Structure of water as a key factor of phase separation in aqueous mixtures of two nonionic polymers

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Phase separation of non-ionic polymers in aqueous mixtures was studied as a function of temperature from 8 to 50°C. The effect of different amounts of urea additive on phase separation was also investigated. Phase diagrams determined in this work are compared with those reported earlier for the polymer systems containing various inorganic salts additives. The effects of temperature, urea and salts additives agree with the previously suggested hypothesis that phase separation is due to the incompatibility of the polymer-modified water structures. Phase diagrams were analysed using the Flory–Huggins theory and the aqueous solution thermodynamics approach. The results of the thermodynamic analysis are in agreement with the above hypothesis.

(Keywords: aqueous two-phase systems; phase separation; polymers' incompatibility; water-soluble polymers; aqueous solutions; water structure)

INTRODUCTION

Aqueous two-phase systems are used successfully for separation and analysis of various biological materials[1–3]. The fundamental principles of partitioning of solutes and particles in the systems, however, are not completely understood at present. It is obvious that the partition process depends on the properties of the coexisting phases which are related to the mechanism of phase separation in aqueous mixtures of two polymers.

Several different explanations for the occurrence of phase separation in aqueous mixtures of two polymers and in those of a single polymer and an inorganic salt are given in the literature[4–9]. Two almost antithetical views are the one advocated by Gustafsson *et al.*[4] and Brooks *et al.*[5] and the one advanced by Zaslavsky *et al.*[9].

It is believed by Gustafsson, Brooks and their coworkers[4,5] that according to the Flory–Huggins theory[10] (developed by Scott[11] and Tompa[12] for non-polar polymers dissolved in non-polar solvents) phase separation in aqueous polymer solutions is due to energetically unfavourable interactions between the segments of the two polymers and that the effect of water interactions with either polymer plays essentially no role in the process. This view[4,5] is at variance with the commonly known fact that the state and/or structure of water is altered in an aqueous solution depending on the chemical nature and concentration of the solutes present[13–15].

It was shown by us previously that the effects of different inorganic salts on the separation of phases in aqueous polymer systems[16–18] are due to the influence of the salts on the structure of water in the systems. It was also found that the physico-chemical properties of water, such as dielectric relaxation time and static dielectric constant[19], relative affinity of water for a CH_2 group[3,20], overall polarity of water as measured by the solvatochromic technique (Zaslavsky *et al.*[33]), etc. in the coexisting phases of the systems are different. On the basis of these findings it was suggested[9] that phase separation in aqueous mixtures of two polymers results from different effects of the polymers on the water structure.

In the present paper the effects of temperature and urea on phase separation of some nonionic polymers in aqueous mixtures were studied by the determination of

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phase diagrams. The phase diagrams obtained, together with those reported previously[18], were examined in terms of the Flory–Huggins theory and in terms of aqueous solution thermodynamics theory to learn which hypothetical mechanism of phase separation is in better agreement with the results of the thermodynamic analysis.

EXPERIMENTAL

The polymers used were Dextran-70 (M_w 57.2 x 10^3; M_n 28.7 x 10^3; Minmedprom, Moscow, USSR; Lot 680480), Poly(ethylene glycol) (PEG)-6000 (M_n *c.* 6 x 10^3; Serva Fine Biochemicals, Heidelberg, FRG; Lot 463-80), Polyvinylpyrrolidone (PVP) (M_w 12.7 x 10^3; Minmedprom, Moscow, USSR; Lot 135-84), and Polyvinyl alcohol (PVA) (M_w 55 x 10^3; 1.3% of acetate groups: Minchimprom, Moscow, USSR; Lot 1246-83). Urea was recrystallized from hot methanol; water was double distilled in quartz.

The binodials of the phase diagrams for the aqueous Dextran–PEG, Dextran–PVP, and Dextran–PVA systems at different temperatures and in the presence of different amounts of urea were constructed from polymer compositions of the coexisting phases of a series of systems in which the total polymer concentrations were varied. The phase systems were made by mixing appropriate weights of polymer stock solutions (and a given amount of urea solution added) and were allowed to separate at the desired temperature for 21–24 h until neither phase showed visible turbidity. The upper and lower phases were analysed for their contents of the phase polymers.

The Dextran concentrations in the phases were determined by polarimetry with a precision of 0.02% (w/w). The PEG concentrations in the bottom Dextran-rich phases of the Dextran–PEG systems were measured using the I_3-binding assay[21] with an accuracy of 0.05% (w/w). The PEG amounts in the upper PEG-rich phases were calculated from the total polymer compositions of the systems and from those of the Dextran-rich phases. The concentrations of PVP in the Dextran-rich phases of the Dextran–PVP systems were determined with a precision of 0.05% (w/w) by the optical density measurements at 270 nm with consideration for the Dextran contents of the phases. The concentrations of PVA in the bottom Dextran-rich phases of the Dextran–PVA systems were determined using the I_3-binding assay[21] with an accuracy of 0.05% (w/w). The amounts of PVP or PVA in the PVP-rich or PVA-rich phases were calculated as noted above for the PEG concentrations. The relative volumes of the coexisting phases were measured using graduated tubes. The densities of the phases were determined by pycnometry with an estimated error of ± 0.2 mg/ml. The critical points of the phase diagrams studied were determined as described elsewhere[1,2].

