

CHROM. 16,376

## THERMODYNAMIC PROPERTIES OF AQUEOUS SOLUTIONS OF MACROMOLECULAR COMPOUNDS

### I. SOLUTIONS OF POLYETHYLENE GLYCOL AND POLYACRYLAMIDE

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(Received October 3rd, 1983)

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#### SUMMARY

Partitioning of a homologous series of DNP-amino acids with aliphatic side-chains in *n*-octanol-aqueous polymer solution and in *n*-octanol-water as a reference system was studied. The effect of polyethylene glycols and polyacrylamides of various molecular weights on the partitioning of the above solutes was examined. The results were used to calculate the relative hydrophobicity of the aqueous polymer solution and the relative hydration power of water in the solution.

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#### INTRODUCTION

It is known<sup>1-6</sup> that some properties of water may be modified by the presence of macromolecules. Previously<sup>4,5</sup> we proposed a new approach to the experimental study of the effect of macromolecules on the thermodynamic properties of aqueous solutions, namely, on the relative hydrophobicity and relative hydration power of water in the solution. The approach is based on the use of the partition technique. Partitioning of a homologous series of solutes with different aliphatic chain lengths in water-organic solvent and in aqueous polymer solution-organic solvent two-phase systems has been studied. The results were used to calculate the free energies of transfer of a CH<sub>2</sub> group and of a polar group of the solute from water to the aqueous solution of the polymer under study.

The above approach was used here to study the effect of polyacrylamide

(PAAm) and polyethylene glycol (PEG) of various molecular weights on the thermodynamic properties of an aqueous solution at pH 7.4.

## EXPERIMENTAL

### Materials

Fractions of polyethylene glycol (PEG) having nominal average molecular weights from 1500 to 40,000 were purchased from Loba (Austria). Fractions of PEG with molecular weights of 300 and 600 were obtained from Merck (F.R.G.). Ethylene glycol was of analytical-reagent grade and was used without further purification.

Polyacrylamide (PAAm) samples were obtained by the fractional precipitation with water (solvent) and methanol (non-solvent) of PAAm prepared as described in ref. 7. The intrinsic viscosity,  $[\eta]$ , in water at  $25 \pm 0.1^\circ\text{C}$  was determined for each fraction with an Ubbelohde dilution viscosimeter and the molecular weights ( $M_w$ ) values of the PAAm fractions were calculated using the equation

$$[\eta] = 2.67 \cdot 10^{-4} M_w^{0.66}$$

Dinitrophenylated amino acids (DNP-Gly, DNP-L-Ala) were obtained from Serva (F.R.G.), 2,4-dinitrofluorobenzene from Calbiochem (U.S.A.), L-norleucine and DL-norvaline from Reanal (Hungary) and DL-2-amino-*n*-octanoic acid from BDH (U.K.). The amino acids were dinitrophenylated as described previously<sup>2</sup>, and their purities were checked by thin-layer chromatography. Their sodium salts were prepared by titration. 1-Octanol and other chemicals and salts were of analytical-reagent grade and were used without further purification.

### Methods

Two-phase systems were prepared by mixing 1–2 ml of *n*-octanol with an equal volume of a stock polymer solution containing buffer, sodium chloride and DNP-amino acid at the desired concentrations. The concentrations of sodium phosphate buffer (pH 7.4) and sodium chloride were adjusted to achieve a composition of the aqueous phase of 0.01 *M* buffer and 0.15 *M* sodium chloride. The solution was mixed with a vortex mixer, incubated at  $25^\circ\text{C}$  for 30–45 min and centrifuged for 5–15 min at 1200 *g* to speed phase settling. Then aliquots of both phases were pipetted from the system and each was analysed for the DNP-amino acid by measuring the absorbance at 360 nm (aqueous phase) or 350 nm (*n*-octanol phase), after appropriate dilution with water (or *n*-octanol), against a correspondingly diluted phase blank.

The absence of the polymer under examination in the *n*-octanol phase was checked in separate experiments by the measurements of the refractive index of the *n*-octanol phase.

