

INFLUENCE OF SOME SALTS ON BINODALINE OF BIPHASIC SYSTEMS FORMED BY NONIONIC POLYMERS

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The effect of salts KSCN, KI, KBr, KCl, KClO₄, KF, K₂SO₄, and NH₄Cl, LiCl, NaCl, KCl, CsCl on the binodials of the phase diagrams for aqueous biphasic dextran-polyvinylpyrrolidone and dextran-polyethylene glycol systems was studied. It is established that the K-salts present at the concentrations of 0.1 and 0.5 mol/kg alter the binodials of the phase diagrams for the above systems. The effect of a salt is found to be related to the lyotropy of the salt quantified by the salt molal surface tension increment. It is assumed that phase separation in an aqueous polymer biphasic system is affected by the presence of a salt mainly due to the effect of the salt on the structure and/or state of water in the system.

Keywords: Phase systems; phase separation; inorganic salts; lyotropic series; partition technique, polymers.

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INTRODUCTION

Aqueous polymer biphasic systems are used successfully for the separation of biological cells, cell organelles and macromolecules [1,2] as well as for their analytical study [3,4]. Partition behaviour of solutes and biological colloids is known to be strongly dependent on the type, molecular weight and concentration of the phase polymers and on the type concentration of salt added to the system [1,4-6].

The most commonly used phase systems contain dextran and polyethylene glycol (PEG) [1-6] or dextran and ficoll [4,7]. A great deal of experimental data collected about these systems seems to be incomplete for the understanding of general factors determining partition of solutes and particles in aqueous polymer biphasic systems. We believe that for better understanding of these factors a comparative study of physico-chemical properties of a series of aqueous biphasic systems is called for. The series which we have recently begun to study includes dextran polyvinylpyrrolidone (PVP), dextran - polyvinyl alcohol (PVA), dextran-ficoll and dextran - PEG. In order to simplify the comparison intended one of the phase polymers used in all the above systems is dextran. The purpose of the present work was to investigate the effects of different inorganic salts on the separation of phases in the above aqueous biphasic systems formed by nonionic polymers.

MATERIALS AND METHODS

Materials

The polymers used are Dextran-70 (M=57,200; M_w=28,700; Minmedprom, Moscow, USSR; Lot 680480), Polyvinylpyrrolidone (PVP) (M_w = 12,700; Minmedprom, Moscow, USSR; Lot 135-84), Polyvinyl alcohol (PVA) (M_w = 55,000; 1.3 % of acetate Groups; Minchimprom, Moscow, USSR; 1246-83), PEG (M_w = 6,000; Serva Fine Biochemicals; Heidelberg, G.F.R.; Lot 419- 80), and Ficoll-400 (M_w 400,000; Pharmacia Fine Chemicals, Uppsala, Sweden; Lot HH-26371). The inorganic salts used

KSCN, KI, KBr, KCl, KNO₃, KF, K₂SO₄, NH₄Cl, CsCl, LiCl, NaCl – were of analytical grade; water was double distilled in quartz.

Methods

Phase diagrams were determined by preparing a number of biphasic systems of various concentrations of the two polymers. The upper and lower phases were analyzed for their contents of the phase polymers. The relative volumes of the two coexisting phases were measured using graduated tubes. The densities of the phases were determined by pycnometry. All the phase diagrams were determined at 23-25°C.

Dextran-PEG system

The systems were centrifuged for 20 min at 4400 g to speed phase settling. The dextran concentrations in both phases were determined by polarimetry and those of PEG refractometrically as described elsewhere [1]. In the presence of a salt the PEG-concentration in the bottom dextran-rich phase was determined using the I₂ binding assay [8]. The salt concentration in the bottom phase was calculated from the refractive index measurements and from the dextran and PEG content of the phase. The PEG and salt concentrations in the upper PEG-rich phase were calculated from the total composition of the system and from that of the dextran-rich phase. Some points on the central part of the binodial curve were determined by turbidimetric titration [1].