The thermodynamic interaction parameters according to the Flory–Huggins theory were calculated from the polymer compositions of the coexisting phases for each system examined on the base of the condition that the chemical potential of each component of the system has to be identical in both coexisting phases. The chemical potentials of the components were expressed using the Scott's approximation[11] of the Flory–Huggins theory. The interaction parameters for the polymer i–solvent and Dextran-solvent interactions, χ_{i-H_2O}, χ_{Dex-H_2O}, and the parameter for the polymer–polymer interactions, χ_{Dex-i}, were calculated from the polymer compositions of the coexisting phases, from that of the critical point of the system, and from the molecular weight of the polymers by the analysis of a set of linear equations as described in refs. 22 and 23. The partial volumes of the polymers used in the calculations are 0.611 cm^3/g for Dextran, 0.800 cm^3/g for PVP, 0.765 cm^3/g for PVA, and 0.833 cm^3/g for PEG[1,2]. An aqueous solution for a given salt or urea was considered as water with altered properties, the partial volume of which was calculated from the density of the solution.

RESULTS

Figure 1 shows the binodials of the phase diagrams for the aqueous Dextran–PEG systems at different

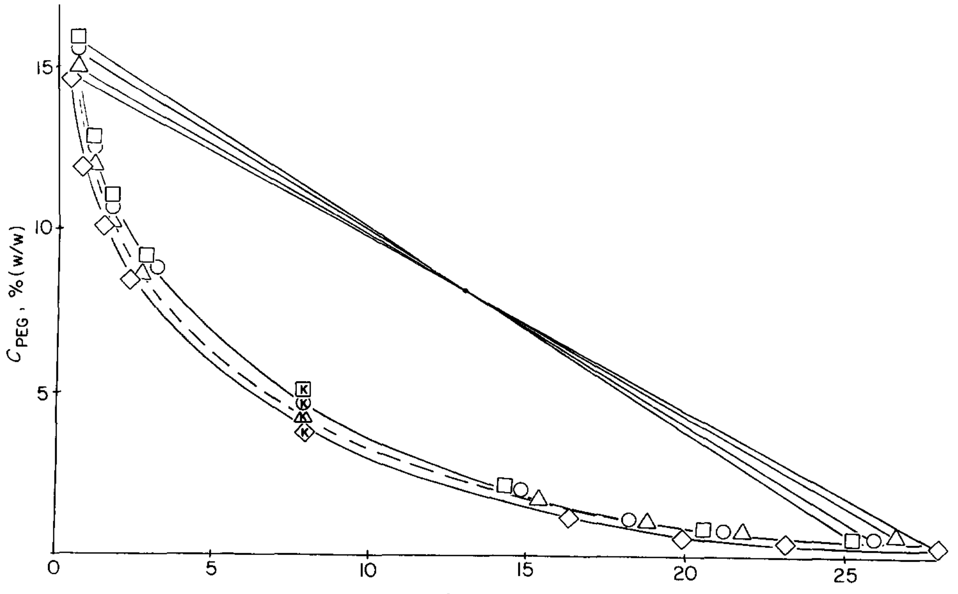

Figure 1 Phase diagrams of the aqueous Dextran–PEG biphasic system at the temperatures: 8°C (◇); 23°C (——△——); 38°C (○) and 50°C (□). The letter K denotes critical points

temperatures in the temperature range from 8°C to 50°C. The binodials for the systems at 38°C and 50°C are so close to each other than only one line was drawn. A parameter which can be used to distinguish the polymer composition of the coexisting phases at different temperatures is the slope of the tie line (STL; tie line is the line which joins the two points on the binodial corresponding to the polymer compositions of the coexisting phases)[18,24]. The STL values for the aqueous Dextran–PEG systems at different temperatures are listed in *Table 1* together with the polymer compositions corresponding to the critical points of the phase diagrams.

The binodials of the phase diagrams for aqueous Dextran–PEG systems at 23°C without urea and containing 2.0 and 4.0 mol/kg urea are shown in *Figure 2*. The binodial of the system is appreciably changed in the presence of urea at the concentrations employed. The STL values are listed in *Table 1* together with the polymer compositions of the systems corresponding to the critical points of the binodials.

