Diffusion in polyacrylamide gels

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Using a novel sorption technique, the diffusion of some series of solutes in polyacrylamide gels has been investigated with regard to: (a) molecular size of solute; (b) concentration of solute and gel polymer; and (c) temperature. The approach used also yields the partition coefficient pertaining to sorption equilibrium. The ratio, D/D_0, where D_0 refers to diffusion in the pure solvent, is found to reflect in part the characteristic interactions between solute and gel polymer. The temperature results indicate that the apparent activation energy for solute diffusion is approximately independent of the polymeric component for dilute gels.

INTRODUCTION

Gels of polyacrylamide are widely used as separation media in electrophoresis and in liquid chromatography where interest is focused on the differing rates of transport of molecules varying in size and/or chemical structure. While partitioning has been extensively studied chromatographically in such systems, diffusion in the gel phase is considerably more difficult to measure with satisfactory precision and hence little investigated.

This work describes measurements of diffusion coefficients for polyhydric alcohols ($CH_2OH \cdot (CHOH)_n \cdot CH_2OH$), oligosaccharides and low molecular weight polyethylene oxide polymers in a series of polyacrylamide gels of different concentration. The approach is based on an equation of a form originally given by Carman and Haul[1] for describing the diffusion of gases and liquids from a constant, finite volume into a porous solid. The technique also yields the partition coefficient characterizing the equilibrium distribution of the diffusing substance between the gel phase and the solution, a quantity which reflects molecular exclusion and/or adsorption should it occur in the system. The solutes used in the present experiments are ones, shown by gel chromatography, having insignificant adsorption to polyacrylamide gels. The experimental arrangement and treatment of the data have been modified substantially in comparison with previously reported measurements[2].

EXPERIMENTAL

The experimental arrangement is shown schematically in *Figures 1* and *2*. Refractive index change of the circulating solution was monitored using a Waters refractive index instrument (model R-403), connected to a digital voltmeter (Newport 2003) and a recorder. A printer (Newport 810) equipped with a clock for registering the signal at a pre-set time interval was coupled to the digital voltmeter.

A 'de-bubbler' was included in the system and was placed prior to the intake ports of the refractive index monitor. Solution/solvent was circulated through the monitor by means of a peristaltic pump (Gilson Minipuls) and the solution returned to the cell holder.

Diffusion measurements

The gel disc (diameter 45 mm, thickness 2.5 mm) was mounted in a plexi-glass cell (*Figure 1*), the cover of which consists of an open stainless mesh, providing point contacts with the gel surface only and which serves to support the gel when the cell is inverted. The disc closely fitted the cell and allowed exposure of a single plane surface of the gel to the solution during a run.

Solvent (10 ml) was initially placed in the thermostated cell holder (*Figure 2*) and circulated through the monitor until a steady base line was established. Concentrated probe solution (2 ml) was then added to the solvent and circulation continued until a constant signal was obtained on the digital voltmeter. Care was taken to restrict the solute concentration to the linear region of the refractive index–concentration relationship. The solution concentration at the start of each experiment was normally 3 mg ml^{-1}. At this juncture the inverted cell (exposed face downwards to preclude convective disturbances due to concentration inversion) was lowered into the solution so that the gel face was immersed. The gel had been previously equilibrated with a large volume of the solvent to be used in the experiment. A magnetic stirrer mixed the probe solution efficiently during the run. The digital voltmeter readings were printed out at three min intervals over a 10 h run. A continuous strip recording of the