Dextran-PVP system

The phases of the biphasic system were allowed to settle for 21- 24 h. The dextran concentrations in both phases were determined by polarimetry. The PVP concentrations in the phases without any salt added were calculated from the dry weight measurements [1]. In the presence of a salt the PVP concentrations in both phases were determined from the intersections of the tie line joining the point representing the total

composition of the system and the lines representing the dextran concentrations in the phases with consideration for that the ratio of the segments of the tie line must be equal to that of the weights of the phases [1]. Some points on the central part of the binodial curve were determined by turbidimetric titration.

Dextran-PVA system

The systems were centrifuged for 15 min at 4400 g and allowed to settle for 21-24 h. The dextran concentrations in both phases were determined by polarimetry. The PVA-concentration in the bottom dextran-rich phase was determined using the Iz-binding assay [8]. The PVA concentration in the upper PVA-rich phase was calculated from the dry weight measurements. The binodials of the phase diagrams for the systems in the presence of salts were determined using the technique described above for the dextran-PVP system. Some points on the central part of the binodial curve were determined using the isoionic dilution of the system of a given polymer and ionic composition and measuring the weight of a system after each step-by-step dilution. The polymer composition of the biphasic system (above binodial curve) and that of the corresponding one-phase system (below binodial curve) was determined. The results were plotted on the graph and the binodial curve was determined [1]

Dextran-Ficoll system

The phases of the biphasic system were allowed to settle for 21- 24 h. The polymers concentrations in both phases were determined by measuring optical rotation and refractive index of the phases [1] with consideration for the total composition of a given system. In the presence of salts the polymers

concentrations in the two phases were calculated from the optical rotation measurements with consideration for the relative weights of the phases as described above for the dextran-PVP system. The above method of the isoionic dilution was used in the presence of a salt added at the fixed ficoll/dextran concentrations ratio of 1.14.

The critical points of the phase diagrams studied were determined as described elsewhere [1].

RESULTS

Figure 1 shows the binodials of the phase diagrams for aqueous Dextran-PVP, Dextran-Ficoll, Dextran- PEG and Dextran-PVA systems. The binodials are different depending upon the type of the phase polymers.

Several typical binodials of the phase diagrams for the Dextrans-PVP systems with 0.10 mol/kg of a salt and without any salt added are shown in Figure 2. It can be seen that the binodial of the aqueous Dextran- PVP system is appreciably changed in the presence of a salt additive at the concentration of 0.10 mol/kg.

Figure 3 shows the binodials of the phase diagrams for the Dextran-PEG systems with 0.25 mol/kg of K_2SO_4 and without any salt. The binodials for the systems containing the other salts at the concentrations up to 0.50 mol/kg and without any salt are so close to each other that only one line can be drawn.

Figure 4 indicates several typical binodials of the phase diagrams for the Dextran-PVA systems with $KSCN$, K_2SO_4 and without any salt added. It appears that the binodial for the Dextran-PVA system is affected by the presence of a salt additive similarly to that for the Dextran-PVP system although the effects observed in the former case are less pronounced.

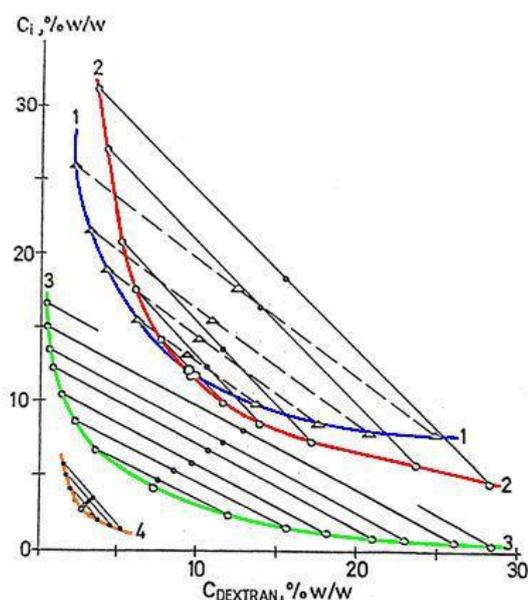


Fig.1. Phase diagrams of aqueous biphasic systems Dextran-PVP [1], Dextran-Ficoll[2], Dextran-PEG[3] and Dextran PVA[4] C_2 denotes concentration of PVP, Ficoll, PEG or PVA.