Figure 3 shows the binodials of the phase diagrams for the aqueous Dextran–PVP systems at different temperatures from 8 to 50°C. The difference between the binodials at different temperatures is more pronounced than that observed in the case of the aqueous Dextran–PEG system. The urea influence on phase separation in

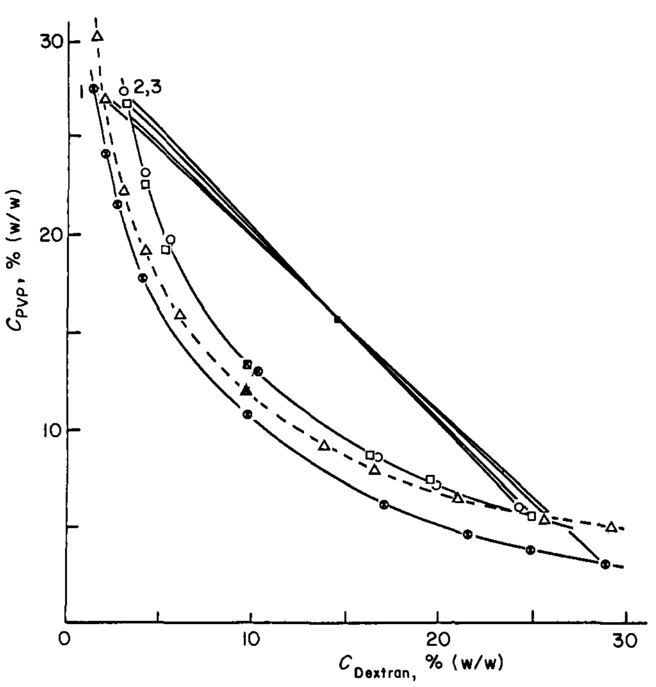

Figure 3 Phase diagrams of aqueous Dextran–PVP biphasic system at the temperatures: 8°C (⊗); 23°C (——△——); 38°C (□) and 50°C (○)

Table 1 Polymer compositions corresponding to the critical points and the slope of tie line (STL) values[a] of phase diagrams for aqueous Dextran–PEG, Dextran–PVP, and Dextran–PVA two-phase systems at different temperatures and in the presence of different amounts of urea (at 23°C)

		Dextran–PEG			Dextran–PVP			Dextran–PVA		
T (°C)	Urea mol/kg	C_{Dex} (% w/w)	C_{PEG} (% w/w)	STL	C_{Dex} (% w/w)	C_{PVP} (% w/w)	STL	C_{Dex} (% w/w)	C_{PVA} (% w/w)	STL
8.0	–	8.0	4.0	0.522	9.8	10.8	0.883	–	–	–
23.0	–	7.9	4.3	0.553	9.6	12.0	0.916	2.65	2.85	1.080
38.0	–	7.9	4.7	0.589	9.7	13.3	0.970	2.50	3.00	1.250
50.0	–	7.8	4.8	0.621	10.0	13.0	1.008	2.37	3.05	1.307
	0.50	–	–	–	9.75	13.1	0.848	2.95	2.65	1.411
	2.00	8.6	4.8	0.547	10.3	13.8	0.938	3.40	2.60	1.310
	4.00	9.0	5.6	0.577	–	–	–	–	–	–

[a] Estimated errors of the STL values given are less than 3%

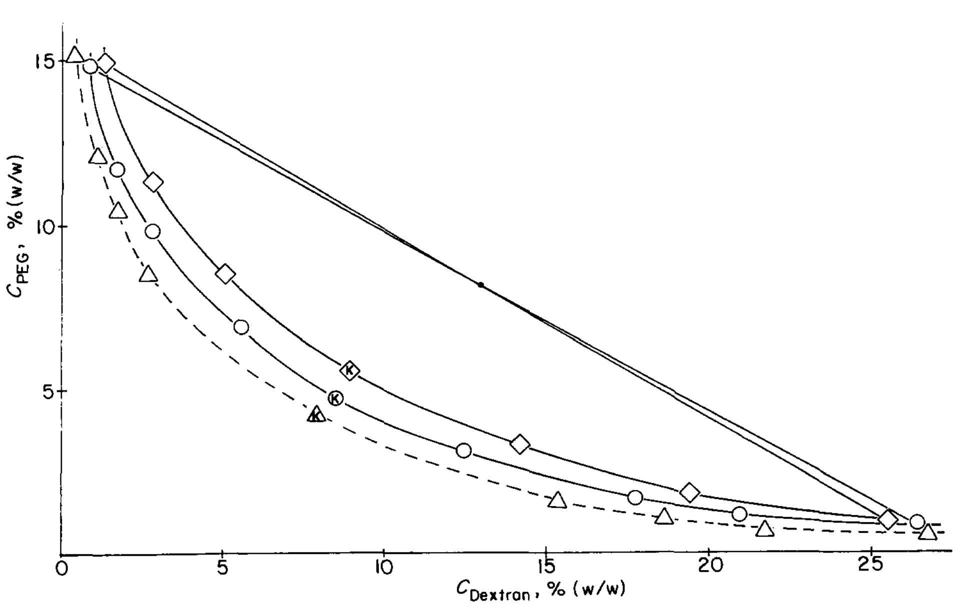

Figure 2 Phase diagrams of the aqueous Dextran–PEG systems without any additive (——△——); in the presence of 2.0 mol/kg urea (○); and in the presence of 4.0 mol/kg urea (◇)

the Dextran–PVP system is shown in *Figure 4*. The STL values and the polymer compositions corresponding to the critical points of the phase diagrams for the aqueous Dextran–PVP systems at different temperatures and in the presence of different amounts of urea are listed in *Table 1*.