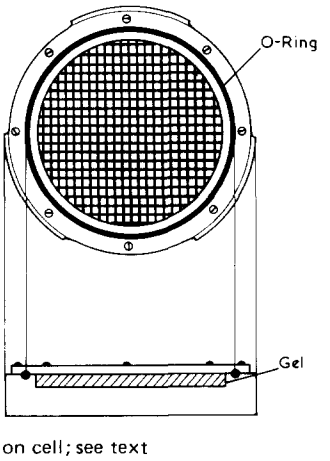

Figure 1 Diffusion cell; see text

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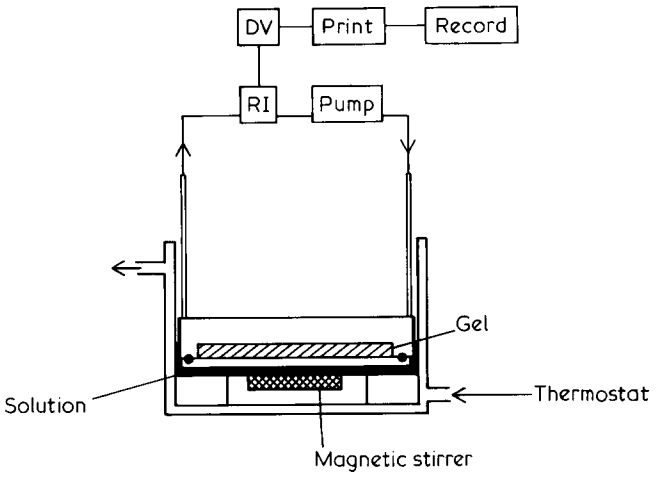

Figure 2 Experimental arrangement (RI-refractive index monitor; DV = digital voltmeter; for details see text)

exponentially decaying output signal was made to check for irregularities during the experiment. Knowledge of the initial and final outputs (concentrations) permitted evaluation of the partition coefficient as well as the diffusion coefficient (see below). All experiments were carried out in a constant temperature room (23.5°C), with the apparatus thermostated at 25°C.

Preparation of gels

Polyacrylamide gel slabs were prepared according to the description given by Pharmacia Fine Chemicals (Uppsala, Sweden) and using a commercially available gel casting apparatus from the same company designated GSC-8. The gels were nominally of 10, 20 and 30% and were prepared using the following solutions:

(a) *10% gel*: mix 2 parts solution A, 1 part solution D and 1 part solution F.

(b) *20% gel*: mix 2 parts solution B, 1 part solution D and 1 part solution F.

(c) *30% gel*: mix 2 parts solution C, 1 part solution D, 1 part solution F.

(A) Acrylamide (19.2 g), bisacrylamide (0.8 g), dissolved in buffer E to 100 ml. Filter.

(B) Acrylamide (38.4 g), bisacrylamide (1.6 g), dissolved in buffer E to 100 ml. Filter.

(C) Acrylamide (57.6 g), bisacrylamide (2.4 g), dissolved in buffer E to 100 ml. Warmed to 30–40° to assist complete dissolution and filtered.

(D) 3-dimethylaminopropionitrile (0.3 g) dissolved in buffer E to 100 ml.

(E) Tris (10.75 g), boric acid (5.04 g), Na_2EDTA (0.93 g), dissolved in distilled water to 1 litre. Final pH 8.3.

(F) Ammonium persulphate (0.3 g) dissolved in buffer E to 100 ml. Prepared fresh daily.

The gel slabs were 7 × 7 cm and 2.5 mm thick. They were stored in the refrigerator in a humid atmosphere.

The gel polymer concentrations were determined gravimetrically on the gels which had first been brought to swelling equilibrium in distilled water (approximately 1 week, with frequent changes of solvent).

(a) 10% nominal — 7.6% w/w; (volume fraction = 0.058)

(b) 20% nominal — 14.2% w/w; (volume fraction = 0.131)

(c) 30% nominal — 17.6% w/w; (volume fraction = 0.178)

Acrylamide and *N,N'*-methylene-bis-acrylamide were from Fluka AG, Buchs SG (Switzerland). The polyhydric alcohols and oligosaccharides were analytical grade reagents.

The polyethylene oxides were from Merck (chromatography grade). The molecular weights determined by vapour pressure osmometry closely agree with the tabulated nominal values. For these low molecular weights one can assume near monodispersity.

THEORY

Calculation of the diffusion coefficient

The diffusion coefficient of a low molecular weight 'probe' in a gel can be estimated by experiments in which the gel, saturated with solvent, is immersed in a cell containing the same solvent, in which the probe is dissolved. As the latter diffuses across a single plane surface into the gel, its concentration decreases from the initial concentrations, C_0, to a final value C_∞. An experimental parameter, λ, is defined such that

$$\lambda = C_\infty/(C_0 - C_\infty) \tag{1}$$

The concentration of probe may be expressed as a function of time by the following equation, derived for gas permeation by Carman and Haul[1] and adapted to liquid diffusion by Brown and Chitumbo[2]; (see also Carslaw and Jaeger[3]).

$$\frac{(C - C_\infty)}{(C_0 - C_\infty)} = \sum_{i=1}^{\infty}\left[\frac{2\lambda(1+\lambda)}{1+\lambda+\lambda^2 q_i^2}\exp\left(\frac{-q_i^2 Dt}{L^2}\right)\right] \tag{2}$$

where $\tan q_i + \lambda q = 0$, L = gel thickness, D = diffusion coefficient.

