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RELATIVE HYDROPHOBICITY OF SYNTHETIC MACROMOLECULES

I. POLYETHYLENE GLYCOL, POLYACRYLAMIDE AND POLYVINYL-PYRROLIDONE

B. Yu. ZASLAVSKY*, A. V. BAEVSKII, S. V. ROGOZHIN, A. V. GEDROVICH, A. V. SHISHKOV,
A. A. GASANOV and A. A. MASIMOV

*Institute of Elemento-organic Compounds, Academy of Sciences of the U.S.S.R., Moscow 117813
(U.S.S.R.)*

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SUMMARY

Partitioning of polyethylene glycol (PEG), polyacrylamide (PAAm) and polyvinylpyrrolidone (PVP) of various molecular weights in the aqueous Ficoll-400-dextran-70 two-phase system was studied at pH 7.4 with varied ionic compositions of the system. The relative hydrophobicities of the polymers were determined. It was established that the hydrophobic character of PEG is equivalent to that of 170 ± 3 CH₂ groups, independent of the number-average molecular weight (M_n) in the range from 1500 to $40 \cdot 10^3$. The relative hydrophobicities of PAAm and PVP depend on the weight-average molecular weight (M_w) of the polymer according to the following equations:

$$\text{PAAm: } n^{\text{CH}_2} = -6.864M_w^{0.217} \quad (M_w \text{ from } 1 \cdot 10^4 \text{ to } 50 \cdot 10^4)$$

$$\text{PVP: } n^{\text{CH}_2} = 429.1M_w^{-0.190} \quad (M_w \text{ from } 5 \cdot 10^3 \text{ to } 2 \cdot 10^5)$$

Possible reasons for the difference observed between PEG, PAAm and PVP are discussed.

INTRODUCTION

The synthesis and study of macromolecular drugs and polymers that may be used as carriers for common pharmaceutical agents are under active investigation¹. From the standpoint of medicinal chemistry, it seems that the hydrophobic character of a given polymer is important as a factor known² to influence the distribution of the polymer throughout the body tissues. The relative hydrophobicity of polyethylene glycol, polyacrylamide and polyvinylpyrrolidone was studied in this work as a function of the molecular weight of the polymers by the partition technique described earlier³⁻⁵.

EXPERIMENTAL

Materials

Ficoll-400 (lot 19069) was obtained from Pharmacia (Sweden) and Dextran-70 (lot 680480) under the trade name Polyglucinum from Minmedprom (U.S.S.R.).

Polyacrylamide (PAAm) samples were obtained by the fractional precipitation with water (solvent) and methanol (non-solvent) of PAAm prepared as described in ref. 6. 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 weight-average molecular weights (M_w) of the PAAm fractions were calculated using the equation⁶

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

Polyethylene glycol (PEG) fractions with number-average molecular weights (M_n) of 1500, 6000, 20,000 and 40,000 were purchased from Loba (Austria) and were used without further purification.

Polyvinylpyrrolidone (PVP) fractions with molecular weights (M_w) of 5000, 12,000, 17,000, 50,000 and 180,000 were kindly provided by Dr. Yu. Kirsch (Institute for Technology of Blood Substitute and Hormone Preparations, Moscow, U.S.S.R.).

Tritium labelling

Tritium labelling of the polymers was carried out as described in ref. 7. A thin, freeze-dried layer of the sample cooled to 77°K was bombarded with ^3H atoms obtained by thermal dissociation of $^3\text{H}_2$ at a tungsten wire heated to 2000°K by an electric current. To obtain an appropriate radioactivity of a sample, about $1 \mu\text{Ci}$ per sample was used.

In order to remove labile ^3H , the samples were dissolved in water and dialyzed against a 50-fold excess of pure water overnight. It was checked in separate experiments that the above treatment achieved complete removal of the labile ^3H .

The samples of the polymers subjected to the above procedure were used in the partition experiments.

Two-phase systems

The aqueous polymeric two-phase systems were prepared as described earlier³ and had the following composition: 12.0% (w/w) Ficoll-400, 11.0% (w/w) Dextran-70 and amounts of sodium chloride and sodium phosphate buffer (pH 7.4) according to

$$C_{\text{buffer}} = 0.11 - 0.67C_{\text{NaCl}}$$

where C_{buffer} is the concentration of buffer, varied from 0.01 to 0.11 M , and C_{NaCl} is the concentration of sodium chloride, varied from 0 to 0.15 M .

The difference in the hydrophobic characteristics of the phases employed was determined by partitioning of DNP-amino acids with aliphatic side-chains as described previously³. It was found to be *ca.* 11.1 cal/mole of CH_2 , expressed in terms of the free energy of transfer of a CH_2 group from the Ficoll-rich phase to the dextran-rich phase of the system. The difference in the hydration properties of the

phases was determined as described earlier⁴ by the C value corresponding to the logarithm of the partition coefficient of DNP-glycine.

Partition experiments

The partition experiments were carried out as described previously³⁻⁵. The phases of the two-phase system were allowed to settle at 25°C for 21-24 h, then aliquots of both phases were pipetted from the system and used for the solute concentration measurements.