Figures 5 and *6* show the binodials of the phase diagrams for the aqueous Dextran–PVA system at different temperatures and in the presence of different amounts of urea, respectively. It was impossible to study the Dextran–PVA system at the temperature below 10°C due to its gelatinization. The binodials for the system at 23, 38 and 50°C are so close to each other that only one line in *Figure 5* could be drawn. The STL values and the critical point compositions for the Dextran–PVA system are given in *Table 1*.

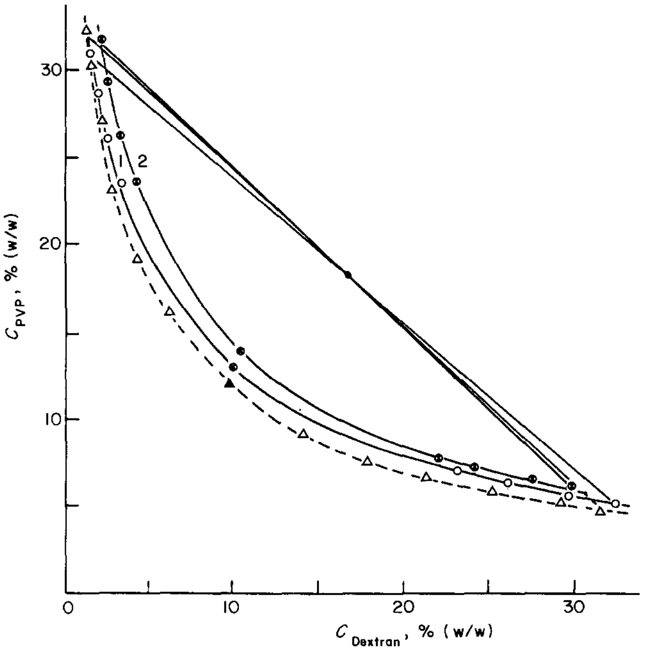

Figure 4 Phase diagrams of aqueous Dextran–PVP biphasic systems without any additive (——△——); in the presence of 0.5 mol/kg urea (○); and in the presence of 2.0 mol/kg urea (⊗)

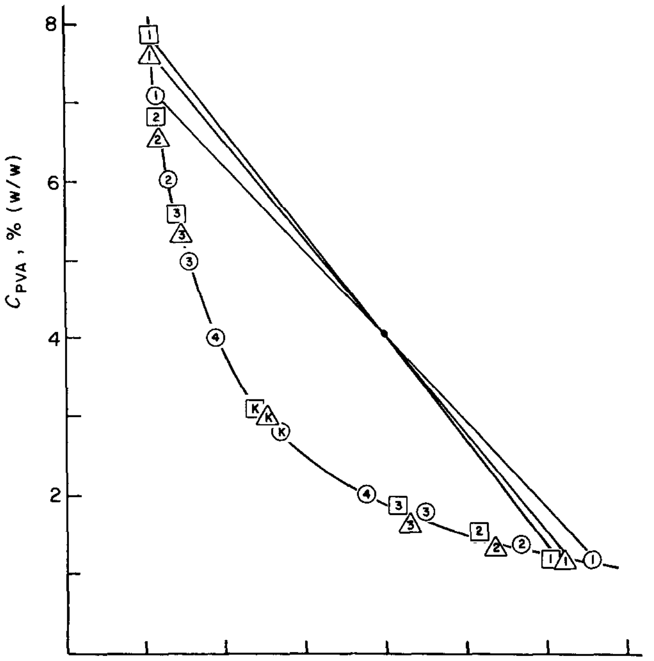

Figure 5 Phase diagrams of aqueous Dextran–PVA biphasic system at the temperatures: 23°C (○); 38°C (△); 50°C (□)

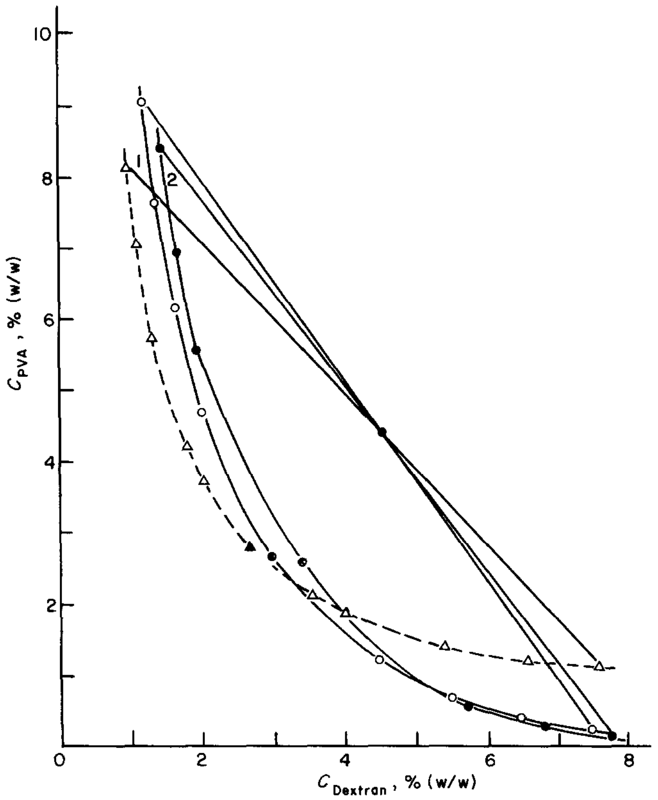

Figure 6 Phase diagrams of aqueous Dextran–PVA biphasic systems without any additive (——△——); in the presence of 0.5 mol/kg urea (○); and in the presence of 2.0 mol/kg urea (●)

