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PECULIARITIES IN GEL PERMEATION CHROMATOGRAPHY OF FLEXIBLE-CHAIN POLYMERS ON MACROPOROUS SWELLING SORBENTS

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SUMMARY

In gel permeation chromatography on macroporous swelling sorbents, deviations from the Benoit principle of universal calibration were observed. It is suggested that these are caused by different degrees of thermodynamic compatibility of the eluted polymers with the sorbent matrix.

INTRODUCTION

The separation of macromolecular components by gel permeation chromatography (GPC) is due to several mechanisms, mainly the molecular sieve mechanism[1–4], the diffusion mechanism[5–9] and the exclusion mechanism (*i.e.*, the mechanism of volume exclusion)[10–12]. As a result of each of these mechanisms, larger macromolecules move along the chromatographic column faster than smaller macromolecules. The molecular sieve mechanism is based on the comparable sizes of the macromolecules and the sorbent pores. The diffusion mechanism is determined by the mobility of macromolecules in the stationary phase of the column and is responsible for the degree of non-equilibrium of the process. The exclusion mechanism is based on the effect of the mutual volume exclusion of polymer segments typical of macromolecules[13].

Usually, GPC should be carried out under conditions close to the equilibrium conditions, when the effect of the diffusion mechanism on the separation of macromolecules becomes negligible. Moreover, if non-swelling solvents such as porous glasses, silica gels and Styrogels are used as packing and the volume interaction of macromolecules with the sorbent matrix is virtually absent, the GPC process is based only on the molecular sieve effect. In this case, the chromatograms can be successfully interpreted in terms of the molecular weight distribution of polymers by using the principle of the Benoit universal calibration graph[14,15]. This principle maintains that the GPC separation of macromolecules occurs according to their hydrodynamic volume, $V \approx M[\eta]$, where M = molecular weight and $[\eta]$ = intrinsic viscosity. This principle is also widely used in GPC on swelling sorbent gels, although this is not always correct. Recently, we have described an important deviation from the universal calibration in GPC on Sephadexes[16].

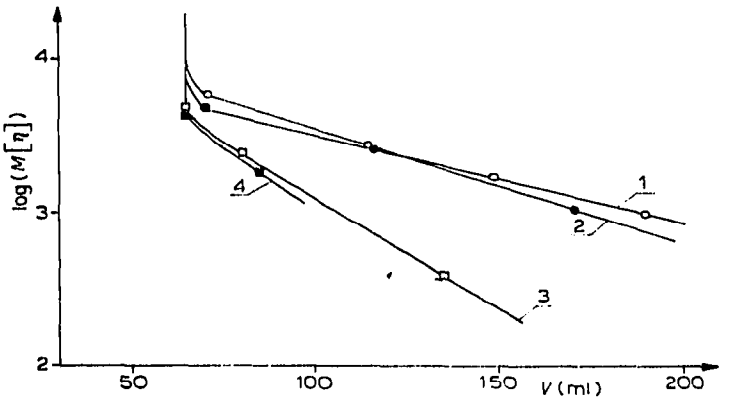

Fig. 1. Retention volumes *versus* logarithm of molecular weight multiplied by intrinsic viscosity of the polymer (obtained on a column packed with Sephadex G-100). 1, Dextran; 2, polyvinylpyrrolidone; 3, polyoxyethylene; 4, polyvinyl alcohol.

EXPERIMENTAL AND RESULTS

GPC for four types of polymers, *viz.*, dextran, polyvinylpyrrolidone (PVP), polyvinyl alcohol (PVA) and polyoxyethylene (POE), was carried out on columns 96 cm long with an I.D. of 20 mm packed with Sephadex G-75 and G-100 (particle diameter 40–120 μm). The flow-rate of the eluent (0.3% sodium chloride solution) was 50 ml/h. A differential flow refractometer with a cell volume of 50 μl and a sensitivity (ΔH) of 10^{-6} was used as a detector. The resulst are shown in Figs. 1 and 2. It is clear that the plots of the retention volume, V, *versus* log ($M[\eta]$) for PVP and dextrans differ