(In the case of diffusion from both sides of the gel $L = \frac{1}{2}$ gel thickness.) Rearranging equation (2) we obtain:

$$C = C_\infty\left[1 + \sum_{i=1}^{\infty}\frac{2(1+\lambda)}{1+\lambda+\lambda^2 q_i^2}\exp\left(\frac{-q_i^2 Dt}{L^2}\right)\right] \tag{3}$$

This can be expressed as

$$C = C_\infty\left[1 + \sum_{i=1}^{\infty}\cdot B_i \exp(-t/\tau_i)\right] \tag{4}$$

where

$$B_i = \frac{2(1+\lambda)}{1+\lambda+\lambda^2 q_i^2}$$

$$\tau_i = \frac{L^2}{q_i^2 D}$$

The value of λ is fixed by the experimental conditions and the g-values may be determined for as many terms as desired. Since $\tan q + \lambda q = 0$, the zeros of this equation may be seen as the intersections of $f_1(g) = \tan q$ and $f_2(q) = -\lambda q$ (see *Figure 3*). For $\lambda = 3.73$, the value appropriate to the majority of the experiments discussed here, $q_1 = 1.725$, $q_2 = 4.769$ and $q_3 = 7.888$. For higher terms, $q_i = (2i-1)(\pi/2)$. The relaxation time, τ_i, for the ith term is related to the relaxation time for the first term by:

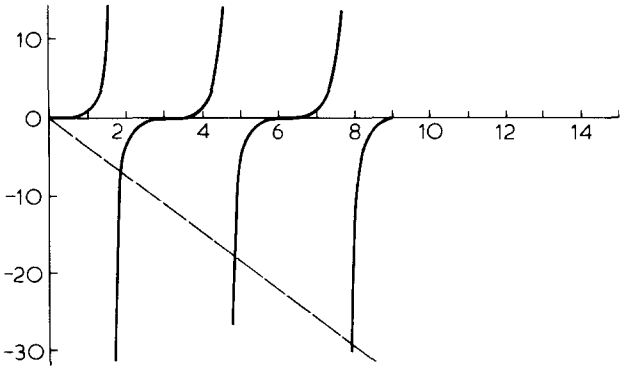

Figure 3 The functions $f_1(q) = \tan q$ (—) and $f_2(q) = -\lambda q$ (— — —). The zeros of $\tan q + \lambda q = 0$ are represented by the intersections of $f_1(q)$ and $f_2(q)$

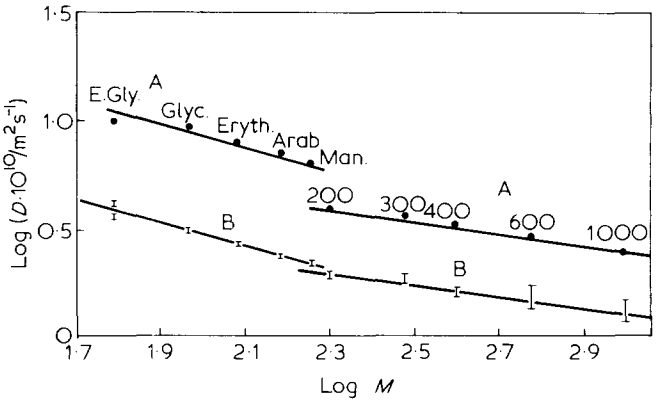

Figure 4 Molecular weight dependence of the diffusion coefficient for polyhydric alcohols and polyethylene oxides in polyacrylamide gel (17.6% w/w); (A) refers to free diffusion and (B) to diffusion in the gel

$$\frac{\tau_i}{\tau_1} = \left(\frac{q_1}{q_i}\right)^2$$

Using the q-values particular to these experiments, yields:

$$\tau_2 = 0.131\ \tau_1$$

$$\tau_3 = 0.0478\ \tau_1$$

It is seen that the relaxation times decrease monotonically. The relative weights of the terms at the start of the experiment are the B_i's. Assuming again a value of 3.73 for λ, we find $B_1 = 0.205$, $B_2 = 0.0295$ and $B_3 = 0.0109$. (The sum of all B's must be $1/\lambda$.) Thus, the terms beyond the first exponential in equation (4) have relatively low weights and disappear rapidly in comparison with the first term. Equation (4) then reduces to

$$C = C_\infty + C_\infty B \exp(-t/\tau) \qquad (5)$$

ignoring measurements at very early times. The data were analysed by fitting equation (5) with a non-linear regression program described in the book by Daniel and Wood[4]. This program yields estimates of the parameters in the model as well as their 95% confidence limits. In order to obtain these confidence limits for quantities of greatest relevance to the present experiments, the model is described in terms of the diffusion coefficient (instead of τ), the partition coefficient (instead of C_∞) and an initial concentration displacement (instead of B).

The partition coefficient, K, is the ratio of the expected λ to the obtained λ. Since the total amount of probe material in the system is constant during the experiment:

$$C_0 V_{sys} = C_\infty V_{sys} + C_\infty V_{gel} K$$

where $V_{gel}K$ is the actual volume within the gel that is available to the probe or $C_\infty K$ is the concentration of the probe within the entire volume of the gel. To ensure internal consistency in the calculations, the extrapolated value of C_0 was used in the calculation of λ. The difference between the measured pre-start concentration and the diffusion-extrapolated C_0 is that referred to above as an initial concentration displacement.

The accuracy of fit has been judged by the root-mean-square difference between measured points and fitted curve, and has fallen in the region 2–4 units for experiments in which the response variable decreases from a starting value of ~7500 units to an asymptotic value of ~6000 units.

RESULTS AND DISCUSSION

Influence of polymer concentration

Figures 4 and *5* illustrate the molecular weight dependence of the diffusion coefficient for the three solute series in the polyacrylamide gel of concentration 17.6% w/w. The slope in each case is indistinguishable from that characterizing the free diffusion of these solutes in the pure solvent. Qualitatively similar data were obtained in the gels of lower polymer concentration and these are summarized in *Figure 6* for the polyhydric alcohols. The common slope shows that solute diffusion in the gels is governed by frictional interactions identical to those found in the pure solvent, a conclusion which is supported by the apparent activation energies (see below).

The ratio D/D_0 decreases with increasing polymer concentration in a linear manner on a semi-logarithmic representation; *Figure 7*. The slope is greatest for the oligosaccharides and least for the polyethylene oxides. An analogous effect is also apparent in the data of Laurent *et al.*[5] and Ogston *et al.*[6] for the diffusion of proteins in concentrated polymer solutions. It may be inferred that

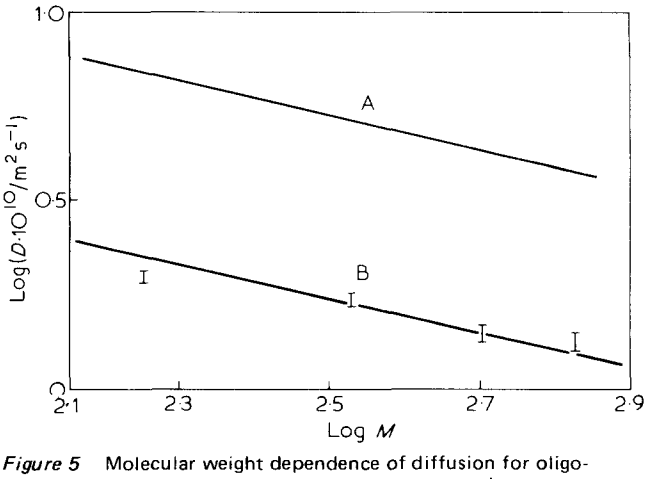

Figure 5 Molecular weight dependence of diffusion for oligosaccharides (glucose, cellobiose, raffinose, stachyose) in polyacrylamide gel (17.6% w/w); (A) — free diffusion; (B) gel diffusion