The partition coefficient, *P*, is defined as the ratio of the solute concentration in the *n*-octanol phase to that in the aqueous phase. The partition coefficients were measured for each solute over approximately ten-fold concentration ranges and were found to be independent of the solute concentration in the two-phase systems. The partition coefficient for each solute was determined as the mean of 2–4 measurements on three dilutions from each partition experiment carried out 2–4 times in a given two-phase system.

## RESULTS

The approach used is based on the linear relationship between the logarithm of the partition coefficient and the number of carbon atoms in the aliphatic chain of the compounds distributed in a given two-phase system<sup>2-6</sup>. Some of the relationships are shown in Fig. 1 and can be described by the equation

$$\ln P = C + En \quad (1)$$

where  $n$  is the equivalent number of  $\text{CH}_2$  groups in the amino acid aliphatic side-chain<sup>2</sup>,  $C$  is the logarithm of the partition coefficient of DNP-glycine and  $E$  represents an average  $\ln P$  increment per  $\text{CH}_2$  group. It is clear that  $E$  is related to the free energy of transfer of a  $\text{CH}_2$  group from one to the other phase of a given two-phase system ( $\Delta G^{\text{CH}_2}$ ) according to the equation  $\Delta G^{\text{CH}_2} = -RTE$ .

A least-squares treatment of the data obtained led to the plots of  $C$  and  $\Delta G^{\text{CH}_2}$  values against concentrations of the polymers studied shown in Figs. 2-5.

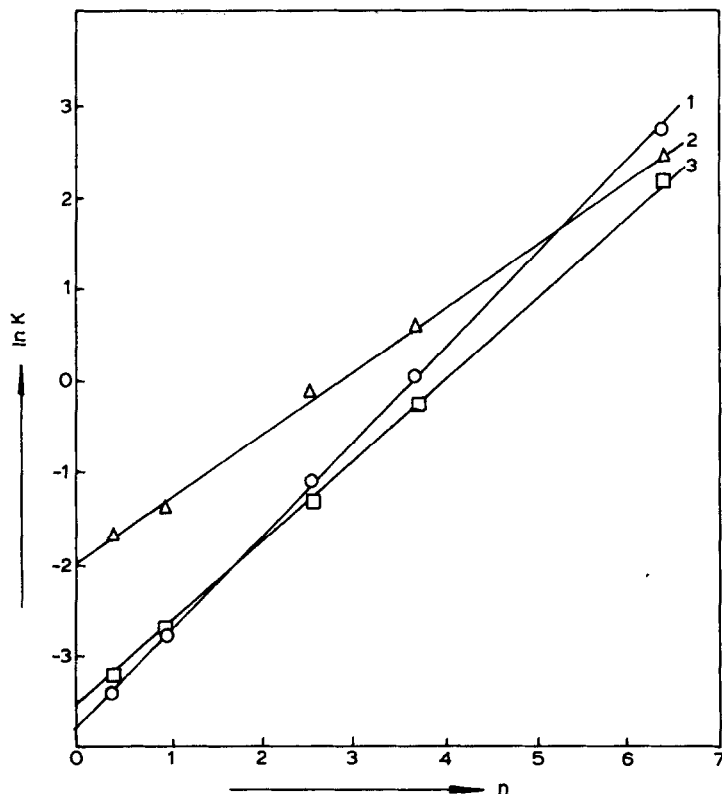


Fig. 1. Logarithm of the partition coefficient as a function of the aliphatic side-chain length ( $n$ ) of dinitrophenylated glycine, alanine, norvaline, norleucine and 2-amino-*n*-octanoic acid in the following two-phase systems: (1) *n*-octanol-0.15 *M* NaCl + 0.01 *M* sodium phosphate buffer (pH 7.4); (2) *n*-octanol-3% polyacrylamide in 0.15 *M* NaCl + 0.01 *M* sodium phosphate buffer (pH 7.4),  $M_w = 3.84 \cdot 10^5$ ; (3) *n*-octanol-25% polyethylene glycol ( $M_n = 2 \cdot 10^4$ ) in 0.15 *M* NaCl + 0.01 *M* sodium phosphate buffer (pH 7.4).

