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INFLUENCE OF INORGANIC ELECTROLYTES ON PARTITIONING OF NON-IONIC SOLUTES IN AN AQUEOUS DEXTRAN–POLY(ETHYLENE GLYCOL) BIPHASIC SYSTEM

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SUMMARY

Partitioning of two non-ionic solutes, 4-nitrophenyl-α-D-mannopyranoside and 4-nitrophenyl-N-acetyl-β--D-glucosaminide, in aqueous dextran–poly(ethylene glycol) (PEG) biphasic systems with a fixed polymer composition but containing various concentrations of KCl, KSCN and K_2SO_4 was studied. It was found that the salt additives alter the polymer composition of the coexisting phases depending on the type and concentration of the salt. The ratio of the thermodynamic activity coefficients of the aqueous medium in the coexisting phases that can be determined from the polymer composition of the phases is suggested as a measure of the solvation properties of the phases. It is shown that the logarithm of the partitition coefficient of a non-ionic glycoside is linearly related to the above ratio. The results obtained imply that the ionic composition of the aqueous dextran–PEG biphasic system influences the partitioning of solutes (both ionic and non-ionic) through its effect on the polymer composition of the phases and on the thermodynamic state and/or structure of water in the phases. It is concluded that the generally accepted hypothesis that the separation capacity of the system can be divided into two independent parts (one related to the solvation properties of the phases and the other to the interfacial potential) is invalid as both are dependent on the ionic compostion of the system.

INTRODUCTION

Aqueous polymer biphasic systems have been employed successfully for the separation, isolation and analysis of various biological solutes and particles[1–3]. The most commonly used systems are those formed by dextran and poly(ethylene glycol) (PEG). An important question when the systems are used for separation is how to influence the partition of the biological material. The various ways in which this can be done[1,4] are related to the polymer and the ionic composition of the two coexisting phases.

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The partition of polyelectrolytes (*e.g.*, proteins) depends on the pH and the type of salt present in the system of a given polymer composition[1,5,6]. The usual explanation[1,5,6] is that the presence of several kinds of ions that differ in their relative affinities for the two coexisting phases gives rise to an electrical potential difference between the phases, the value and sign of which govern the partitioning of ionic solutes. It is believed[1,5,6] that the separation capacity of the aqueous dextran–PEG biphasic system can be divided into two independent parts. One part depends on the difference in the net charge of the co-partitioned polyelectrolytes and is sensitive to the ionic composition of the system, *i.e.*, to the interfacial potential, and the other is determined by the solvation properties of the phases for the solutes being partitioned, *i.e.*, it depends on the polymer composition of the phases, and is independent of the ionic composition of the system. This hypothesis[1,5,6] is clearly based on the concept that the ionic composition of the system does not affect the polymer composition of the phases known[4,7] to influence the partitining of solutes in the system.

We have recently established[8] that the presence of inorganic salts can alter the polymer composition of the phases of aqueous biphasic systems formed by non-ionic polymers. In order to check the assumption that inorganic salts may influence the partitioning of solutes in the aqueous dextran–PEG phase system as a result of their effect on the polymer composition of the phases, the partition of two non-ionic glycosides in systems containing different amounts of KCl, KSCN and K_2SO_4 was studied and the results are presented here.

EXPERIMENTAL

Materials

The polymers used were dextran-70 ($\bar{M}_w$ 57.2 · 10^3; $\bar{M}_n$ 28.7 · 10^3) (Minmedprom, Moscow, U.S.S.R; Lot 680480) and PEG-6000 (M_n *ca.* 6 · 10^3) (Serva, Heidelberg, F.R.G.; Lot 419-80). 4-Nitrophenyl-α-D-mannopyranoside and 4-nitrophenyl-N-acetyl-β-D-glucosaminide were obtained from Chemapol (Prague, Czechoslovakia). Inorganic salts of analytical-reagent grade were used. Water was doubly distilled in quartz.

Methods

The aqueous dextran–PEG biphasic systems were prepared as described elsewhere[1,4,7,8]. All of the phase systems used had the same polymer composition, containing 12.8% (w/w) of dextran and 8.0% (w/w) of PEG, but differed in their salt compositions as indicated below.

