

THE EFFECT OF SOME INORGANIC SALTS ON THE PEG-DEXTRAN-WATER AQUEOUS TWO-PHASE SYSTEM

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Abstract. The presented work is devoted to the search, preparation, and investigation of aqueous two-phase polymer systems possessing properties suitable for specific application areas. In this study, the effects of a number of inorganic salts (NiSO_4 , CoSO_4 and MnSO_4) on the properties of the PEG-dextran-water aqueous two-phase system, and on the parameters characterizing these properties, were examined. The results of the study demonstrate that the addition of these salts causes the binodal curve of the aqueous polymer two-phase system to shift away from the origin of coordinates, the area of the homogeneous region increases, polymer incompatibility in water decreases, and phase separation does not occur. These phenomena arise due to the disruption of the structure of water by the salts in the PEG-dextran-water system. The study also investigated the effect of these salts on the surface tension coefficient of water. The observed decrease in surface tension with increasing salt concentration is consistent with the behavior of water structure-breaking salts.

Keywords: Aqueous two-phase system (ATPS), polyethylene glycol (PEG), dextran, inorganic salts, surface tension, binodal curve, critical point.

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Received: 21 September 2025; Revised: 5 November 2025; Accepted: 15 December 2025;

Published: 18 February 2026.

1 Introduction

As is well known, in order to study each constituent of biological mixtures (cells, viruses, proteins, etc.) individually, it is first necessary to separate them from one another (Nasir et al., 2020). Although numerous chemical methods exist for the separation and purification of biological particles, these methods are not suitable for the separation of biological materials. This is because the application of such methods often leads to denaturation of biological objects under the influence of various factors, resulting in the loss of their biological activity. On the other hand, biological methods are complex, labor-intensive, and not economically viable.

More recently, the Swedish scientist P.A. Albertsson developed a new, simple, economically efficient, and convenient method for the separation and purification of biological materials, which does not require large equipment (Albertson, 1970). This method is based on the unequal distribution of substances in aqueous two-phase polymer systems. The essence of the method lies in the fact that when two chemically distinct polymers are co-dissolved in water, at certain concentrations exceeding specific threshold values, the homogeneous solution separates into two immiscible liquid phases that are in equilibrium and possess different physicochemical properties due to hydrophobic and hydrophilic interactions - i.e., a transition from a homogeneous to a heterogeneous system occurs (Iqbal et al., 2016). In such equilibrium systems, one phase becomes enriched with one of the components, while the other is enriched with the second

component. In both phases, water constitutes the majority (70–90%) Kontogeorgis et al. (2022). The physicochemical properties of the phases differ, and the affinity for the phases of substances distributed in the system varies. Consequently, substances are unequally distributed between the phases. Since water is the main component of both phases, the structure of biomaterials is not damaged during the distribution process, thereby preserving their biological functions. One of the major advantages of this method is its easy scalability from laboratory settings to biotechnological and industrial processes.

It should be specifically noted that this modified method has broad application potential in biomedical and pharmacological fields. It has eliminated the primitive understanding of important concepts such as hydrophilicity, hydrophobicity, hydrophobic effect, and hydrophobic interaction, and enables the quantitative evaluation of relative hydrophobicity of substances that cannot otherwise be determined. Furthermore, this method is widely used in preliminary medical diagnostics and helps to understand the reasons for the existence of numerous energetic barriers within living organisms.

From the above, it is evident that aqueous two-phase polymer systems have a broad range of applications (Castro et al., 2020)). Therefore, the identification, study, and practical application of two-phase systems tailored to specific applications are not only important but also of theoretical significance. To describe aqueous two-phase polymer systems (Yao & Yan, 2024), a type of phase diagram different from conventional ones is used - namely, the phase diagram (Silva et al., 2020). This diagram is characterized by several parameters (binodal curve, critical point, tie line, slope of the tie line, etc.). The binodal curve represents the geometric locus of points separating the homogeneous region from the heterogeneous region. The tie line is a parameter that shows the volume ratios and compositions of the two liquid phases in equilibrium at any given point on the binodal curve. The critical point, a characteristic point on the phase diagram, corresponds to the condition where the volumes and concentrations of the phases are equal.

The optimal method for obtaining aqueous two-phase systems suitable for different applications and for controlling their properties is the addition of various small-molecule compounds (salts, alcohols, sugars, etc.) to the system. At present, the most widely used additives in the biotechnology industry are organic and inorganic salts.

2 Experiments

The present study is devoted to investigating the effect of some inorganic salts (NiSO_4 , CoSO_4 , MnSO_4) on the properties of the PEG-dextran-water aqueous two-phase system (ATPS) and on the parameters characterizing these properties.

