Steric exclusion and partial exclusion by polymer incompatibility in gel permeation chromatography

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The early elution of some polymers in gel permeation chromatography is represented by a network-limited mechanism involving a distribution coefficient, K_D, for steric exclusion and a distribution coefficient, K_P, for solute–gel interactions. Values of K_P below unity correspond to partial exclusion by polymer incompatibility with the gel. A thermodynamic treatment for K_P is presented, predicting that K_P decreases as molecular weight increases. Values of K_P determined from experimental results reported for separations of poly(vinyl acetate) and polyoxyethylene are in the direction predicted by the theoretical treatment for partial exclusion by polymer incompatibility.

INTRODUCTION

In a previous paper[1], the retention volume, V_R, for a polymer in gel permeation chromatography (g.p.c.) was defined by:

$$V_R = V_0 + K_D K_P V_i \tag{1}$$

where V_0 is the interstitial or void volume, V_i is the total volume of solvent within the gel, K_D is the distribution coefficient for steric exclusion and K_P is the distribution coefficient for solute–gel interactions. Equation (1) was derived assuming that the g.p.c. mechanism was a network-limited separation, as proposed by Heitz and Kern[2,3]. For polymers separating solely by steric exclusion K_P is unity. Then a plot of log(hydrodynamic volume) *versus* V_R is the same for random coil polymers, as demonstrated by Benoit and coworkers[4] on a semilogarithmic plot of $[\eta]M$ *versus* V_R. Here, $[\eta]$ is the intrinsic viscosity (100 cm^3/g) of the polymer in the g.p.c. eluent and M is the molecular weight of the polymer. Dawkins and Hemming[1] proposed a simple relation between K_D and $[\eta]M$, which when substituted into equation (1) gave:

$$(V_R - V_0)/K_P = V_i(-A \log[\eta]M + B) \tag{2}$$

where A and B are constants.

Equations (1) and (2) successfully represented the retardation of polystyrene ($K_P > 1$) in poor and θ solvents with semirigid crosslinked polystyrene gels[1,5,6]. Altgelt and Moore[7] observed that, whilst steric exclusion generally dominates g.p.c. separations, adsorption, partition and incompatibility mechanisms may result from solute–gel interaction effects. Adsorption and partition mechanisms will both give $K_P > 1.0$; however, a distinction between the two mechanisms was difficult for the polystyrene experiments[1,5]. The representation of the experimental data[1,5,6] over a wide molecular size range by a single value of K_P is consistent with an adsorption mechanism, because studies[8] of polymer adsorption in the absence of size exclusion effects have suggested little or no dependence on molecular weight. Furthermore, a thermodynamic interpretation of equation (1) for polystyrene in poor and θ solvents also suggested an adsorption mechanism because of the dependence of the results for K_P on solvent and temperature[9].

The observations of the early elution of some polymers in g.p.c. have been explained by polymer incompatibility with the gel[7,10–13]. Such experimental results have been reported for both semirigid and soft gels and with organic and aqueous eluents. In polymer–polymer mixtures incompatibility is highly dependent on the molecular weight, M, of each polymer and on the positive value of the interaction parameter χ_{23} between the two polymers[14]. Here the eluent, i.e. solvent, polymeric solute and gel are represented by 1, 2 and 3, respectively. Early elution of a solute results when $K_P < 1$ in equation (1). In the thermodynamic interpretation of equation (1)[9], it was observed that a positive enthalpy change on transferring solute from the mobile phase to the stationary phase corresponded to $K_P < 1.0$. In this paper we show that equations (1) and (2) represent g.p.c. experimental data[11,13,15] for partial exclusion by incompatibility between a polymeric solute and the gel.