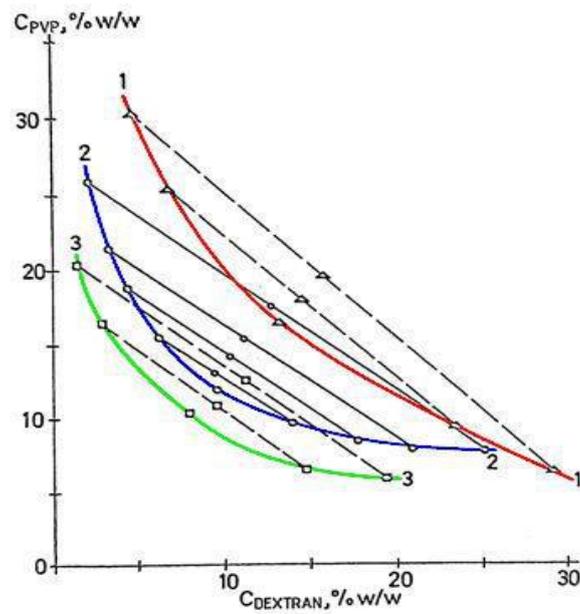


Fig.2. Phase diagrams of aqueous biphasic systems of Dextran and PVP with 0.10 mol/kg KSCN[1], with no salt added [2], and with 0.10 mol/kg K_2SO_4 [3].

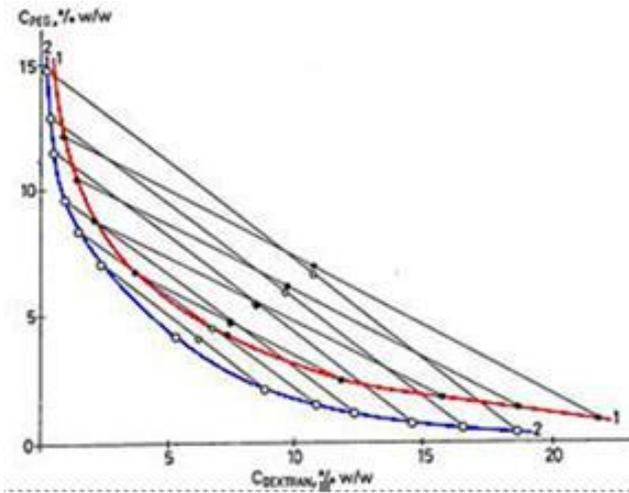


Fig.3. Phase diagrams of aqueous biphasic system of Dextran and PEG with no salt added [1] and with 0.25 mol/kg K_2SO_4 [2].

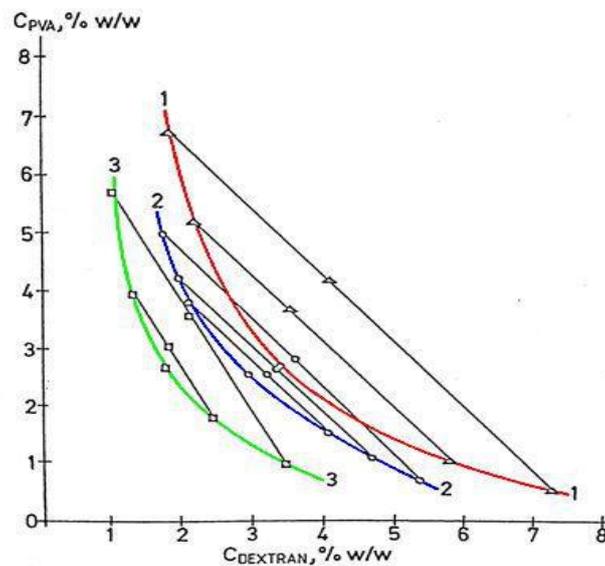


Fig.4. Phase diagrams of aqueous biphasic system of Dextran and PVA with 0.50 mol/kg KSCN [1], with no salt added [2], and with 0.10 mol/kg K_2SO_4 [3].