Figure 1 shows the phase diagram and characteristic parameters of the PEG-dextran-water two-phase system without any additives. Polymer concentrations are expressed in weight percent.

Figure 2 presents the phase diagrams of the PEG-dextran-water two-phase system upon addition of the studied salts. As can be seen from the figures, the binodal curve in the phase diagram shifts away from the origin under the influence of all three salts, and the extent of the shift increases with increasing salt concentration. The area of the homogeneous region expands, indicating that polymer-polymer incompatibility decreases and their mutual compatibility improves. This result shows that all three salts (NiSO_4 , CoSO_4 and MnSO_4) disrupt the structure of water, thereby making phase separation more difficult. To restore phase separation, the concentrations of the water-structuring polymers (both PEG and dextran, which are water-structuring polymers) must be increased.

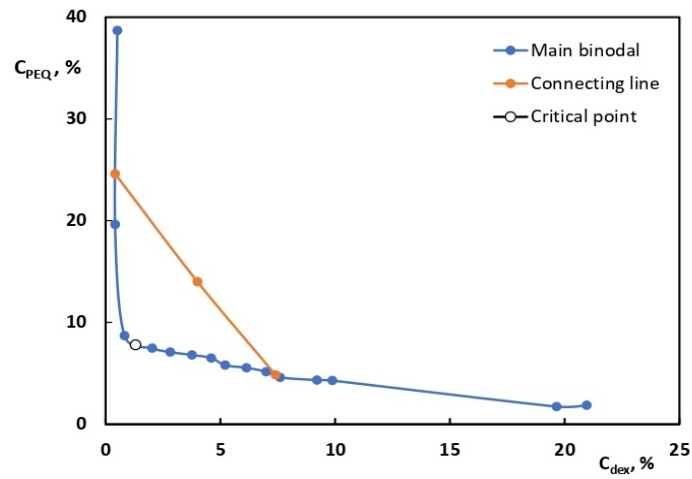


Figure 1: Phase diagram of the two-phase system PEG-dextran-water.

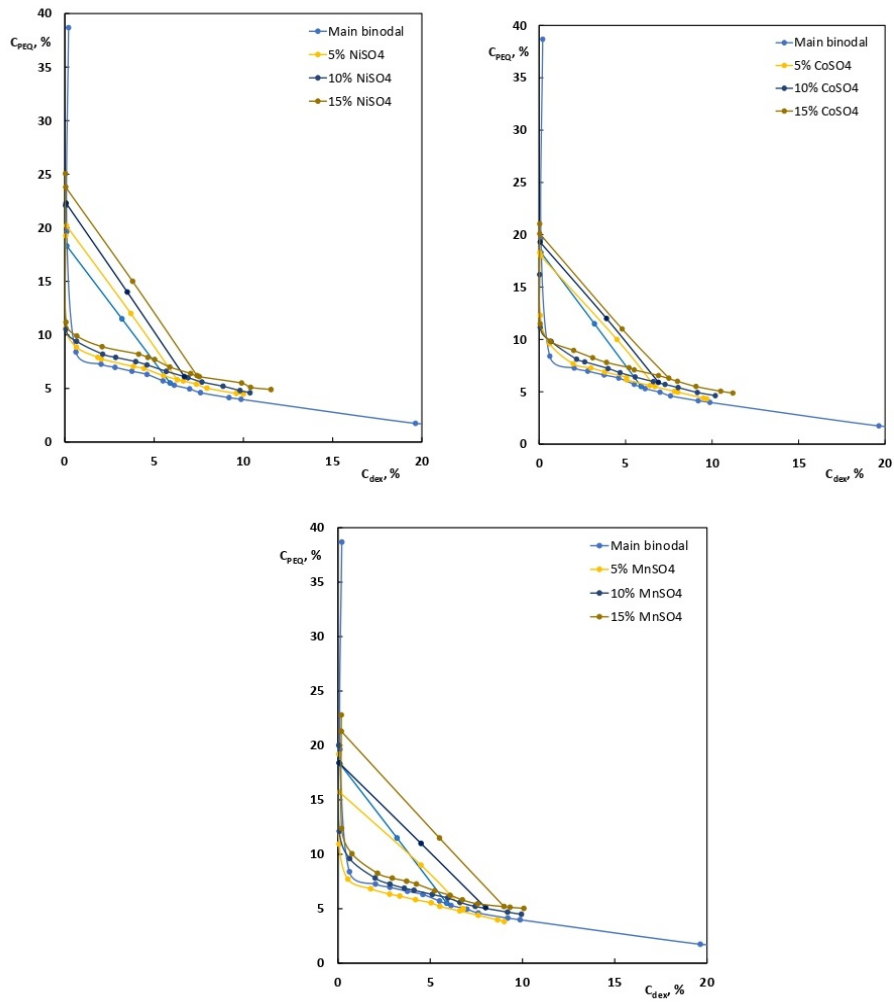


Figure 2: Effect of inorganic salts NiSO_4 , CoSO_4 and MnSO_4 on the phase diagram of the two-phase system PEG-dextran-water.