DISCUSSION

The binodials of the phase diagrams for the systems examined shown in *Figures 1, 3* and *5* and the data presented in *Table 1* indicate that an increase of the temperature causes an increase in the amount required for phase separation of at least one of the polymers. It should be noted also that the ratio between the differences of the polymers' concentrations between the two phases, characterized by the STL value, increases as the temperature is increased. It follows from the results obtained that the most temperature-sensitive system is the Dextran–PVP system and the least sensitive one is the Dextran–PVA system.

The binodials shown in *Figures 2, 4* and *6* indicate that an addition of urea to the systems under study produces a shift of the binodial (or an alteration of the STL value in the case of the Dextran–PVA system) similar to that induced by the temperature increase. It should be noted also that the temperature and urea effects are similar to those observed when the water structure-breaking salts are added to the systems[16–18].

The similarity between the effects produced by essentially different factors (temperature, urea, inorganic salts), which have in common only that all these factors are known to affect the structure of water, suggests that the structure and/or state of water in an aqueous polymer system is of fundamental importance for phase separation in such a system[9,16–20]. This conclusion is clearly at variance with the aforementioned view[4,5] that water plays essentially no role in phase separation in aqueous polymer systems. The above conclusion is tested below by the results of the thermodynamic analysis of the phase diagrams for the systems studied.

Phase diagram analysis using the Flory–Huggins theory

The treatment of the phase diagrams according to the Flory–Huggins theory as indicated above was performed on the basis of two assumptions. The first one is that in contrast to the known experimental evidence the polymer–solvent interaction parameters are assumed to be independent of a given polymer concentration over the concentration range used. This assumption seems to be reasonable for PVP in water up to the PVP concentration of *c.* 30% (w/w)[25] but it may be incorrect for the other polymers under examination. The second assumption is that a four component system (Dextran–polymer i–water–salt/urea) can be treated as a pseudo three component system while it is known[18] that salt additives distribute unequally between the coexisting phases, i.e. the ionic composition of the two phases is different. It should be noted, however, that phase separation occurs in a given solvent, i.e. in water with a given total initial content of a salt or urea. It is believed[26,27] that in an aqueous polymer solution the ions are usually excluded from the water regions immediately adjacent to the nonionic polymer macromolecules and in the case of PEG it was shown by Boucher *et al.*[28,29] that in aqueous PEG solutions containing different salts the PEG–H_2O interaction parameter χ_{PEG-H_2O} is independent of the type and concentration of a salt present and amounts to 0.490 ± 0.004 (the salts examined were 0.3 M K_2CO_3, 2.4 M NaCl, 0.39 M $MgSO_4$, 0.3 M $ZnSO_4$ and K_3PO_4, 0.8 M KF, etc.). Thus, as a first approximation the above assumptions can be regarded as acceptable.

The thermodynamic interaction parameters values for the systems examined (including those studied previously[18]) are listed in *Tables 2–4*.

Table 2 Thermodynamic interaction parameters for the aqueous Dextran–PVP systems in the presence of different additives[a]

Additive (temperature °C)[b]		χ_{Dex-H_2O}	χ_{PVP-H_2O}	$\chi_{Dex-PVP}$
8	–	0.542	0.564	0.013
23	–	0.573	0.592	0.011
38	–	0.620	0.629	0.011
50	–	0.631	0.637	0.012
NH_4SCN	(0.10)	0.615	0.640	0.011
NaSCN	(0.10)	0.590	0.620	0.010
KSCN	(0.10)	0.612	0.640	0.009
$KClO_4$	(0.05)	0.562	0.581	0.010
KBr	(0.10)	0.506	0.537	0.010
KCl	(0.10)	0.613	0.628	0.011
KF	(0.10)	0.682	0.681	0.013
$(NH_4)_2SO_4$	(0.10)	0.651	0.661	0.013
Na_2SO_4	(0.10)	0.633	0.649	0.014
Cs_2SO_4	(0.10)	0.688	0.689	0.014
K_2SO_4	(0.10)	0.615	0.628	0.014
NaCl + PBS[c]		0.584	0.592	0.011
PBS[c]	(0.11)	0.638	0.637	0.015
urea	(0.50)	0.638	0.638	0.010
urea	(2.00)	0.631	0.646	0.010

[a] Estimated errors are less than 5% for all the χ-values given

[b] Temperature is 23°C unless otherwise specified; concentrations of additives are given in parentheses in mol/kg