The concentrations of the polymers examined were determined by radioactivity measurements as described in detail in ref. 8.

The partition coefficient of a polymer, K , is defined as the ratio of the polymer concentration in the Ficoll-rich phase to that in the dextran-rich phase. The K values were measured for each solute over approximately ten-fold concentration ranges and were found to be independent of the solute concentration under all the conditions employed. Each partition coefficient was determined as the mean of three measurements on four dilutions from each partition experiment carried out three or four times at a given ionic composition. The deviation from the mean K value did not exceed 3% for any of the polymers examined.

RESULTS

It has been shown previously⁹ that the estimates of the hydrophobic character of solutes depend on the particular two-phase system used. As the system containing 12.5% (w/w) Ficoll-400 (lot 11069, Pharmacia) and 10.8% (w/w) Dextran-70 (lot 580870, Minmedprom) was previously chosen as a reference system⁹, the results obtained in this work were transformed to the reference system according to

$$\ln K_0 = a_i \ln K_i + b_i \quad (1)$$

where K_i is the partition coefficient of a solute in the two-phase system employed, K_0 is the partition coefficient of the solute in the reference system and a_i and b_i are constants. The a_i and b_i values were determined as described earlier¹⁰ and were found to be 1.416 and -0.139 , respectively, and to be independent of the ionic composition of the systems compared under the conditions employed.

It has been shown recently^{3,5} that the partition coefficient of a non-ionic solute can be described by

$$\ln K = n^{\text{CH}_2} E \quad (2)$$

where E characterizes the difference in the hydrophobic characters of the two phases of the system (according to the equation $\Delta G_{\text{tr}}^{\text{CH}_2} = -RTE$, where $\Delta G_{\text{tr}}^{\text{CH}_2}$ is the free energy of transfer of a CH_2 group between the phases³) and n^{CH_2} is the equivalent number of CH_2 groups which characterizes the intensity of the interactions of the solute with an aqueous environment relative to that of a CH_2 group, *i.e.*, is a measure of the relative hydrophobicity of the solute under the given conditions of the aqueous medium^{3,5,9-11}.

E is independent of the ionic composition of the Ficoll-dextran two-phase

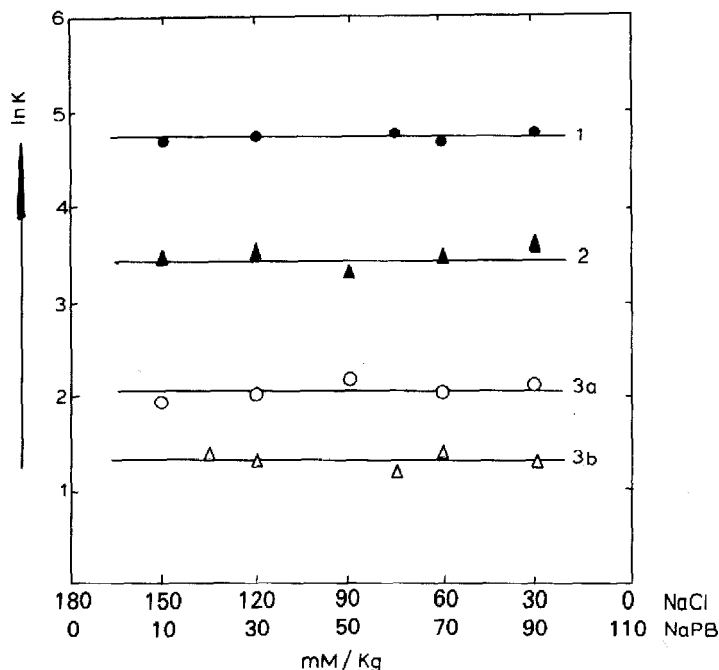


Fig. 1. Logarithm of the partition coefficient of a polymer fraction as a function of the ionic composition of the Ficoll-400-dextran-70 two-phase system. 1, PEG, $M_n = 20 \cdot 10^3$; 2, PAAm, $M_w = 4.9 \cdot 10^5$; 3a, PVP, $M_w = 12 \cdot 10^3$; 3b, PVP, $M_w = 180 \cdot 10^3$. NaPB = sodium phosphate buffer (pH 7.4).

system under the conditions used^{3,9}. Some of the typical relationships between the logarithm of the partition coefficient of the polymers examined and the ionic composition of the system are presented in Fig. 1. It can be seen that all the relationships

TABLE I

HYDROPHOBIC CHARACTER OF POLYETHYLENE GLYCOL (PEG), POLYACRYLAMIDE (PAAm) AND POLYVINYLPIRROLIDONE (PVP) OF VARIOUS MOLECULAR WEIGHTS

Parameter E in eqn. 2 is 0.027.