3 Results and Discussions

The effect of the salts also influences the concentrations of the phase-forming components at the critical point - a characteristic point of the phase diagram. Figure 3 illustrates the dependence of the total concentration of the phase-forming components at the critical point on the concentration of the added salts.

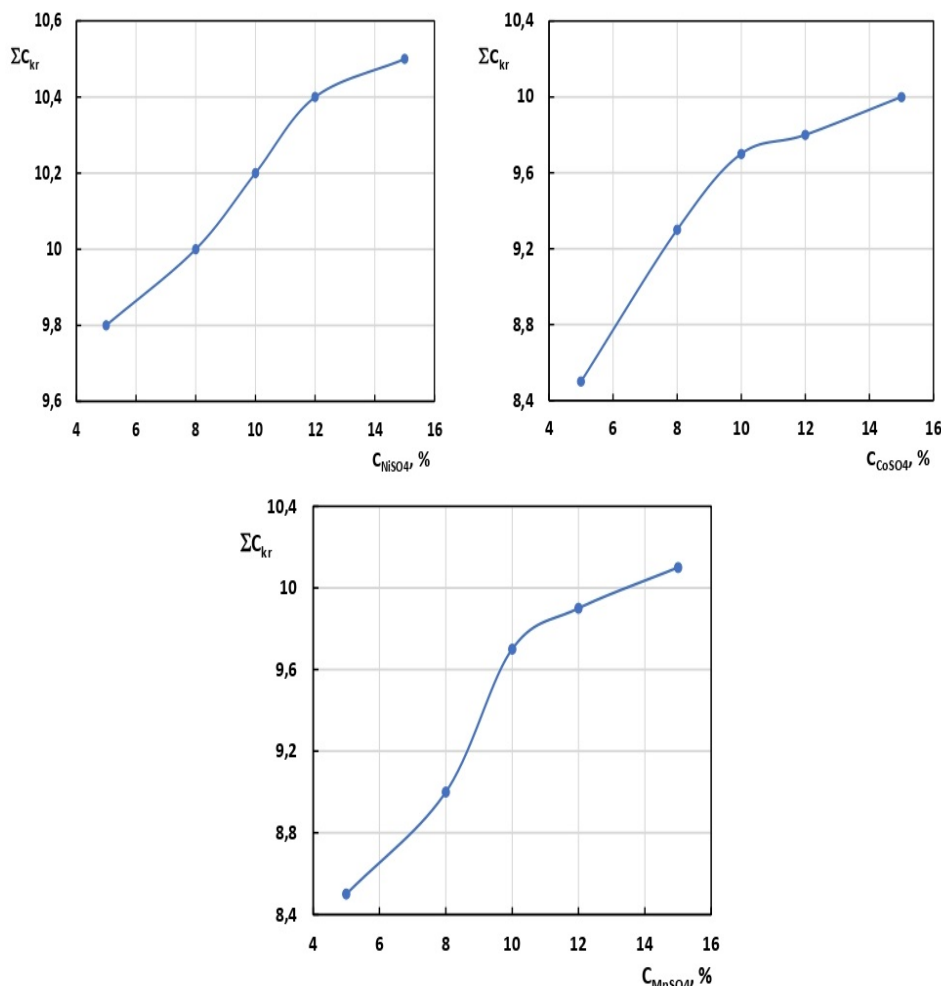


Figure 3: Dependence of the total concentration corresponding to the critical points on the concentration of salts.

The results can be explained based on O.Y. Samoilov's hydration theory (Rodnikova, 2021). It should be especially emphasized that all structural and energetic changes occurring in aqueous solutions are associated with hydration processes.

According to Samoilov's theory, due to the influence of ions dissociated from electrolytes (salts), the translational mobility of water molecules in the vicinity of the ion changes (Masimov et al., 2023). Depending on the nature of the ions, mobility may either increase or decrease. As a result, the overall structure of water changes. This translational alteration depends on the ion's polarizing ability, its radius, and the surface charge density of the ion. It is particularly important to note that since the basis of aqueous two-phase systems is water, any change in the properties of these systems caused by additives is primarily due to the interaction of the additive with water. The ability of additives to modify the structure of water affects how water interacts

with the phase-forming polymers. In turn, this influences the compatibility or incompatibility of the polymers in water, thereby affecting the phase separation process.