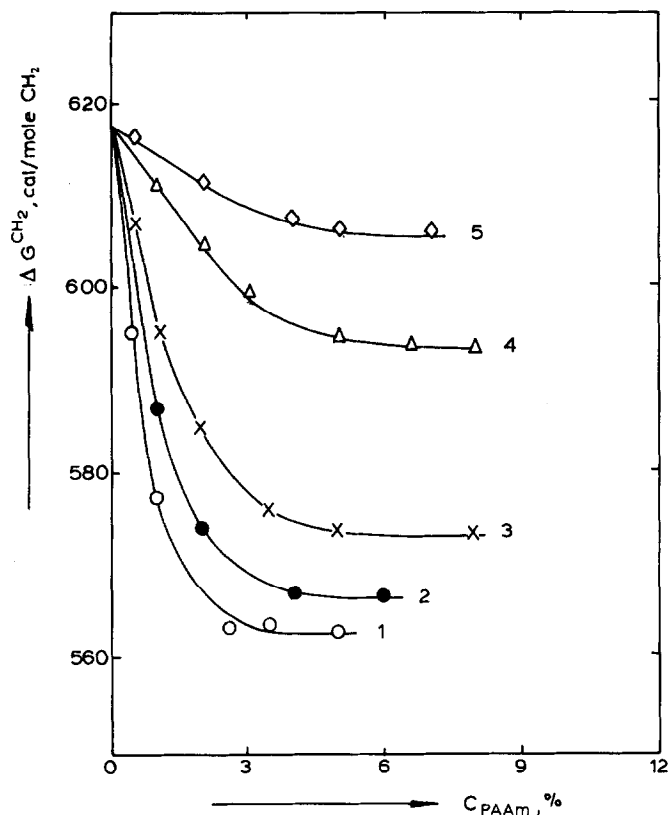


Fig. 2. Free energy of transfer of a  $\text{CH}_2$  group from *n*-octanol to a solution of polyacrylamide (PAAm) in 0.15 *M* NaCl + 0.01 *M* sodium phosphate buffer (pH 7.4) ( $\Delta G^{\text{CH}_2}$ ) as a function of the polymer concentration ( $C_{\text{PAAm}}$ ). Molecular weight of the polymer: 1,  $4.54 \cdot 10^5$ ; 2,  $4.42 \cdot 10^5$ ; 3,  $3.84 \cdot 10^5$ ; 4,  $6.65 \cdot 10^4$ ; 5,  $1.06 \cdot 10^4$ .

## DISCUSSION

It is known<sup>2,5,6,8</sup> that the free energy of transfer of a  $\text{CH}_2$  group between the two phases of a given two-phase system,  $\Delta G^{\text{CH}_2}$ , can be used as a measure of the difference in the relative hydrophobicities between the phases. When this criterion is used for comparison of different aqueous phases with a given organic phase, it can be taken as a measure of the relative hydrophobicity of these phases. The same parameter can be applied for comparison of different organic solvents with water or any salt solution. *n*-Octanol was chosen<sup>2,5,6</sup> as the reference organic solvent. The  $\Delta G^{\text{CH}_2}$  value specific for the 0.15 *M* sodium chloride in 0.01 *M* sodium phosphate buffer (pH 7.4)–*n*-octanol two-phase system is known<sup>5,6</sup> to be 618 cal/mole of  $\text{CH}_2$ . Hence, it is possible to calculate the free energy of transfer of a  $\text{CH}_2$  group between the phases of the hypothetical polymer solution–buffer two-phase system,  $\Delta g^{\text{CH}_2}$ .