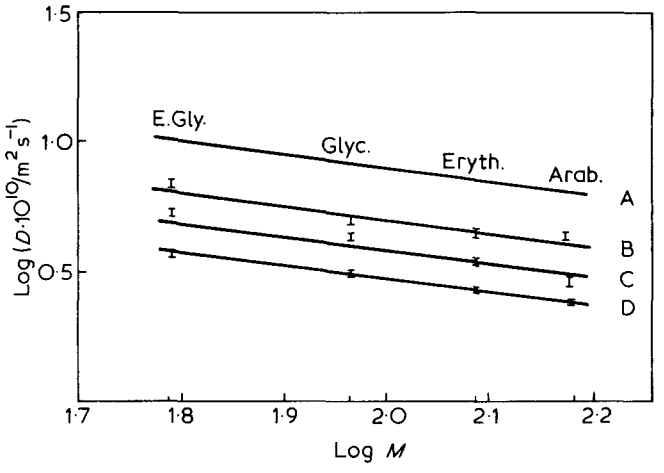

Figure 6 Diffusion data for polyhydric alcohols in (A) pure solvent and (B), (C), (D), gels of concentration 7.6%, 14.2% and 17.6%, respectively

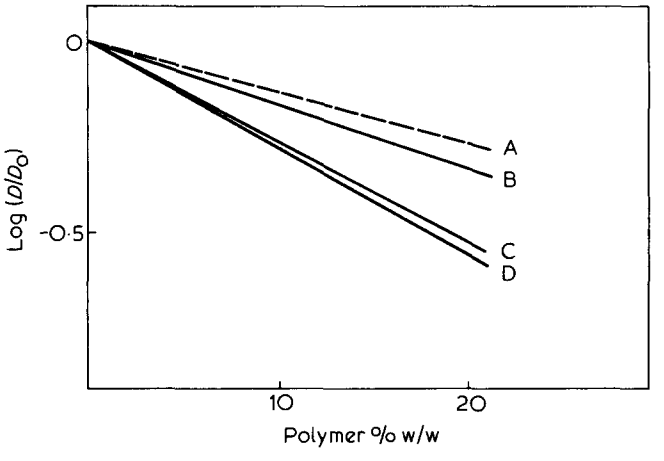

Figure 7 The diffusion ratio D/D_0 as a function of polymer concentration (A) according to the expression of Mackie and Meares[7]. (B) For polyethylene oxides; (C) for polyhydric alcohols; (D) for oligosaccharides

D/D_0 reflects in part the characteristic interactions of a given solute type with the gel polymer. This variation in D/D_0 is not predicted by the 'inert obstacle' theories, based on the volume fraction of polymer, such as that of Mackie and Meares[7] (line A in *Figure 7*) and Prager[8] which presumably estimate a minimum value for the reduction in the diffusion coefficient in an idealized system. It is to be expected that the solute–polymer interactions will influence the transport process. An extreme case in point is the very different D/D_0 values for the isomeric α- and β-cyclodextrins in poly-(methacrylic acid); Iijima *et al.*[9] conclude that the very low D/D_0 ratios for the β-compound are due to this substance forming a complex with the polymer.

In the present system the oligosaccharides and polyhydric alcohols, possessing essentially the same functional groups, have nearly identical D/D_0 values at a given polymer concentration. The polyethylene oxides are considerably less polar substances. Thus the ratio D/D_0 will only exceptionally be a statistically predictable quantity and in most cases will be uniquely defined by the particular solute–polymer pair.

The sensitivity of the ratio D/D_0 may have some relevance in our understanding of the mechanism underlying liquid chromatography since the separation on column elution may in some cases partly reflect differences in diffusion as well as the partition coefficient prevailing under practical flow rates.

The molecular weight dependence of the equilibrium partition coefficient differs greatly for the solute series. For the low molecular weight polyhydric alcohols K is approximately independent of M. The exclusion of the polyethylene oxides increases strongly with increasing molecular weight in comparison with the oligosaccharides as shown in *Figure 8*. In aqueous solution the polyethylene oxide chain is apparently, at least partly, helical[10] and thus should have rod-like character at low molecular weight, whereas the oligosaccharides are relatively compact structures.

Concentration dependence

Figure 9 illustrates the insignificant influence of the solute concentration on the diffusion coefficient for glycerol and PEG 600 in the 17.6% gel. In this concentration interval the partition coefficient is also independent of concentration (*Figure 10*) for both solutes. In the case of glycerol the theoretical value of K is 0.86, assuming that all solvent is accessible to this small solute.