The effects of the salts on the binodial of the phase diagram for the Dextran-Ficoll system were studied at the fixed Ficoll/Dextran concentrations ratio of 1.14 corresponding to the critical point of the system in water and at the salts concentrations of 0.10

and 0.50 mol/kg. The results obtained are listed in Table 1. The concentrations of the phase polymers representing the critical points of the binodials of the phase diagrams for the systems studied with and without salt additives are listed in table 1.

Table 1.

Compozition of the systems corresponding to the critical points of the dextran-PVP-H₂O-salt (0.10mol/kg), dextran-PVA-H₂O salt(0.50mol/kg)systems, concentrations of dextran and Fikoll required for phase separation in the dextran-fikoll-H₂O system in the presence of 0.10 mol/kg) and 0.50mol/kg) of a salt additive and molal surface tension increments ($\Delta\sigma$) of the salts.

Salt	C _{dex}	C _{pvp}	C _{dex}	C _{pva}	C _{dex} ^a	C _{fikoll} ^a	C _{dex} ^b	C _{fikoll} ^b	$\Delta\alpha \cdot 10^3$ dyn·g/cm·mol
–	9.50	12.10	3.05	2.45	9.96	11.35	9.96	11.05	-
KSCN	14,00	15,50	3,35	2,70	11,28	12,86	12,02	13,70	0,45
KL	13.80	15.50	3.00	3.00	11.08	12.63	11.47	13.08	0.84
KBr	12.50	14.30	2.63	3.08	10.31	11.75	10.38	11.83	1.31
KNO ₃	-	-	-	-	10.20	11,62	9,96	11,35	1,67
KCl	10.00	13.40	2.68	2.25	10.18	11.61	9.94	11.33	1.88
KF	9.50	11.30	-	-	9.81	11.18	8.94	10.19	2.35
K ₂ SO ₄	8.10	10.10	1.75	2.65	9.04	10.31	-	-	2.58
CsCl	10.00	12.40	2.25	3.00	9.94	11.33	9.51	10,85	1,85
NaCl	10.80	12.60	2.40	2.55	9.96	1.35	9.96	11.35	1.75
LiCl	10.00	12.30	2.45	2.65	9.96	11.35	9.96	11.35	1.65
NH ₄ Cl	9.80	12.20	2.55	3.15	10.08	11.49	10.19	11.62	1.39

DISCUSSION

The data listed in Table 1 and the binodials of the phase diagrams determined indicate that the effect of an inorganic salt additive on the phase separation in the systems under study is related to the position of the salt in the Hofmeister's lyotropic series [9]. The lyotropy of a salt can be quantified according to Melander et al. [9] by the salt molal surface tension increment $\Delta\sigma$. The molal surface tension increments of the salts used are given in the Table.

To describe the salt-dependent shift in the binodial of a given phase diagram the concentrations of the phase polymers required for phase separation with and without a given salt additive were determined at the fixed polymersconcentrations ratio $C_i/C_{Dex}=1.14$ (C_i is the concentration of PVP, Ficoll or PVA; C_{Dex} is the concentration of Dextran). The above ratio corresponds to the critical point of the Dextran-Ficoll-water biphasic system. It was chosen for all the systems under consideration to simplify their comparison, as if the critical points for each of the systems are considered the data can be presented only as the three-dimensional plot. The polymers concentrations providing phase separation at the chosen constant polymer/Dextran ratio allow to compare the dependence of Dextran compatibility with a given polymer (PVA, PVP or Ficoll) on the nature of the polymer and to study the effect of salts on the Dextran-polymer compatibility. The difference between the C_i values corresponding to the systems with and without salt additive (ΔC_i) was used as a measure of the salt-induced shift of the binodial.

Figure 5 shows that the effect of a salt on the phase separation in the aqueous Dextran-PVP, Dextran-ficoll and Dextran-PVA systems is linearly related to the lyotropy ($\Delta\sigma$) of the salt. It should be particularly noted that a salt can induce an increase

(positive ΔC_i - values) as well as a decrease (negative ΔC_i -values) in the polymers' compatibility depending on the type of the salt. It can also be seen from the relationships plotted in figure 5 that the salt susceptibility of the systems under study decreases as follows: Dextran-PVP (0.1 mol/kg salt) > Dextran-Ficoll (0.1 mol/kg salt) > Dextran-Ficoll (0.5 mol/kg salt) > Dextran-PVA (0.5 mol/kg salt) > Dextran-PEG (0.5 mol/kg salt). It should be noted, however, that the above conclusion seems to hold for the case of the K-salts. The addition of the 1:1 chlorides studied to the above systems has little or no effect upon the binodials of the phase diagrams for the systems under consideration (see Table).