Parameter *C* in eqn. 1, in accordance with the previously proposed model of the partitioning process<sup>9,10</sup>, can be used as a measure of the hydration power of

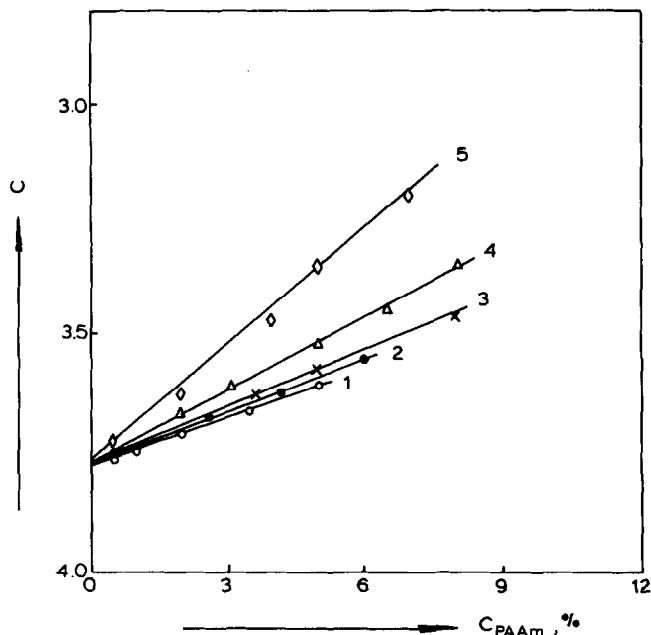


Fig. 3. Parameter  $C$  as a function of the concentration of polyacrylamide in aqueous solution. Molecular weight of PAAm as in Fig. 2. For explanation, see text.

water in a given phase of the two-phase system. The hydration power of water has been defined<sup>9,10</sup> as the ability of water to participate in interactions with ionogenic polar groups, which is mediated by the presence of neighbouring polymers and ions. Hence, the variation in the  $C$  value observed at various concentrations of the polymers of different molecular weights are considered under the assumption that these variations specify the effect of the polymers on the hydration power of water in the solutions under study.

#### *Solutions of polyacrylamide (PAAm)*

The data presented in Fig. 2 indicate that the relative hydrophobicity of the aqueous solution of PAAm at pH 7.4 depends on the molecular weight and the concentration of the polymer. The curves in Fig. 2 include two main ranges. The initial linear range seems to characterize the effect of the polymer concentration on the hydrophobic character of the solution under study. This range can be described as:

$$\Delta g^{\text{CH}_2} = \beta c \quad (2)$$

where  $c$  is the concentration of the polymer (in mole/l),  $\Delta g^{\text{CH}_2}$  is the free energy of the hypothetical transfer of a  $\text{CH}_2$  group from the polymer solution in the above buffer to the buffer and  $\beta$  is a constant. Values of  $\beta$  for all the fractions of PAAm examined are given in Table I.