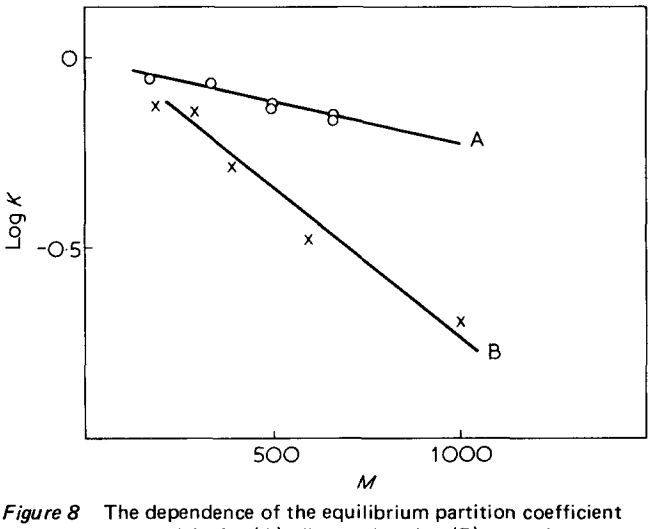

Figure 8 The dependence of the equilibrium partition coefficient K on molecular weight for (A) oligosaccharides; (B) polyethylene oxides

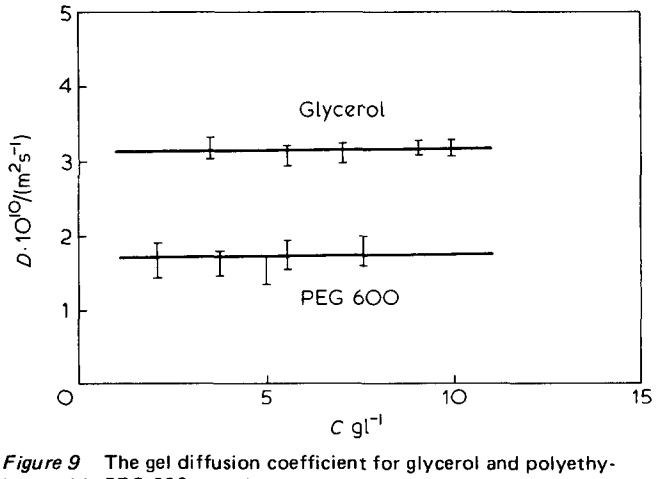

Figure 9 The gel diffusion coefficient for glycerol and polyethylene oxide PEG 600 at various concentrations

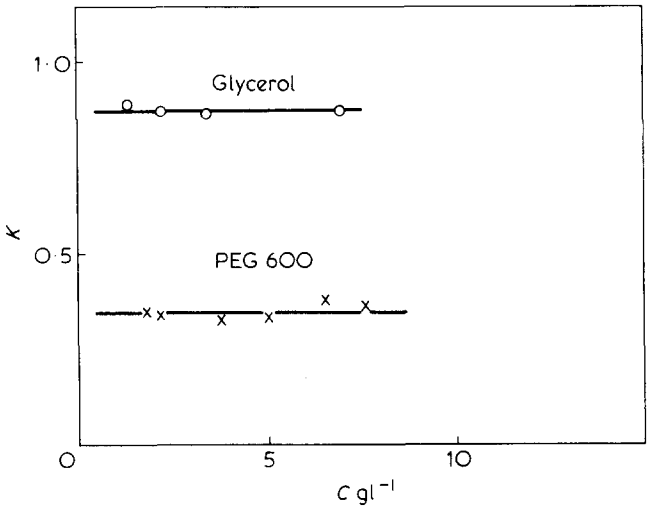

Figure 10 The equilibrium partition coefficient, K, for glycerol and polyethylene oxide PEG 600 at the same concentration as *Figure 9*