RESULTS

From the data of Altgelt[11,15] for polystyrene and poly(vinyl acetate) in tetrachloroethylene separating on crosslinked polystyrene gels, plots were constructed according to equation (2) as shown in *Figure 1*. The value of V_0 was taken as 40.1 cm^3, as suggested by the calibration curve in the paper by Altgelt[11]. We shall assume that polystyrene separates on polystyrene gel by steric exclusion alone, i.e. $K_P = 1.0$. When K_P is taken as unity for both polymers, a universal plot of $\log[\eta]M$ *versus* $(V_R - V_0)$ is not obtained in *Figure 1*. With $K_P = 1.0$ for polystyrene, a universal calibration is

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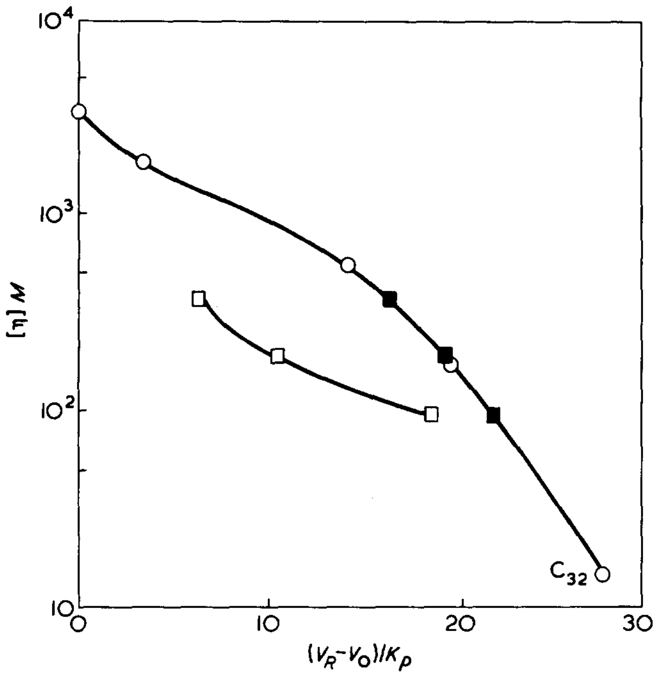

Figure 1 Plot according to equation (2) for g.p.c. data in ref 11. ○, Polystyrene (K_p = 1); □, poly(vinyl acetate) (K_p = 1); ■, poly(vinyl acetate) (K_p values in *Table 1*)

Table 1 K_p values for poly(vinyl acetate) from *Figure 1*

$[\eta]M$	K_p
374	0.38
194	0.55
98.5	0.86

obtained with the values of K_P in *Table 1* for poly(vinyl acetate) at the experimental values of $[\eta]M$.

In *Figure 2*, data for dextran and polyoxyethylene in 0.3% aqueous sodium chloride solution, separating on crosslinked dextran gels (Sephadex G–100)[13], are presented according to equation (2), taking V_0 = 65 cm^3 as suggested by the calibration curve in the paper by Belenkii and coworkers[13]. We shall assume that dextran separates on dextran gel by steric exclusion alone (K_P = 1). In *Figure 2* a universal plot of $\log[\eta]M$ *versus* $(V_R - V_0)$ is not obtained with K_P = 1 for both polymers. With K_P = 0.274 for polyoxyethylene at $[\eta]M$ = 2700 a universal calibration with dextran (K_P = 1) is obtained. Values of K_P were not determined for the other two polyoxyethylene samples. Polyoxyethylene with $[\eta]M$ = 5250 elutes at the exclusion limit and polyoxyethylene with $[\eta]M$ = 420 is much smaller in size than the dextran standards.

DISCUSSION

The plots in *Figures 1* and *2* demonstrate that equation (2) does not account for differences in the behaviour between the pairs of polymers considered when K_P is taken as unity for both polymers. The data in *Table 1* suggest that K_P is dependent on molecular weight whereas our previous studies[1,5,6] of solute retardation ($K_P > 1.0$) only required a single value of K_P over a wide molecular size range. Therefore, a network-limited mechanism with $K_P < 1$ in equation (1) may be used to explain the polymer separations involving steric exclusion and partial exclusion by incompatibility between the polymeric solute and the gel. Because of the dependence of K_P on molecular size when $K_P < 1.0$, the thermodynamic interpretation of equation (1) must be more rigorous than the treatment given elsewhere[9].

For an equilibrium separation, the standard free energy change ΔG^0 for the transfer of solute molecules from the mobile phase to the stationary phase is given by:

$$\Delta G^0 = \Delta G_D + \Delta G_P \qquad (3)$$

where ΔG_D is the free energy change for steric exclusion and ΔG_P is the free energy change for solute–gel interactions. Therefore, equation (1) becomes:

$$V_R = V_0 + V_i \exp(-\Delta G_D/kT)\exp(-\Delta G_P/kT) \qquad (4)$$

where k is Boltzmann's constant and T is temperature. The entropy and enthalpy contributions to ΔG_D have been reviewed elsewhere[9] and will not be considered further. Moreover adsorption and partition mechanisms arising from solute–gel interactions will be assumed to be absent.