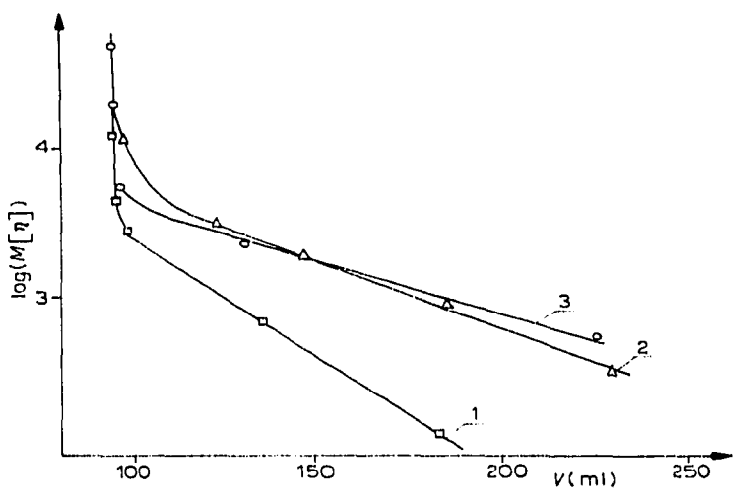

Fig. 2. Retention volumes *versus* logarithm of molecular weight multiplied by intrinsic viscosity of the polymer (obtained on a column packed with Sephadex G-75). 1, Experimental curve for polyoxyethylene; 2, curve for dextran calculated by using curve 1 and eqns. 6 and 7 in which χ^* is found from the data obtained with Sephadex G-100 and shown in Fig. 1; 3, experimental curve for dextran.

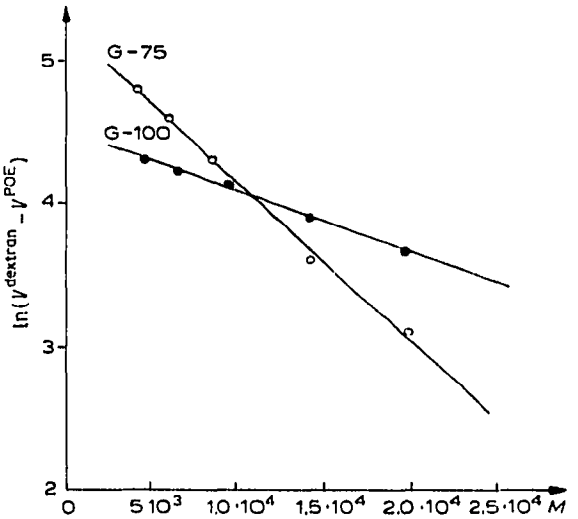

Fig. 3. Logarithm of difference between retention volumes of POE and dextran *versus* molecular weight of dextran (obtained on columns packed with Sephadex G-100 and G-75).

greatly from those for POE. Moreover, the logarithm of the difference in the V values is inversely proportional to the molecular weight of PVP or dextran (Fig. 3).

These results can be interpreted as follows. In GPC on swelling sorbents, macromolecules permeate into the macropores of the sorbent according to the molecular sieve mechanism with different probabilities determined by their hydrodynamic size. The value of the sorbent volume accessible to macromolecules, V^{acc}, and therefore the value of the retention volume, V, depends on the total pore volume. This dependence is universal, *i.e.*, it is general for all types of macromolecules. Nevertheless, the walls of macropores of the swelling sorbent are permeable to macromolecular units and, in accordance with the exclusion mechanism, there is a certain probability that these units may penetrate through them into dense sorbent regions, *i.e.*, into micropores, increasing the accessible volume of the sorbent by a certain value, ΔV^{acc}. This ability of macromolecular chains to diffuse into dense regions of the swelling sorbent is closely related to the thermodynamic compatibility of macromolecules with the sorbent matrix and can be treated on the basis of the concept of the excluded volume[13] as a property supplementary to the molecular sieve factor. Possible arrangements of macromolecules in pores of a swollen sorbent are shown schematically in Fig. 4.