O.Y. Samoilov introduced the concepts of “hydrophobic” or “positive” hydration to describe the stabilizing, structuring effect of substances, including electrolytes, on water structure. Conversely, he used the terms “hydrophilic” or “negative” hydration to describe the structure-breaking effect of additives on water (Rodnikova, 2021).

When the frequency of exchange of water molecules surrounding ions $[(\nu_{water})_{ion}]$ with free water molecules (bulk water) $[(\nu_{water})_0]$ is less than the frequency of exchange of pure water molecules with each other or when their residence time in the ionic environment (τ_i) is greater than the residence time of pure water molecules next to each other (τ), the salt introduced into the system structures the water in the system. In this case, the water surrounding the ion receives activation energy for the subsequent transition. This hydration is called *hydrophobic* or *positive hydration*.

$$(\nu_{water})_{ion} \leftrightarrow (\nu_{water})_0 < (\nu_{water})_0 \leftrightarrow (\nu_{water})_0, \quad (1)$$

In another case, if the frequency of exchange of water molecules surrounding ions with free water molecules (bulk water) is greater than the frequency of exchange of pure water molecules with each other or if their residence time in the ionic environment (τ_i) is shorter than the residence time of pure water molecules (τ) ($\tau_i < \tau$), then the salt introduced into the system disrupts the structure of water in the system. In this case, the water surrounding the ion more quickly acquires the activation energy for the transition. Such hydration is called *hydrophilic* or *negative hydration*.

$$(\nu_{water})_{ion} \leftrightarrow (\nu_{water})_0 > (\nu_{water})_0 \leftrightarrow (\nu_{water})_0 \quad (2)$$

Thus, the analysis of the results in the context of Samoylov’s hydration theory shows that the condition (2) is fulfilled upon the addition of all three salts to water - that is, a structure-breaking effect on water is observed (?). It should be noted that the phase-forming polymers and the common anion (the sulfonic group) exert a structuring effect on water. At the same time, the cations form complexes with the ether oxygen of polyethylene glycol, and these complexes interact with water molecules in a way that weakens the effect of disrupting existing hydrogen bonds. This, in turn, enhances the water-structuring effect of polyethylene glycol (Masimov et al., 2013, 2019). However, the experimental results - namely, the increase in the total concentration of phase-forming polymers at the critical point depending on the salt concentration (Figure 3) - indicate that the water structure-breaking effects of all three cations outweigh the water structuring effects caused by the sulfonic group and the formed complexes (Masimov & Teymurova, 2025).

In the present work, the influence of the studied salts on the surface tension coefficient of water was also investigated. The surface tension measurement of the investigated systems were carried out on a KRUS DSA30 tensiometer with the pendant drop method. The obtained results confirm the influence of salts on the structure of water and the phase formation process in the PEG-dextran-water aqueous two-phase system. Figure 4 presents the effect of the studied salts on the surface tension of water - that is, the dependence of the surface tension coefficient of water on the salt concentration ($\sigma - C_{salt}$ dependence).

As seen in Figure 4, the surface tension coefficient of water decreases as the concentration of each of the three salts increases.

In general, the effect of different ions on the physical properties of water depends on the type of ion, the surface charge density of the ion, temperature, and other factors. The change in the surface tension coefficient of water under the influence of ions provides insight into the structural or thermodynamic state of water (Marcus, 2009).

When a strong electrolyte is added to water, it dissociates under the influence of water, forming ions surrounded by a hydration shell. These ions cannot migrate to the surface; therefore, more work is required to increase the surface area of the water by a unit length (e.g., 1 cm), and

the surface tension coefficient increases. In contrast, when a weak electrolyte is added to water, the degree of hydration is weak. This allows the ions to more easily rise to the surface, requiring less work to increase the surface area by 1 cm, and the surface tension coefficient decreases. Dipole–dipole and hydrogen bonds are weakened, water becomes somewhat freer, its mobility increases, and the structure of water is disrupted.