The theoretical treatment of polymer incompatibility suggests that the solvent is of secondary importance[14]. Since the eluent is chosen to be highly compatible with the gel and to be a good solvent for the polymer, we shall assume athermal mixing, i.e. $\chi_{12} = \chi_{13} = 0$. An expression for K_P from ΔG_P for solute–gel interactions leading to partial exclusion by polymer incompatibility is derived by following the thermodynamic methods described elsewhere[16,17]. The activity of polymer a'_2 in the mobile phase (signified by a prime) is given by[18]:

$$\ln a'_2 = \ln\phi'_2 - (x_2 - 1)(1 - \phi'_2) \qquad (5)$$

where ϕ'_2 is the volume fraction of polymer in the mobile

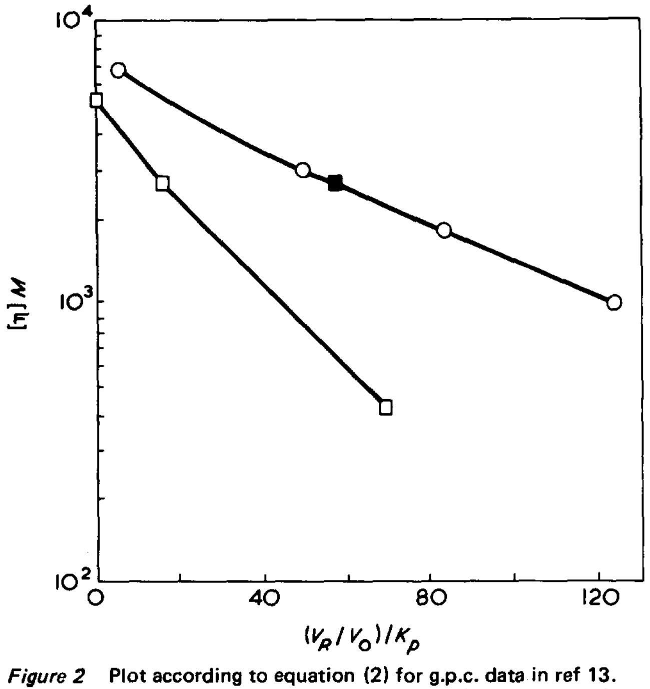

Figure 2 Plot according to equation (2) for g.p.c. data in ref 13. ○, Dextran (K_p = 1); □, polyoxyethylene (K_p = 1); ■, polyoxyethylene (K_p = 0.274)

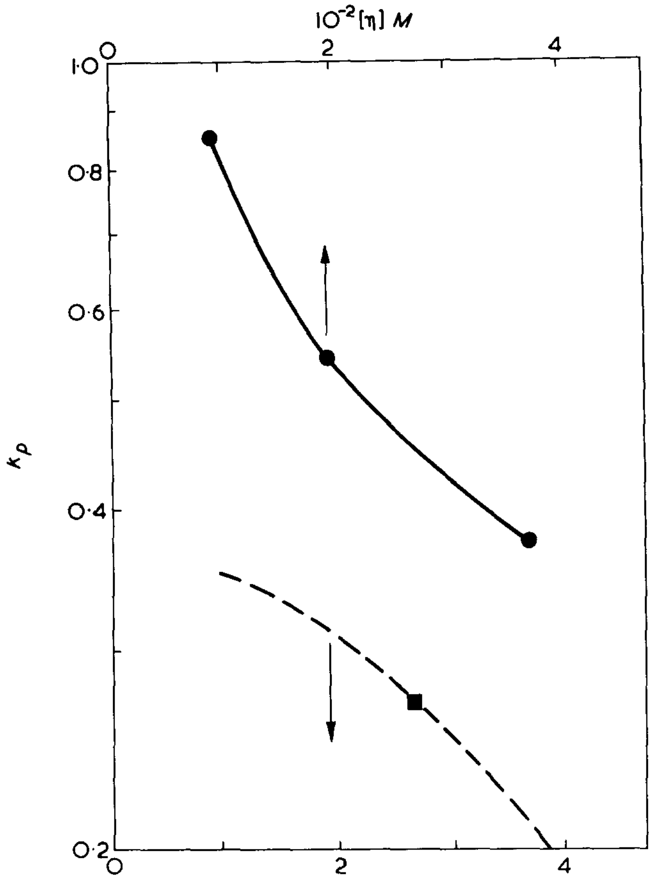

Figure 3 Dependence of ln K_p on molecular size. ●, Poly(vinyl acetate) data in *Table 1*; ■, polyoxyethylene (K_p = 0.274) from *Figure 2*; – – –, calculated K_p values for polyoxyethylene to give a universal calibration for polyoxyethylene and dextran (K_p = 1) in *Figure 2*