Polymer	Molecular weight	n^{CH_2}
PEG	$1.5 \cdot 10^3$	170 ± 2.0
	$6.0 \cdot 10^3$	168 ± 3.5
	$20.0 \cdot 10^3$	171 ± 2.4
	$40.0 \cdot 10^3$	169 ± 2.0
PAAm	$1.06 \cdot 10^4$	-51.0 ± 1.2
	$6.65 \cdot 10^4$	-76.4 ± 3.1
	$4.54 \cdot 10^5$	-106.3 ± 1.2
	$4.90 \cdot 10^5$	-126.8 ± 2.2
PVP	$5.0 \cdot 10^3$	102.5 ± 7.1
	$12.0 \cdot 10^3$	75.8 ± 2.4
	$17.0 \cdot 10^3$	71.0 ± 1.5
	$50.0 \cdot 10^3$	64.8 ± 1.6
	$180.0 \cdot 10^3$	49.3 ± 3.6

established are in agreement with eqn. 2. The n^{CH_2} values for the polymer fractions examined are given in Table I.

DISCUSSION

The results in Table I indicate that the relative hydrophobicity of PEG is independent of the molecular weight (M_n) of the sample in the range from 1500 to $40 \cdot 10^3$ and amounts up to 169.5 ± 2.6 equivalent CH_2 groups. This observed independence seems to agree with the fact that the hydrophobic substituent constants, π_x , are 0.5 for a CH_2 group and -0.98 for a $-\text{O}-$ moiety¹², resulting in *ca.* zero for the PEG monomeric unit. Hence it appears reasonable that the effect of the molecular weight on the relative hydrophobicity of PEG is negligible or absent. It should be noted that the results obtained in the study of the effect of PEG on the thermodynamic properties of aqueous solutions¹³ agree with those reported here in the sense that the effect of PEG is also independent of the molecular weight of the polymer.

The relative hydrophobicities of PAAm and PVP appear to depend on the molecular weight of the polymers, as shown in Fig. 2. The relationships established are as follows:

for PAAm with M_w from $1 \cdot 10^4$ to $5 \cdot 10^5$:

$$n^{\text{CH}_2} = -6.864M_w^{0.217} \quad (r^2 = 0.986) \quad (3)$$

and for PVP with M_w from $5 \cdot 10^3$ to $2 \cdot 10^5$:

$$n^{\text{CH}_2} = 429.1M_w^{-0.190} \quad (r^2 = 0.981) \quad (4)$$

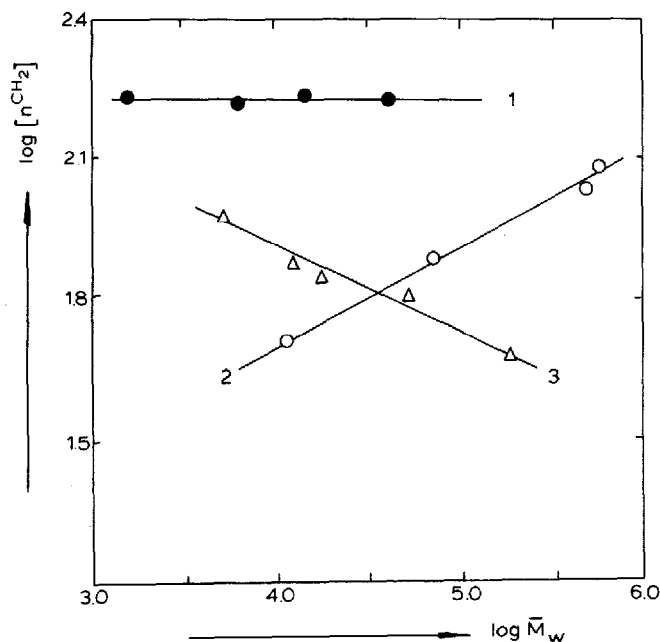


Fig. 2. Relative hydrophobicity of (1) PEG, (2) PAAm and (3) PVP as a function of the molecular weight of the polymer.

where r^2 is correlation coefficient.

It follows from eqns. 3 and 4 that PVP is a much more hydrophobic polymer than PAAm, although not as hydrophobic as PEG, in the range of molecular weights examined. It should be noted that the relative hydrophobicity of both PVP and PAAm decreases with increase in the molecular weight of the polymer. Assuming that the relative hydrophobicity of a polymer is a function of its chain length, eqns. 3 and 4 can be connected into the following equations:

for PAAm:

$$n^{\text{CH}_2} = -17.310p^{0.217} \quad (5)$$

and for PVP:

$$n^{\text{CH}_2} = 201.114p^{-0.190} \quad (6)$$

where p is the degree of polymerization.

From eqns. 5 and 6, it follows that the relative hydrophobicities of a monomeric unit of PAAm and PVP are -17.3 and 201.1 equivalent CH_2 groups, respectively. These values appear to be unexpectedly large, probably owing to the effect of the specific features of the folding of the polymers under study. It is interesting that the hydrophobic character of the polymers examined appears to be of a similar order of magnitude to that reported for proteins¹⁰. This fact is important with regard to the possibility of simulating the behaviour of biological macromolecules in an aqueous environment by that of synthetic polymers.

It should be noted also that the relative hydrophobicity of the polymers examined seems to be related to the effect of the polymers on the thermodynamic properties of aqueous solutions¹³. This relationship is under study at present.

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