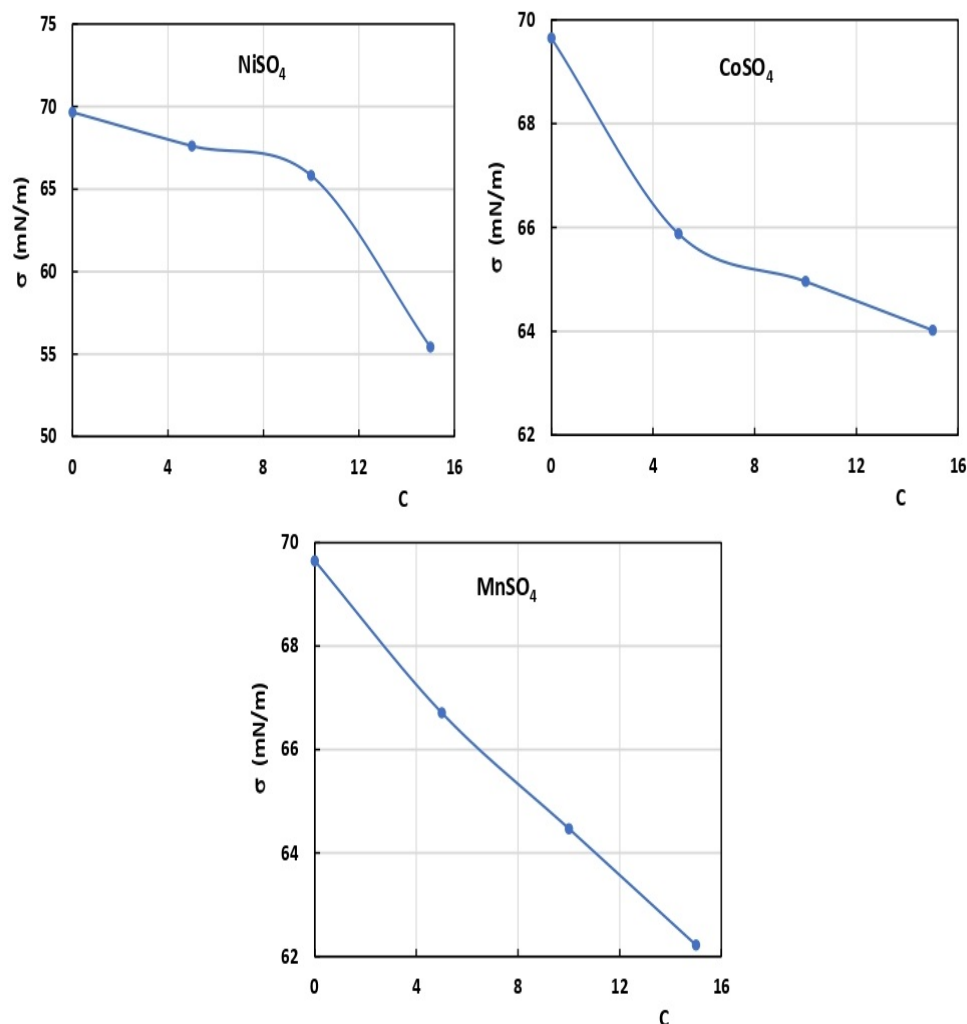


Figure 4: Variation of the surface tension coefficient of the solution depending on the salt concentration (Weight concentrations of all three salts were used).

As observed from the results (Figure 4), the surface tension coefficient of water decreases under the influence of the investigated salts. This behavior is characteristic of water structure-breaking electrolytes. For such electrolytes, due to weak dissociation, ions can more easily migrate to the surface. The ions that reach the surface act as surfactants, facilitating the mobility of water molecules: they disrupt the structure of water (Shchukin et al., 2001; Petersen & Saykally, 2006).

4 Conclusion

In this study, the effect of inorganic salts such as NiSO₄, CoSO₄ and MnSO₄ on the phase diagram and surface properties of the polyethylene glycol-dextran-water two-phase system was

comprehensively investigated. The results obtained show that the addition of NiSO_4 , CoSO_4 and MnSO_4 salts causes the binodal curve to move away from the coordinate origin, which results in a decrease in the incompatibility in water of PEG and dextran and thus an increase in their mutual solubility in water. This effect is associated with the destructive effect of the mentioned salts on the water structure, as explained by Samoilov's hydration theory. As the salt concentration increases, the total polymer concentration at the critical point also increases, indicating that the water-polymer interactions are strengthened and the phase separation becomes more difficult.

Surface tension measurements performed using the pendant drop method showed that surface tension decreases as the salt concentration increases, which corresponds to the lyotropic series. This result confirms that the studied salts act as agents that disrupt the structure of water. Overall, these findings allow for a deeper understanding of how inorganic salts influence aqueous two-phase systems at the molecular level and provide useful insights for optimizing these systems for biotechnological and biomedical applications.

Thus, the results regarding the effects of NiSO_4 , CoSO_4 and MnSO_4 on the water-polymer two-phase system, along with the observed decrease in surface tension caused by these salts, clearly demonstrate that the studied salts have a structure-breaking effect on water.

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