The data in Fig. 2 indicate that the relative hydrophobicity of the polymer

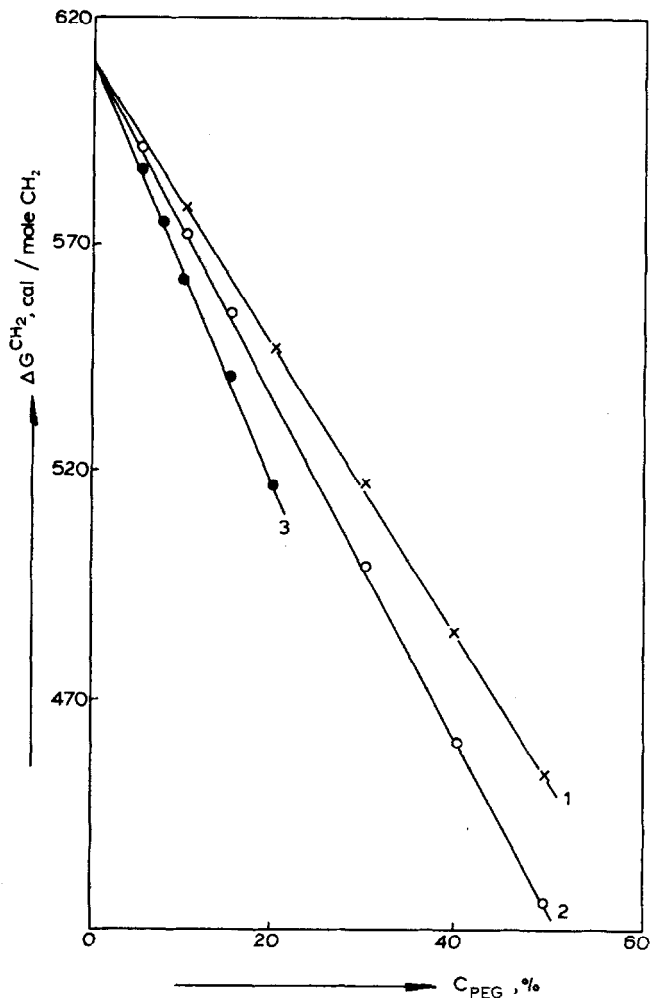


Fig. 4. Free energy of transfer of a  $\text{CH}_2$  group from *n*-octanol to a solution of polyethylene glycol or ethylene glycol in 0.15 *M* NaCl + 0.01 *M* sodium phosphate buffer (pH 7.4) ( $\Delta G^{\text{CH}_2}$ ) as a function of the concentration of the solute (*c*). 1, Ethylene glycol; 2, polyethylene glycol ( $M_n = 300$ ); 3, polyethylene glycol ( $M_n = 2 \cdot 10^4$ ).

solution attains the limit,  $\delta(\Delta g^{\text{CH}_2})$ , specific for the given molecular weight of the polymer at a certain concentration of PAAM. The  $\delta(\Delta g^{\text{CH}_2})$  values determined from the data shown in Fig. 2 are presented in Table I.

The results in Table I indicate that both  $\beta$  and  $\delta(\Delta g^{\text{CH}_2})$ , characterizing the effect of PAAM on the hydrophobic character of the aqueous polymer solution, depend on the molecular weight of the polymer, as shown in Fig. 6. The relationships plotted in Fig. 6 are described by

$$\beta = 0.030M_w^{1.364} \quad (r^2 = 0.997) \quad (3)$$

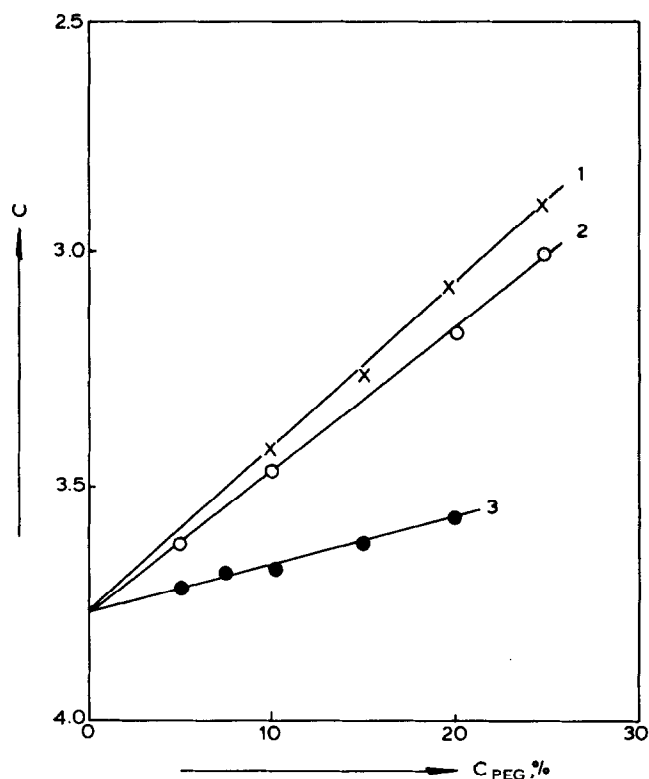


Fig. 5. Parameter  $C$  as a function of the concentration of ethylene glycol (1) and of polyethylene glycols with  $M_n = 300$  (2) and  $2 \cdot 10^4$  (3) in aqueous solution. For explanation, see text.

and

$$\delta(\Delta g^{CH_2}) = 0.260 M_w^{0.406} \quad (r^2 = 0.939) \quad (4)$$

where  $r^2$  is the correlation coefficient.