phase and x_2 is the degree of polymerization of the polymer. The activity of polymer, a_2, in the stationary phase (no prime) is given by[14]:

$$\ln a_2 = \ln\phi_2 + (1 - x_2)\phi_1 + [1 - (x_2/x_3)]\,\phi_3 + x_2\chi_{23}(\phi_3^2 + \phi_1\phi_3) \qquad (6)$$

where ϕ_1, ϕ_2 and ϕ_3 are volume fractions, x_3 is the degree of polymerization of the gel, and χ_{23} is the polymer–gel interaction parameter. The value of x_3 must be ∞. As the activity of the polymer in the two phases will be the same, K_P is calculated from equations (5) and (6) with:

$$\ln K_P = \ln\frac{\phi_2}{\phi_2'} \qquad (7)$$

giving:

$$\ln K_P = -(x_2 - 1)(1 - \phi_2') - (1 - x_2)\phi_1 - \phi_3 - x_2\chi_{23}(\phi_3^2 + \phi_1\phi_3) \qquad (8)$$

Equation (8) can be simplified. Dilute polymer solutions are generally separated in g.p.c., so it is reasonable to assume $\phi_2' \approx \phi_2 \approx 0$. Then, if we assume $\phi_1 = (1 - \phi_3)$, equation (8) becomes:

$$\ln K_P = -x_2\phi_3(1 + \chi_{23}) \qquad (9)$$

The value of ϕ_3 which appears as a constant in equation (9) will depend on the gel type and the eluent. Equation (9) is a simplified version of expressions which have been proposed for partition mechanisms with soft highly-swollen gels[16,17]. Strong polymer–gel interaction in a partition mechanism results in a negative value of χ_{23}, whereas the incompatibility effect leads to a positive value of χ_{23}. Therefore, K_P in equation (9) decreases as molecular weight increases, i.e. greater exclusion as polymer incompatibility increases.

A plot of the data in *Table 1* is shown in *Figure 3*. For polyoxyethylene in *Figure 2*, K_P values giving a universal calibration of polyoxyethylene with dextran standards (K_P = 1) were determined and are plotted in *Figure 3* together with the experimental point at $[\eta]M = 2700$. In spite of the assumptions and simplifications in deriving equation (9), it is clear that the molecular size dependence of the K_P values for poly(vinyl acetate) and polyoxyethylene is consistent with an increase in incompatibility as molecular weight rises. If values of M are calculated from the values of $[\eta]M$ in *Figures 1* and *2*, assuming literature values for the Mark–Houwink constants[19], then the dependence of $\ln K_P$ on M shows the same behaviour as the curves drawn in *Figure 3*. Our treatment has considered the gel network as a polymer of infinite molecular weight, and so our expressions do not contain a parameter characterizing the swelling of the network which will give rise to a free energy contribution. Further improvements in the prediction of K_P are possible by including a term for the dependence of $\ln a_2$ on gel structure, e.g. for polyoxyethylene separating on a swollen gel network. Belenkii and coworkers[13] have given an alternative and more complicated thermodynamic treatment for the dependence of V_R on the compatibility of polymeric solutes with swollen gel networks. Their analysis of the experimental data involves the calculation of χ_{12} and χ_{13} rather than χ_{23}. It is possible that another mechanism contributing to V_R for polyoxyethylene is an osmotic effect, i.e. deswelling of the gel because of polymer incompatibility; see for example the osmotic behaviour of dextran gels reported by Edmond and coworkers[20]. Semirigid gels, such as those used for poly(vinyl acetate), exhibit only slight swelling and our treatment is undoubtedly a simplification of the thermodynamics of the polymeric network constituting the internal surface of a pore.

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