. As eqn. 1 shows in both sequences, the limit of K is unity when the molecular weight of the solute decreases. Eqn. 1 cannot be applied quantitatively to Marsden's results because the polymer, solvent and solutes are strongly polar.

Even if the importance of the χ_{32} term does not lead to increasing elution volumes with increasing size of the solute, it may induce divergence from Benoit's et al. universal calibration[32]. The effect of such a "parasite" retentions was always attributed to the gel–solute interactions[23,24,33–35], but it was never explained quantitatively.

A more complete check was recently performed in our laboratory[36,37] using a sequence of cross-linked triazinic polycondensates whose structures are known. The elution of polystyrene samples of various molecular weights and of some alkanes with different solvents corroborated our proposed theory.

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