The results in Fig. 3 indicate that the relative hydration power of water in the

TABLE I

CHARACTERISTICS OF THE EFFECT OF POLYACRYLAMIDE (PAAm) ON THE RELATIVE HYDROPHOBICITY AND THE RELATIVE HYDRATION POWER OF WATER IN THE PRESENCE OF 0.15 M NaCl IN 0.01 M SODIUM PHOSPHATE BUFFER (pH 7.4)

$M_w$	$\alpha$ (l/mole)	$\beta$ (cal/mole $CH_2 \cdot l \cdot mole$ )	$\delta(\Delta g^{CH_2})$ (cal/mole $CH_2$ )
$4.54 \cdot 10^5$	1700	$1.56 \cdot 10^6$	55.0
$4.42 \cdot 10^5$	1660	$1.49 \cdot 10^6$	51.0
$3.84 \cdot 10^5$	1514	$1.24 \cdot 10^6$	46.0
$6.65 \cdot 10^4$	355	$1.13 \cdot 10^5$	24.0
$1.06 \cdot 10^4$	87	$9.27 \cdot 10^3$	11.0

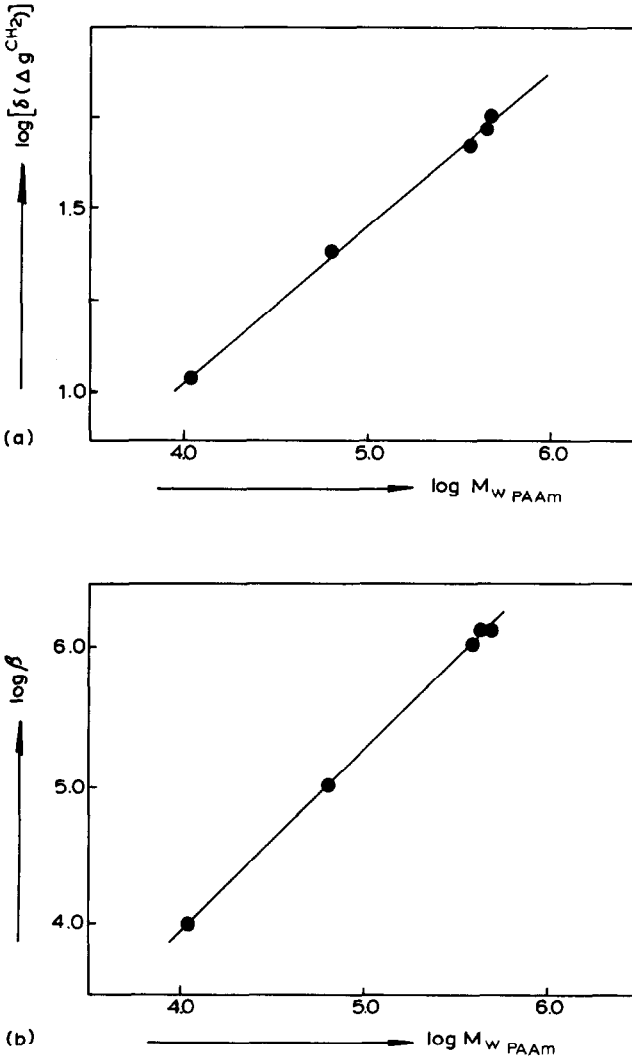


Fig. 6. (a) Parameter  $\delta(\Delta g^{\text{CH}_2})$  and (b) parameter  $\beta$  as a function of the molecular weight ( $M_w$ ) of polyacrylamide. For explanation, see text.

PAAm solution also depends on the concentration and molecular weight of the polymer. All the relationships observed are described by

$$C = a + \alpha c \quad (5)$$

where  $a$  is a constant ( $a = -3.776 \pm 0.019$ ) characterizing the relative hydration power of water in relation to the  $\alpha$ -carboxyl group of DNP-amino acid in the presence of 0.15  $M$  sodium chloride in 0.01  $M$  sodium phosphate buffer (pH 7.4), and  $\alpha$  specifies the concentration effect of PAAm on the hydration power of water under the above conditions.