[c] 0.15 mol/kg NaCl in 0.01 mol/kg PBS; PBS–sodium phosphate buffer, pH 7.4

Table 3 Thermodynamic interaction parameters for the aqueous Dextran–PEG systems in the presence of different additives[a]

Additive (temperature °C)[b]		χ_{Dex-H_2O}	χ_{PEG-H_2O}	$\chi_{Dex-PEG}$
8	–	0.502	0.436	0.041
23[c]	–	0.502	0.467	0.042
23	–	0.500	0.456	0.038
38	–	0.504	0.480	0.047
50	–	0.509	0.490	0.052
NH_4SCN	(0.10)	0.502	0.457	0.043
NaSCN	(0.10)	0.509	0.487	0.048
KSCN	(0.10)	0.504	0.481	0.041
KSCN	(0.50)	0.511	0.517	0.048
KSCN	(0.75)	0.552	0.583	0.056
KCl	(0.10)	0.508	0.496	0.052
KCl	(0.50)	0.507	0.497	0.048
KCl	(0.75)	0.524	0.531	0.048
$(NH_4)_2SO_4$	(0.10)	0.514	0.514	0.056
Na_2SO_4	(0.10)	0.558	0.594	0.061
Cs_2SO_4	(0.10)	0.517	0.529	0.055
K_2SO_4	(0.05)	0.511	0.500	0.047
K_2SO_4	(0.10)	0.521	0.539	0.054
K_2SO_4	(0.25)	0.531	0.565	0.050
NaCl + PBS[d]		0.515	0.520	0.061
PBS[d]	(0.11)	0.527	0.543	0.056
urea	(2.00)	0.491	0.419	0.027
urea	(4.00)	0.495	0.441	0.025

[a] Estimated errors are less than 5% for all the χ-values given

[b] Temperature is 23°C unless otherwise specified; concentrations of additives are given in parentheses in mol/kg

[c] PEG lot no. 419-80 (see refs 18 and 22)

[d] 0.15 mol/kg NaCl in 0.01 mol/kg PBS; PBS–sodium phosphate buffer, pH 7.4

Table 4 Thermodynamic interaction parameters for the aqueous Dextran–PVA systems in the presence of different additives[a]

Additive (temperature °C)[b]		χ_{Dex-H_2O}	χ_{PVA-H_2O}	$\chi_{Dex-PVA}$
23	–	0.805	0.683	0.040
38	–	0.674	0.590	0.039
50	–	0.693	0.592	0.046
KSCN	(0.50)	0.554	0.546	0.022
K_2SO_4	(0.10)	0.597	0.539	0.047
NaCl + PBS[c]		0.579	0.545	0.040
PBS[c]	(0.11)	0.596	0.548	0.042
urea	(0.50)	0.561	0.546	0.025
urea	(2.00)	0.547	0.543	0.021

[a] Estimated errors are less than 5% for all the χ-values given

[b] Temperature is 23°C unless otherwise specified; concentrations of additives are given in parentheses in mol/kg

[c] 0.15 mol/kg NaCl in 0.01 mol/kg PBS; PBS–sodium phosphate buffer, pH 7.4

The data for the aqueous Dextran–PVP systems presented in *Table 2* furnish the most illustrative example of the general trends observed from the analysis of the phase diagrams examined using the Flory–Huggins theory. The data given in *Table 2* indicate that an addition of a salt[18] or urea, or an increase in the temperature producing significant alterations of the phase diagram does not affect the parameter $\chi_{Dex-PVP}$ value which amounts to 0.012 ± 0.002 for all the systems examined. On the one hand, this agrees with the fact that there are no direct interactions between the phase polymers and the ions or urea present in the systems. On the other hand, it indicates that the observed alterations of the polymers' compatibility in aqueous solution induced by the salt or urea present or by the temperature increase are not due to any changes in the Dextran–PVP interactions (if these interactions occur at all). Hence, it can be assumed that the additives and the temperature alterations affect the interactions of the phase polymers with the solvent–water.

It can be seen from the data in *Table 2* that the interaction parameters for the polymer–solvent interactions χ_{Dex-H_2O} and χ_{PVP-H_2O} values depend on the type of additive present and on the temperature of the system. Analysis of the polymer–solvent interaction parameters values given in *Table 2* indicates that there is a relationship between these parameters described as:

$$\chi_{Dex-H_2O} = A + B\chi_{PVP-H_2O} \tag{1}$$

where $A = -0.112 \pm 0.034$; $B = 1.157 \pm 0.054$ are constants; the correlation coefficient $r^2 = 0.964$ for $N = 19$ (number of the systems examined).