The calculation of ΔV^{acc} for macromolecules compatible with the sorbent can be carried out as follows. We will consider the swollen sorbent gel in the solvent as a solution of macromolecules represented by dense regions in each gel grain. For this solution, the free energy of mixing of the polymer with the solvent can be calculated by a standard method[17]. Then, the free energy change related to the permeation of segments of macromolecules into dense gel regions can readily be estimated by using the analogy with the excluded volume of macromolecules[13]. If we assume that each dense gel region is a sphere of radius R, uniformly filled with units and of a much greater size than the macromolecule regarded as a Gaussian coil [$R \gg (\overline{r^2})^{\frac{1}{2}}$, where

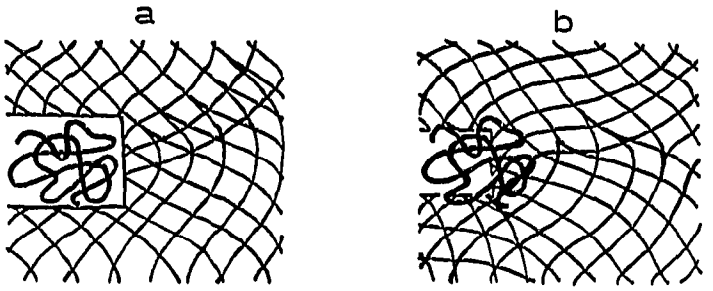

Fig. 4. Schematic arrangement of macromolecule in a pore of the swollen sorbent. (a) Macromolecule incompatible with the sorbent; (b) macromolecule compatible with the sorbent.

$(\overline{r^2})^{\frac{1}{2}}$ is the radius of gyration of macromolecules], then the expression for the total free energy change in the system gel–macromolecule–solvent when the macromolecule and the dense gel region approach and the distance between them becomes a, by analogy with theory[17], can be written as

$$\Delta F_a = kT \cdot \frac{V_g V_m}{V_1}\left[\int \varrho_g \varrho_m \mathrm{d}v + \sum_{k=2}^{\infty}\sum_{n=1}^{k} \frac{(k-1)!}{n!(k+1-n)!} \cdot V_g^{n-1} V_m^{k-n} \int \varrho_g^{n} \varrho_m^{k+1-n} \mathrm{d}v - (\chi_{1g} + \chi_{1m}) \int \varrho_m \varrho_g \mathrm{d}v + \chi_{gm} \cdot \frac{V_1}{V_g} \int \varrho_m \varrho_g \mathrm{d}v\right] \quad (1)$$

where V_1 is the volume of the solvent molecule, V_g and V_m are the volumes of the units of the gel and the macromolecule, ϱ_g and ϱ_m are the densities of these units in a volume element δV, a is the distance between the centres of the dense gel region and of the macromolecule, χ_{1g} and χ_{1m} are the constants of interaction of the units of the gel and of the macromolecule with the solvent, χ_{gm} is a constant characterizing the interaction of these units in a particular solvent and the value of ϱ_g outside the dense gel regions is reduced to zero.

Integrating eqn. 1 and, for simplicity, neglecting the series included in it (because of its low value), we obtain

$$\Delta F_a = \begin{cases} 0 \text{ if } a > R \\ kT \cdot \dfrac{V_g V_m}{V_1}\left[1 + \chi_{gm} \cdot \dfrac{V_1}{V_g} - (\chi_{1g} + \chi_{1m})\right] \varrho_g z \text{ if } a < R \end{cases} \quad (2)$$

where $z = M/M_0$ is the number of units in a macromolecule and M_0 is the weight of one unit.

The free energy change, ΔFa, determines the probability, $\exp(-\Delta F_a/kT)$, that the macromolecule is at a distance a from the dense gel region. Thus, the product $\exp(-\Delta F_a/kT)\, 4\pi a^2 \mathrm{d}a$ represents the part of the volume of the spherical gel layer that is accessible to the macromolecule, $4\pi a^2 \mathrm{d}a$. Extending the integration over a to the whole gel grain and taking into account the fraction of dense regions in each grain, we obtain

$$\Delta V^{\mathrm{acc}} = \frac{V_g - V_{\mathrm{sieve}}^{\mathrm{acc}}}{V_g} \int_0^{R_g} 4\pi a^2 \exp(-\Delta F_a/kT)\, \mathrm{d}a \quad (3)$$