The composition of the coexisting phases was determined as follows. The systems were centrifuged for 20 min at 4400 *g* to speed phase settling. The dextran concentrations in both phases were determined by polarimetry[1] with an accuracy of 0.018% (w/w). The PEG concentration in the dextran-rich phase was measured using the I_3-binding assay[9] with an accuracy of 0.028% (w/w). The concentration of a given salt in the dextran-rich phase was calculated from refractive index measurements and from the dextran and PEG contents of the phase. The PEG and salt concentrations in the PEG-rich phase were calculated from the total composition of the system and from that of the dextran-rich phase. The salt concentration was determined with an accuracy of 0.045% (w/w) and the concentration of water with an accuracy of 0.056% (w/w) The concentrations of salts in the two phases were also determined in separate experiments with an accuracy of 0.003% (w/w) from measurements of the potassium concentrations by atomic-absorption spectrometry. The relative volumes of the two coexisting phases were measured using graduated tubes. The densities of the phases were determined by pyknometry.

Partition experiments were carried.out as described elsewhere[10,11]. Aliquots of the settled phases were pipetted from a given system and each was used for measurements of the solute concentration. The absorbance of each aliquot, appropriately diluted with water, was measured at 300 nm against a correspondingly diluted top- or bottom-phase blank.

The partition coefficient, *K*, defined as the ratio of the solute concentration in the PEG-rich phase to that in the dextran-rich phase, was measured for each solute over approximately a 10-fold concentration range and was found to be independent of the solute concentration in a system of given ionic composition. The partition coefficient for each solute was determined as the mean of 2–4 measurements on 4–5 dilutions from each partition experiment carried out 3–4 times in a given biphasic system.

RESULTS AND DISCUSSION

The polymer compositions of the two coexisting phases of aqueous dextran–PEG systems with different salt compositions are shown in Table I, together with the partition coefficients for the glycosides studied. The results indicate that the logarithm of the partition coefficient of a glycoside is linearly related to the concentration of the salt present in the system. As the glycosides studied are non-ionic this linear relationship seems to have no physical meaning. It implies that there is a linear relationship between the salt concentration and the solvation properties of the coexisting phases governing the partitioning of solutes in the system.

It follows from the thermodynamic equilibrium of the coexisting phases that the chemical potentials of the solvent in both phases are identical. This condition can be written as

$$C^{D}_{H_2O}/C^{PEG}_{H_2O} = f^{PEG}_{H_2O}/f^{D}_{H_2O} = \alpha \qquad (1)$$

where C_{H_2O} is the concentration of the aqueous solvent in a given phase of the system, f_{H_2O} is the activity coefficient of the solvent in a given phase; and the superscripts D and PEG denote the dextran and the PEG-rich phase, respectively. It should be particularly noted that the solvent in each phase is the aqueous medium of a given ionic composition, *i.e.*, it is implied that the main role of the salt consists in the alteration of the solvation properties of the phase medium owing to its effect on the structure of water and on the phase polymer–water interactions[8,12–14].

According to eqn. 1, α characterizes the ratio of the activity coefficients of the aqueous solvents in the coexisting phases, *i.e.*, that of the thermodynamic states of the medium in the phases. The α values determined from the composition of the phases in accordance to eqn. 1 are listed in Table I.

The results in Table I indicate that α is linearly related to the concentration of a given salt in the system. This seems to explain the aforementioned linear relation-

TABLE I

COMPOSITION OF THE COEXISTING PHASES OF THE AQUEOUS DEXTRAN–PEG PHASE SYSTEM CONTAINING 12.8% (w/w) OF DEXTRAN, 8.0% (w/w) OF PEG AND DIFFERENT AMOUNTS OF INORGANIC SALTS, DISTRIBUTION COEFFICIENT OF PEG (K_{PEG}), RATIO OF THE THERMODYNAMIC ACTIVITY COEFFICIENTS OF THE AQUEOUS SOLVENT IN THE PHASES (α) AND LOGARITHMS OF THE PARTITION COEFFICIENT OF 4-NITROPHENYL-α-D-MANNOPYRANOSIDE (K_{man}) AND 4-NITROPHENYL-N-ACETYL-β-D-GLUCOSAMINIDE (K_{gl})