The results in Fig. 3 were treated according to eqn. 5 and the  $\alpha$ -values found are given in Table I. It follows from the results obtained that  $\alpha$  depends on the molecular weight of PAAm according to the equation

$$\alpha = 5.151 \cdot M_w^{-1.234} \quad (r^2 = 0.971) \quad (6)$$

Hence the results obtained indicate the different effect of polyacrylamide on the thermodynamic properties of the aqueous solutions under examination. The observed increase in the relative hydrophobicity of the PAAm solution with increase in the concentration and molecular weight of the polymer seems to agree with the conclusion<sup>1</sup> that PAAm is a strong water-structure breaker. The relative hydration power of water in the presence of PAAm appears to decrease with increase in the polymer concentration. The effect of the polymer on this parameter, however, seems to decrease with increase in molecular weight of the polymer.

#### *Solutions of polyethylene glycol (PEG)*

Fig. 4 indicates that, as with PAAm, the relative hydrophobicity of a PEG solution increases with increase in the polymer concentration. The relationships observed (Fig. 4) do not achieve any limit, probably because it is impossible to obtain the concentration of PEG required to reach the  $\delta(\Delta g^{CH_2})$  value under the experimental conditions employed. The data in Fig. 4 were treated according to eqn. 2 and the  $\beta$ -values obtained are given in Table II.

The  $\beta$  values obtained are plotted against the molecular weight of PEG in Fig. 7. It should be noted that the  $\beta$ -value determined for the solution of ethylene glycol fits the relationship observed. The relationship is

$$\beta = 0.304M_n^{1.016} = 21.46p^{1.016} \quad (r^2 = 0.994) \quad (7)$$

where  $p$  is the degree of polymerization.

It seems possible to conclude from the fact that the effect of ethylene glycol and PEG with molecular weights up to  $4 \cdot 10^4$  on the hydrophobic character of the

TABLE II

CHARACTERISTICS OF THE EFFECT OF ETHYLENE GLYCOL AND POLYETHYLENE GLYCOL ON THE RELATIVE HYDROPHOBICITY AND THE RELATIVE HYDRATION POWER OF WATER IN THE PRESENCE OF 0.15 M NaCl IN 0.01 M SODIUM PHOSPHATE BUFFER (pH 7.4)

$M_n$	$\alpha$ (l/mole)	$\beta$ (cal/mole $CH_2$ /l/mole)
$4 \cdot 10^4$	32.0	$1.405 \cdot 10^4$
$2 \cdot 10^4$	19.8	$8.232 \cdot 10^3$
$6 \cdot 10^3$	5.40	$1.890 \cdot 10^3$
$3 \cdot 10^3$	2.61	$1.169 \cdot 10^3$
$1.5 \cdot 10^3$	1.42	$4.98 \cdot 10^2$
$6 \cdot 10^2$	1.98	$2.02 \cdot 10^2$
$3 \cdot 10^2$	1.05	$1.07 \cdot 10^2$
62.07	0.192	19.9