where R_0 is the radius of swollen gel grains, V_0 is its total volume and V_{sieve}^{acc} is the gel volume that is accessible to macromolecules according to the molecular sieve mechanism. If the macromolecules undergoing chromatography are incompatible with the gel matrix under the conditions of a particular GPC experiment, then for these macromolecules the value of the retention volume, V^{incomp}, is completely determined by the value of V_{sieve}^{acc}:

$$V^{incomp} = V_0 + V_{sieve}^{acc} \tag{4}$$

For a polymer compatible with the gel, we have another dependence:

$$V^{comp} = V_0 + V_{sieve}^{acc} + \Delta V^{acc} = V^{incomp} + \Delta V^{acc} \tag{5}$$

where ΔV^{acc} is determined by eqn. 3 and V_0 is the mobile phase in the chromatographic system.

Eqns. 2–5 show that if we know the values of the Flory–Huggins constants, χ_{1m}, χ_{1g} and χ_{gm}, and the retention volumes for polymers that are definitely incompatible with the sorbent under the conditions used, it is easy to calculate the retention volumes for compatible polymers. We can adopt the opposite procedure and determine the χ_{ij} constants from the values of V^{comp} and V^{incomp}. In our experiment, POE and PVA were incompatible with the sorbent, whereas PVP and dextran were compatible with it. The choice of a dextran gel (Sephadex) as sorbent made it possible in calculating V^{acc} to put for dextran macromolecules $\chi_{1g} = \chi_{1m} \equiv \chi^*$, $\chi_{gm} = 0$. The calculations were carried out according to the equation

$$V^{comp} = V^{incomp} + (V_c - V^{incomp}) \exp(-k_c M_c) \tag{6}$$

which is obtained by substituting into eqn. 5 the value of ΔV^{acc} from eqn. 3; V_c is the total volume of the chromatographic system, M_c is the molecular weight of dextran and k_c is the parameter characterizing the system Sephadex–dextran–aqueous sodium chloride solution; $k_c = (1/M_c)\cdot(\Delta F_a/kT)$ for $a < R$.

Eqn. 2 gives

$$\chi^* = \frac{\lambda}{2}\left(1 - k_c \cdot \frac{M_0 V_1}{V_g V_m Q_g}\right) \tag{7}$$

The values of the retention volumes obtained in experiments on a column with Sephadex G-100 were substituted into eqn. 6. The k_c parameter was found and the χ^* constant was calculated by using eqn. 7. The results are shown in Table I. The average value of the Flory–Huggins constant found by this method for dextran, $\chi^*_{av} = 0.47$, was used for calculating the retention volume of dextran macromolecules during their elution through a column packed with Sephadex G-75, which differs from Sephadex G-100 in its density, porosity and degree of swelling. As in the preceding case, the values of the retention volumes for polymers incompatible with Sephadex were identified with the retention volumes of POE. The results of the calculations are shown in Fig. 2. The experimental results agree with the calculated values to within 15%.

TABLE I

CALCULATED χ^* VALUES

χ^* was calculated by using eqn. 7 and data from experiments on Sephadex G-100.

Molecular weight of dextran, M_c	χ^*
$5 \cdot 10^3$	0.45
$7 \cdot 10^3$	0.48
$1 \cdot 10^4$	0.47
$5 \cdot 10^4$	0.49
Average	0.47

These results, in combination with the dependence of ΔV^{acc} on the molecular weight of dextran shown in Fig. 3 and consistent with the dependence in eqn. 6 predicted by theory, permit us to assume the adequacy of our model of the GPC of flexible-chain polymers on swelling sorbents.

Results obtained in GPC on columns filled with Enzacryl K_1 and K_2 gels have been published recently[18]. In our opinion, the arguments used by the authors of that paper for the interpretation of the results should be supplemented by taking into account the difference in thermodynamic compatibility between POE on one hand and oligosaccharides on the other hand with the poly(acrylomorpholine) matrix of these gels in aqueous solutions and the difference in thermodynamic compatibility between POE with Enzacryl gels in aqueous solutions and in chloroform.

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