Salt	C_s^* *(mol/kg)*	*Composition of upper phase (%, w/w)*				*Composition of bottom phase (%, w/w)*				α^{**}	K_{PEG}	*Ln* K_{man}	*Ln* K_{gl}
		C_{Dex}	C_{PEG}	C_s	C_{H_2O}	C_{Dex}	C_{PEG}	C_s	C_{H_2O}				
–	–	0.44	14.95	–	84.61	25.94	0.57	–	73.49	0.869	26.23	0.144	0.276
KCl	0.10	0.44	15.11	0.48	83.97	26.04	0.13	1.02	72.81	0.874	116.23	0.160	0.304
	0.50	0.34	15.83	3.21	80.62	25.06	0.11	3.97	70.86	0.893	143.91	0.222	0.356
	1.00	0.29	16.63	5.80	77.28	23.76	0.06	8.00	68.18	0.917	277.17	0.298	0.463
KSCN	0.10	0.53	15.23	1.00	83.24	25.57	0.39	0.94	73.10	0.879	39.05	0.186	0.315
	0.50	0.37	16.14	5.07	78.42	24.10	0.24	5.58	70.08	0.906	67.25	0.358	0.469
	1.00	0.18	17.85	10.54	71.43	22.30	0.16	9.78	67.76	0.946	111.56	0.583	0.664
K_2SO_4	0.05	0.42	15.30	0.56	83.72	25.18	0.62	1.18	73.02	0.880	24.68	0.186	0.329
	0.10	0.30	15.37	1.49	82.84	24.66	0.28	1.99	73.07	0.890	54.89	0.228	0.382
	0.25	0.03	17.70	3.50	78.77	24.05	0.10	5.00	70.85	0.922	177.00	0.353	0.541

* C_s = Total amount of the salt additive in the system.

** Calculated according to eqn. 1.

ship between the logarithm of the partition coefficient of a glycoside and the concentration of an inorganic electrolyte in the biphasic system. The physical meaning of the α–ln$K$ relationship is obvious as the affinities of a solute for the phases are governed by the thermodynamic state and/or structure of water in the phases, which determines the solvation properties of the phases. As yet there is no general theory of water structure that permits the quantitative characterization of the alteration of the structure and/or thermodynamic state of water under the influence of phase polymers and inorganic ions present in the system. It should also be noted that inorganic salts are known[12,13] to interact directly with PEG and these interactions may alter the conformation of PEG macromolecule, affecting the polymer–water interactions. It should be emphasized that the relationship between the logarithm of the partition coefficient of a glycoside and the distribution coefficient of PEG, K_{PEG}, is poor, indicating that the solvation properties of the phases are governed not only by the phase polymers but also by the ionic composition of the phases.

It can be seen from the data in Table I that the partition coefficients of a given non-ionic solute differ at the same α value provided by different inorganic electrolytes. It seems that different salts induce different alterations of the water structure[14] in the phases, producing the same ratio of the solvent activity coefficients. Even so, α seems to be preferable to the length of the tie line (TLL) (length of the line connecting the top- and bottom-phase system compositions on the phase diagram) used by several workers[4,15] as a measure of the difference in composition between coexisting phases. It can be seen from Table I that TLL is almost constant for all the systems of different ionic composition used here. The partition coefficients of the glycosides, however, vary significantly.

It should be noted that by plotting the logarithm of the partition coefficient of mannopyranoside in the presence of KCl, KSCN or K_2SO_4 on the ordinate against α on the abscissa, linear graphs are obtained that have a common point of intersection corresponding to $\alpha = 0.872 \pm 0.002$. For the corresponding point of intersection for glucosaminide $\alpha = 0.860 \pm 0.020$. The physical meaning of the points is obscure at present. The results in Table I indicate that the partition coefficients of the glycosides under study are sensitive to α to different extents, depending on the type of inorganic electrolyte used. Partitioning of mannopyranoside in the presence of KSCN, which is a water-structure breaker[14], depends on the ratio of the solvent activity coefficients more than that of glucosaminide. The opposite situation is observed in the presence of K_2SO_4, which is a water-structure promoter[14].

The results obtained, even though they cannot be explained precisely, indicate unambiguously that the presence of an inorganic electrolyte in the aqueous dextran–PEG biphasic system alters the polymer composition of the phases and the thermodynamic state (and structure) of water in the phases. These alterations affect the solvation properties of the phases and influence the partitioning of solutes in the system. The above results obtained for non-ionic solutes imply that partitioning of ionic solutes, *e.g.*, polyelectrolytes, is at least partially influenced by the same factors. The results of this study disagree with the aforementioned hypothesis[1,5,6] that the separation capacity of the system can be divided into two independent parts, as both of these parts depend on the ionic composition of the system. It is concluded that the interfacial potential should not be considered as the only critical factor governing the partitioning of biological materials in aqueous biphasic systems formed by non-ionic polymers and containing inorganic electrolyte additives.

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