Using the familiar general expressions for the polymer–solvent interaction parameter given by the Flory–Huggins theory the Dextran–water interaction parameter can be written as:

$$\chi_{Dex-H_2O} = \frac{z_{Dex}\,\Delta w_{Dex-H_2O}}{kT} = \frac{z_{Dex}}{kT}\left[1/2(w_{Dex-Dex} + w_{H_2O-H_2O}) - w_{Dex-H_2O}\right] \tag{2}$$

where z_{Dex} is the number of the Dextran repeating units–water contacts; Δw_{Dex-H_2O} is the energy change associated with creating a new Dextran repeating unit–water molecule contact; k is the Boltzmann constant; T is the absolute temperature; $w_{Dex-Dex}$ is the free energy of the Dextran–Dextran repeating units interactions; $w_{H_2O-H_2O}$ is the free energy of water–water interactions; w_{Dex-H_2O} is the free energy of the Dextran repeating unit–water interaction.

From the similar expression for the PVP–water interaction parameter it is derived:

$$(1/2)(w_{H_2O-H_2O}) = (kT/z_{PVP})(\chi_{PVP-H_2O}) + w_{PVP-H_2O} - (1/2)(w_{PVP-PVP}) \tag{3}$$

where the meaning of all the terms is the same as above but they are related to PVP.

Taking into account that the aqueous Dextran–PVP biphasic system is formed in a given aqueous medium equations (2) and (3) can be combined and we obtain:

$$\chi_{Dex-H_2O} = A + B\chi_{PVP-H_2O} \tag{1a}$$

where

$$A = \frac{z_{Dex}}{kT}\left[1/2(w_{Dex-Dex} - w_{PVP-PVP}) - (w_{Dex-H_2O} - w_{PVP-H_2O})\right] \tag{4}$$

and

$$B = z_{Dex}/z_{PVP} \tag{5}$$

The constancy of the A and B coefficients in equation (1) and the expressions (4) and (5) imply that the observed alterations of the phase diagrams for the Dextran–PVP–water system induced by the temperature change or by an addition of an inorganic salt[18] or urea occur at the constant value of $(w_{Dex-H_2O} - w_{PVP-H_2O})$ term as the $(w_{Dex-Dex} - w_{PVP-PVP})$ term value is obviously independent of the solvent.

Taking into account equation (2), it can be concluded that the observed alterations of the χ_{Dex-H_2O} and χ_{PVP-H_2O} values (see *Table 2*) are governed by the changes of the parameter $w_{H_2O-H_2O}$ value induced by the temperature alteration or by addition of urea or inorganic salt[16–18] affecting the structure and/or state of water in the system.

It should be noted that the Flory–Huggins interaction parameters determined for the aqueous Dextran–PEG and Dextran–PVA systems (see *Tables 3* and *4*) fit the above equation (1). In the case of the Dextran–PEG system the constants are: $A = 0.345 \pm 0.013$ and $B = 0.336 \pm 0.027$ ($r^2 = 0.883$, $N = 23$) and in the case of the Dextran–PVA system $A = -0.376 \pm 0.108$ and $B = 1.751 \pm 0.188$ ($r^2 = 0.925$, $N = 9$). The fact that equation (1) is valid for the Dextran–PEG and Dextran–PVA systems seems to imply that the above reasoning can be applied to these systems as well as to the Dextran–PVP system.

Thus, the empirical relationship (1) between the polymer–solvent interaction parameters found for the systems examined can be taken to imply that phase separation depends on the state and/or structure of water in the systems. This conclusion clearly agrees with the aforementioned experimental observations reported previously[16–20] and is consistent with the hypothesis[9] that phase separation in aqueous polymer systems results from different effects of the polymers on the water structure.

The standard state of a component of a system in the Flory–Huggins theory is that of the pure component. This choice of the standard state implies similar character of intermolecular interactions for a given component in its pure state and in a solvent, which is quite reasonable for the van der Waals model used in the Flory–Huggins theory. The state of a polar polymer in aqueous solution is very different from that in the pure polymer. In this case it seems more reasonable to choose the standard state for the polymer as its state in aqueous solution at the infinite dilution. It is known that the standard state choice does not affect the qualitative conclusions drawn from the experimental results obtained. Hence, the above conclusion was checked by the treatment of the phase diagrams determined using the procedure suggested previously[9].

Phase diagram analysis using the aqueous solution thermodynamics approach

When two phases are in equilibrium the chemical potential of a given component of the system is the same in both phases. For water, which is one of the system's components, it can be written:

$$f_1^{H_2O} \times C_1^{H_2O} = f_2^{H_2O} \times C_2^{H_2O} \tag{6}$$

where C^{H_2O} is the concentration of water in a given phase; f^{H_2O} is the activity coefficient for water in a given phase; indexes 1 and 2 denote the top and bottom phase, respectively.

Assuming that the activity coefficient of water in a given phase is determined by the amounts of both polymers present in the phase, by their specific effects on the local structure of water, and by an artificially discriminated factor K characterizing the structure and/or state of water in the bulk phase, we may write:

$$f^{H_2O} = C_P a_P + C_Q a_Q + K \tag{7}$$

where C_P and C_Q are the concentrations of the polymers

P and Q, respectively; a_P and a_Q characterize the effects of the polymers on the structure of water surrounding the macromolecules. It should be noted that in order to simplify the calculation procedure we assume that a_P and a_Q are constants independent of the polymers P and Q concentrations and of the presence of the second polymer Q (or polymer P, respectively). It is clear that the factor $K = 1$ in the absence of the polymers.