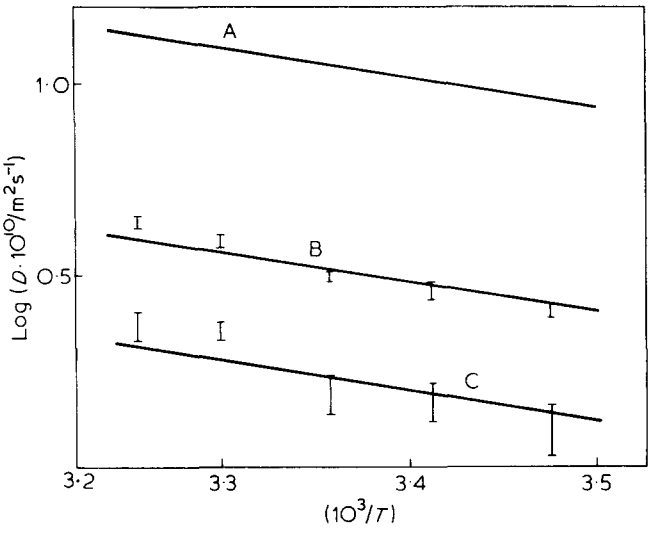

Figure 11 Arrhenius plots for (A) inverse viscosity of water; (B) diffusion data for glycerol; (C) diffusion data for polyethylene oxide PEG 600

These results show that adsorption effects are insignificant in this case.

Temperature dependence

These data are shown in *Figure 11* as Arrhenius plots. The lines are drawn with slopes corresponding to the temperature dependence of the inverse viscosity of water. Since this slope also applies for these solutes diffusing in water alone, one concludes that, at these gel polymer concentrations, the frictional behaviour is determined solely by the solute-solvent interaction. The polymer segments simply impose a more tortuous path in diffusion. Since, in addition, there are interactions between the polar groups of solute and gel polymer, a unique factor for each solute must be introduced to quantitatively describe its diffusion with a given network.

ACKNOWLEDGEMENTS

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APPENDIX

Summary of diffusion coefficients. The values are given together with the 95% confidence limits and these are also indicated in the figures.

	$D.10^{10}/m^2\,s^{-1}$	Gel c (17.6% w/w)
Temperature	PEG 600	Glycerol
15°C	$1.2_8 \pm 0.19$	$2.5_8 \pm 0.07$
20°C	$1.5_4 \pm 0.17$	$2.9_0 \pm 0.09$
25°C	$1.7_8 \pm 0.19$	$3.1_3 \pm 0.04$
30°C	$2.2_8 \pm 0.12$	$3.8_2 \pm 0.08$
34°C	$2.3_4 \pm 0.21$	$4.3_1 \pm 0.08$

PEG 600		Glycerol	
C/kg m^{-3}	$D_{25}.10^{10}/m^2\,s^{-1}$	C/kg m^{-3}	$D_{25}.10^{10}/m^2\,s^{-1}$
2.20	$1.6_9 \pm 0.23$	1.47	$4.5_2 \pm 0.73$
3.80	$1.5_9 \pm 0.19$	2.24	$3.7_3 \pm 0.17$
5.01	$1.5_5 \pm 0.18$	3.50	$3.1_6 \pm 0.10$
5.57	$1.5_4 \pm 0.17$	5.54	$3.0_5 \pm 0.07$
7.60	$1.7_9 \pm 0.17$	6.98	$3.1_3 \pm 0.08$
		9.10	$3.2_0 \pm 0.05$
		9.90	$3.1_8 \pm 0.04$

	D_{25} $10^{10}/m^2\,s^{-1}$		
Probe	Gel a (7.6% w/w)	Gel b (14.2% w/w)	Gel c (17.6% w/w)
E. Glycol	$6.4_7 \pm 0.14$	$5.5_7 \pm 0.11$	$4.1_0 \pm 0.05$
Glycerol	$5.3_1 \pm 0.16$	$4.2_5 \pm 0.08$	$3.1_9 \pm 0.05$
Erythritol	$4.5_4 \pm 0.09$	$3.5_6 \pm 0.06$	$2.6_0 \pm 0.05$
Arabitol	$4.3_2 \pm 0.05$	$2.9_3 \pm 0.05$	$2.4_7 \pm 0.03$
Mannitol	$3.3_4 \pm 0.05$	$2.6_8 \pm 0.04$	$1.9_7 \pm 0.04$
PEG 200	–	–	$2.0_9 \pm 0.07$
300	–	–	$1.9_4 \pm 0.08$
400	–	–	$1.7_0 \pm 0.08$
600	–	–	$1.5_9 \pm 0.19$
1000	–	–	$1.1_5 \pm 0.26$
Glucose	–	–	$1.9_8 \pm 0.06$
Cellobiose	–	–	$1.7_2 \pm 0.07$
Raffinose	–	–	$1.3_7 \pm 0.11$
Stachyose	–	–	$1.3_2 \pm 0.07$

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