The effects of the 1:1 chlorides on the Dextran-PVA system (see inset in Fig. 5) follow the relation similar to those observed in the case of the K-salts. It should be noted also that the data given in Figure 5 for the Dextran-Ficoll system seem to indicate that the studied effect of salts is concentration-dependent. This observation and particularly that the lines representing the effects of 0.10 mol/kg salt and those of 0.50 mol/kg salt in Figure 5 are intersected is hard to explain and further experimental study seems to be called for. The effects of salts on the intrinsic viscosity, cloud point, swelling and solubility of various nonionic polymers in aqueous media have been reported in the literature [10-12]. These effects are usually in line with Hofmeister's lyotropic series and are attributed either to direct macromolecule-ion interactions (ion binding to the polar groups of a given polymer) or to the saltinduced changes in the water structure [10-12]. The observed effects of both anions and cations upon the Dextran-PVA biphasic system seems to disagree with the direct ion-polymer binding hypothesis.

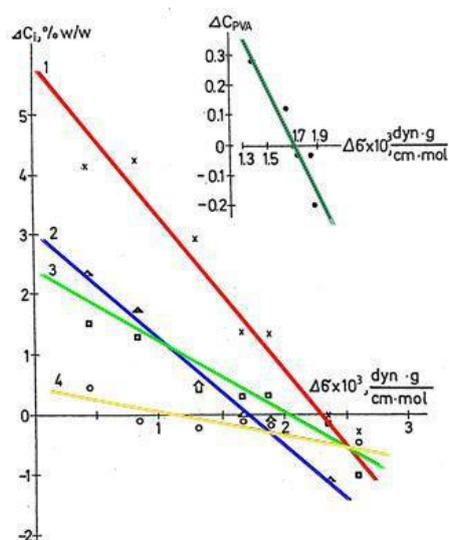


Fig. 5. The shift in the compatibility (ΔC_i) of Dextran with PVP [1], Ficoll [2,3] and PVA [4] at the concentrations ratio of polymer/Dextran=1.14 induced by the presence of 0.10 mol/kg [1,2] and 0.50 mol/kg [3,4] salt: KSCN, KI, KBr, KNO₃, KCl, KF, K₂SO₄ as a function of the salt lyotropy ($\Delta\sigma$). Insert: The shift in the compatibility of Dextran with PVA induced by the presence of 0.50 mol/kg of KCl, CsCl, NaCl, LiCl, NH₄Cl as a function of the salt lyotropy.

It should be noted also that an analysis of the Flory-Huggins χ interaction parameters for all the systems under consideration (Zaslavsky et al., in preparation) reveals that the ion-polymer binding affects the Dextran-PEG but not the other biphasic systems under study. Hence, we suggest that the effects of inorganic salts upon the phase separation in the systems under study are mainly due to the effect of the salts on the structure and/or state of water in the systems. It is known that the structure and/or thermodynamic state of water is affected by the presence of macromolecules [13-15] as well as by that of inorganic salts [9]. It is possible to assume that separation of phases in the aqueous biphasic systems formed by nonionic polymers is related to the effects of the polymers on the structure and/or state of water

in the mixture and these effects may be altered in the presence of a salt additive.

The results presented here indicate that the salts added to an aqueous polymer biphasic system should be considered as the phase forming constituents of the mixture and not just as the additives affecting partition of solutes and colloids in the system through the electrostatic interfacial potential difference [1]. It follows from the results obtained that not only ionic but the polymeric composition of the phases of a given aqueous biphasic system can be significantly altered by the presence of an inorganic salt in the system, and this possibility should be taken into account when the steering effect of salts upon the partitioning of solutes and biological particles in aqueous polymer biphasic systems are considered.

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