Combining equations (6) and (7) we obtain:

$$(C_{P_1}\beta - C_{P_2}) = (a_Q/a_P)(C_{Q_2} - C_{Q_1}\beta) + \Delta \qquad (8)$$

where

$$\beta = C_1^{H_2O}/C_2^{H_2O}$$

and

$$\Delta = (K_2 - K_1\beta)/a_P$$

All the terms but a_Q/a_P and Δ are known from the polymer composition of the coexisting phases. The a_Q/a_P and Δ values can be easily calculated from the composition of the two phases using the least-squares method. It has been shown previously[9] that the phase diagrams reported in the literature[1] can be treated successfully by this procedure. The a_Q/a_P values calculated from the phase diagrams determined in this work and those reported earlier[18] are listed in *Table 5*.

Comprehensive examination of the a_Q/a_P values listed in *Table 5* is beyond the scope of the present report. The a_i/a_{Dex} values determined in the presence of 0.15 mol/kg NaCl in 0.01 mol/kg sodium phosphate buffer (SPB), pH 7.4 should be noted, however. The relative hydrophobicities of PEG, PVA, and PVP at this ionic composition of the aqueous medium have been estimated by the partition technique previously[30,31]. The relative hydrophobicity of PEG is equivalent to that of 170 CH_2 groups[30], that of PVA amounts to 33.4 equivalent CH_2 groups[31], and that of PVP is 75.8 equivalent CH_2 groups[30]. It can be seen that there is a relationship described as

$$\begin{gathered}\ln(a_i/a_{Dex}) = -1.063 + 0.262\ln(n_i^{CH_2}) \\ r^2 = 0.999 \qquad N = 3\end{gathered} \qquad (9)$$

where a_i/a_{Dex} as defined above; $n_i^{CH_2}$ is the equivalent number of CH_2 groups which characterizes the intensity of the interactions of the polymer i with an aqueous environment relative to that of a CH_2 group[3,32].

Table 5 Ratios of thermodynamic interaction parameters for the aqueous Dextran–polymer i biphasic systems (polymer i: PEG, PVP, and PVA) in the presence of different additives[a]

Additive (temperature °C)[b]		a_{PEG}/a_{Dex}	a_{PVP}/a_{Dex}	a_{PVA}/a_{Dex}
8 –		1.39	1.04	–
23 –		1.36	1.033	0.85
38 –		1.33	1.013	0.82
50 –		1.03	0.97	0.79
NH_4SCN	(0.10)	1.37	0.999	–
NaSCN	(0.10)	1.33	1.002	–
KSCN	(0.10)	1.26	0.981	–
KSCN	(0.50)	1.27	–	0.90
KSCN	(0.75)	1.09	–	–
$KClO_4$	(0.05)	–	1.067	–
KBr	(0.10)	–	1.034	–
KCl	(0.10)	1.46	1.01	–
KCl	(0.50)	1.41	–	–
KCl	(0.75)	1.39	–	–
KF	(0.10)	–	1.02	–
$(NH_4)_2SO_4$	(0.10)	1.28	1.002	–
Na_2SO_4	(0.10)	1.25	0.99	–
Cs_2SO_4	(0.10)	1.29	1.002	–
K_2SO_4	(0.05)	1.38	–	–
K_2SO_4	(0.10)	1.38	1.013	0.55
K_2SO_4	(0.25)	1.18	–	–
NaCl + PBS[c]		1.33	1.07	0.87
PBS[c]	(0.11)	1.39	1.005	0.60
urea	(0.50)	–	1.01	0.73
urea	(2.00)	1.37	1.009	0.794
urea	(4.00)	1.44	–	–

[a] Estimated errors are less than 7% for all the values given

[b] Temperature is 23°C unless otherwise specified; concentrations of additives are given in parentheses in mol/kg

[c] 0.15 mol/kg NaCl in 0.01 mol/kg PBS; PBS–sodium phosphate buffer, pH 7.4

The relationship between the relative intensity of the polymer i–water interactions ($n_2^{CH_2}$) and the a_i/a_{Dex} ratio seems to support the above hypothesis[9] that phase separation in an aqueous mixture of two polymers is related to the effects of the polymers on the structure and/or state of water in the mixture. Thus, the analysis of the phase diagrams using the aqueous solutions thermodynamics approach leads to the conclusion identical to the one drawn above from the analysis using the Flory–Huggins theory. The results obtained appear to be in line with the aforementioned hypothesis[9] that phase separation in aqueous polymer systems is due to the incompatibility of the polymer-modified water structures.

CONCLUSIONS

Examination of the influence of temperature and urea on phase diagrams for aqueous mixtures of two non-ionic polymers indicates that these factors affect the mixtures in agreement with the previously suggested hypothesis that phase separation depends on the structure and/or state of water in a given mixture. The results of analysis of phase diagrams for the mixtures in terms of the Flory–Huggins theory and in terms of the aqueous solution thermodynamics are consistent with the hypothesis.

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