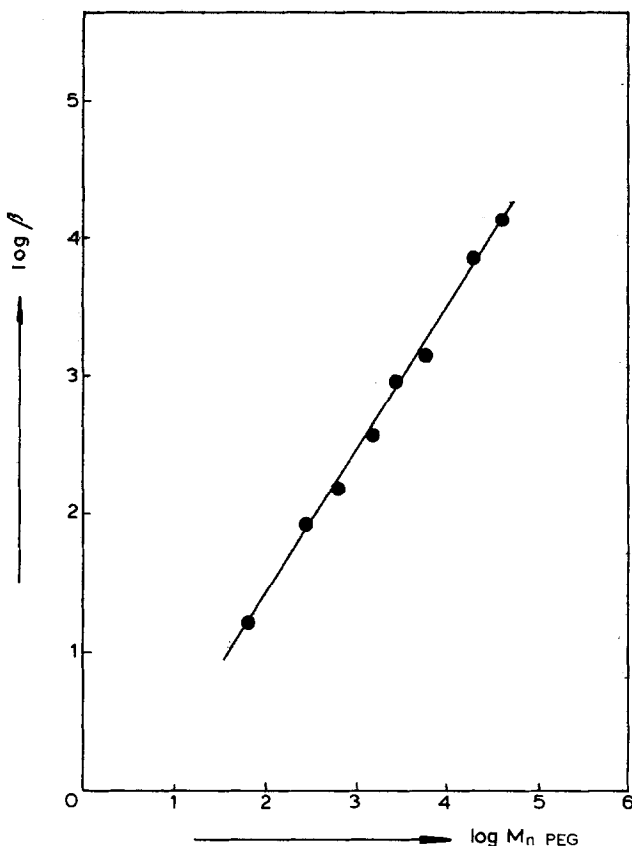


Fig. 7. Parameter  $\beta$  as a function of the molecular weight ( $M_n$ ) of polyethylene glycol.

aqueous solution are described by the same equation (eqn. 7) that the relative hydrophobicity of the PEG solution is governed not by the molecular weight of the polymer but by the concentration of the monomeric unit.

The results in Fig. 5 were treated according to eqn. 5 and the  $\alpha$ -values determined are given in Table II and Fig. 8. It can be seen that the effect of ethylene glycol and PEG with molecular weights up to 600 on the relative hydration power of water can be described by

$$\alpha = 2.683 \cdot 10^{-3} M_n^{1.038} = 0.136p^{1.038} \quad (r^2 = 0.999) \quad (8)$$

A similar effect of PEG with molecular weights from 1500 up to  $4 \cdot 10^4$  is described by

$$\alpha = 1.097 \cdot 10^{-3} M_n^{0.978} \quad (r^2 = 0.998) \quad (9)$$

It should be noted that as with polyacrylamide, an increase in the PEG concentration results in a decrease in the relative hydration power of water in the solution. It seems from eqns. 8 and 9 that the effect of PEG on the hydration power of water is ap-

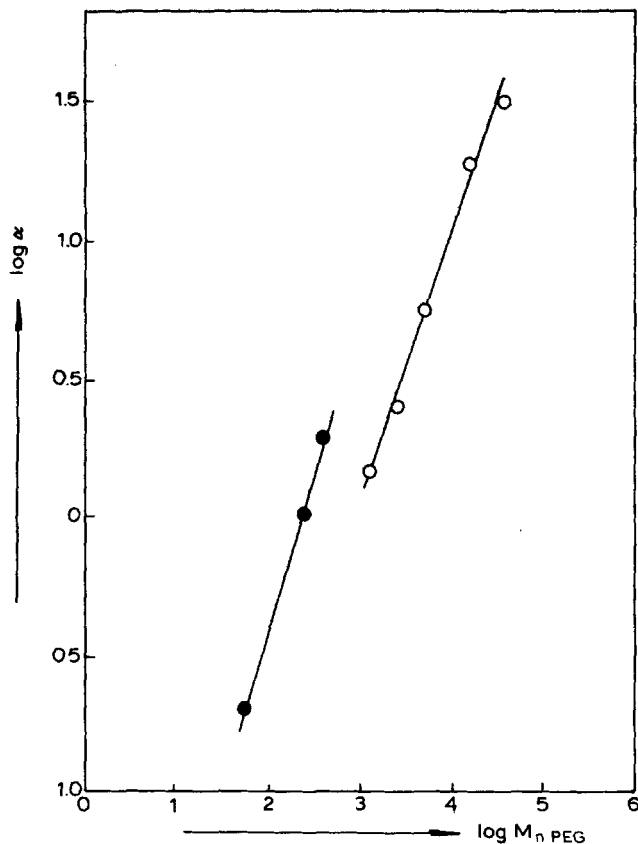


Fig. 8. Parameter  $\alpha$  as a function of the molecular weight ( $M_n$ ) of polyethylene glycol. For explanation, see text.

proximately proportional to the molecular weight and/or the degree of polymerization of the polymer.

The results obtained are clearly insufficient for any far reaching generalizations. It seems, however, that the approach used here is promising for obtaining a greater insight into various aspects of the physical chemistry of polymer solutions and the biological effects of polymers.

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