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THE INFLUENCE OF LOW-MOLECULAR SUBSTANCES ON THE PHASE SEPARATION CAPACITY OF A TWO-PHASE PEG – SODIUM CITRATE – WATER SYSTEM

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Abstract. As is known, two-phase polymer-polymer-water and polymer-electrolyte-water systems (SPIS) are widely used in the separation and purification of biological objects in biotechnology and pharmacology, as well as in the quality control of medicinal products. Depending on the nature of these processes, it is important to purposefully change the separation capacity of SPIS. The aim of the presented research is to ensure that it is adapted to each application object by using different additives to separate and purify biological objects. The presented study investigated the phase diagrams of the water-polymer two-phase system PEG - sodium citrate - water in the presence of certain additives. The influence of additives on the separating ability of the two-phase system PEG - sodium citrate - water was determined. Analysis of the provided data shows that the variation in the parameters of the phase diagram and the different values of the separating ability n* of the two-phase system depending on the nature of the additives is associated with changes in the structure of water under the influence of these factors. This leads to changes in the interaction of the phase-forming components of the two-phase system with water, resulting in differences in the physicochemical properties, particularly the relative hydrophobicities of the two-phase system.

1. Introduction

The method of uneven distribution of substances in water-two-phase systems is widely used for the separation and purification of biological particles, fractionation of high-molecular-weight compounds and in various biotechnological and biochemical research (Can *et al*., 2018; Hosseini & Pirdashti, 2021; Masimov *et al*., 2020; Zaslavsky *et al*., 1988; Zaslavsky *et al*., 1989; Shahbazova & Masimov, 2022). According to modern scientific understanding, the uneven distribution of substances in two-phase systems (TPS) is related to differences in physicochemical properties, particularly the relative hydrophobicities of the coexisting phases in two-phase systems (Zaslavsky *et al*., 1988; Zaslavsky *et al*., 1989; Shahbazova & Masimov, 2022). These differences, due to the absence of direct interaction of the distributed compounds with the phase-forming components of the two-phase system, are mainly mediated by their interactions with

water, which have different structures (Silverio *et al*., 2012; Johansson *et al*., 2011; Masimov *et al*., 2021; Porto *et al*., 2008; Masimov *et al*., 2019b).

As is known, water is the basis of all living systems, determines the biologically active conformation of high-molecular-weight compounds in the organism, influences enzymatic reactions, provides substance transport in the body, etc. (Pashayev, 2019a; Grilo *et al*., 2016; Iqbal *et al*., 2016; Masimov *et al*., 2019c; Pashayev, 2019b; Zhang *et al*., 2020; Masimov *et al*., 2019a). As mentioned above, the separation and purification of biological objects are of great importance in medicine, pharmacology and biotechnology (Čížová *et al*., 2017; Almeida *et al*., 2014; Iqbal *et al*., 2016; Diuzheva *et al*., 2018; Tang *et al*., 2014; Ruiz-Ruiz *et al*., 2012). Therefore, the search for and research of two-phase systems with optimal characteristics (phase diagrams, separating ability, chemical nature and molecular weight of phase-forming polymers, selection of additives, etc.) are of great interest (Grilo *et al*., 2016; Iqbal *et al*., 2016; Masimov *et al*., 2019c).

2.   Methods and materials (Experimental)

To obtain two-phase systems, polyethylene glycol (PEG) with a molecular weight of M=6000 (produced by the Spanish company "Panreac") and sodium citrate were used. Additionally, a series of salts (Na_2SO_4, K_2SO_4, Na_2CO_3, KCl, KBr, KJ) and α-amino acids were used as additives. Solutions were prepared using bidistilled water. Analytical scales produced by the "KERN 770" company, allowing for samples with an accuracy of 0.0001g, were used for preparing solutions. A spectrophotometer was used to determine the concentration of the distributed substance. To determine the separating ability of the two-phase PEG-sodium citrate-water system, the distribution of α-amino acids in this system was studied. The obtained results were processed using the method of non-linear squares.

3.   Results and discussion

To characterize the difference in the ability of phase media to participate in the mentioned interactions, it was proposed (Zaslavsky *et al*., 1988; Zaslavsky *et al*., 1989) to study the dependencies of the distribution coefficient K=CI/CII (CI-concentration of the distributed compound in the I phase, CII-in the second phase) of a homologous series of substances - markers, differing in the length of the aliphatic radical from the number of carbon atoms in the aliphatic chain. Experimentally obtained results obtained when studying dextran-phicol-water systems and dextran-PEG-water are described by a general comparison (Zaslavsky *et al*., 1988): (1) nC - the number of carbon atoms in the aliphatic chain of the marker substance molecule; E-characterizes the contribution of the methylene group to the total value of lnK; the parameter characterizes a similar contribution of the polar group. Similarly, it can be said that universal equations (1) were obtained for the distribution of phenylated (DNF) amino acids with unbranched aliphatic series of sodium salts of dinitrophenylated (DNF) amino acids with unbranched aliphatic chains in the aqueous two-phase system PEG-sodium citrate-water (Figure 1).
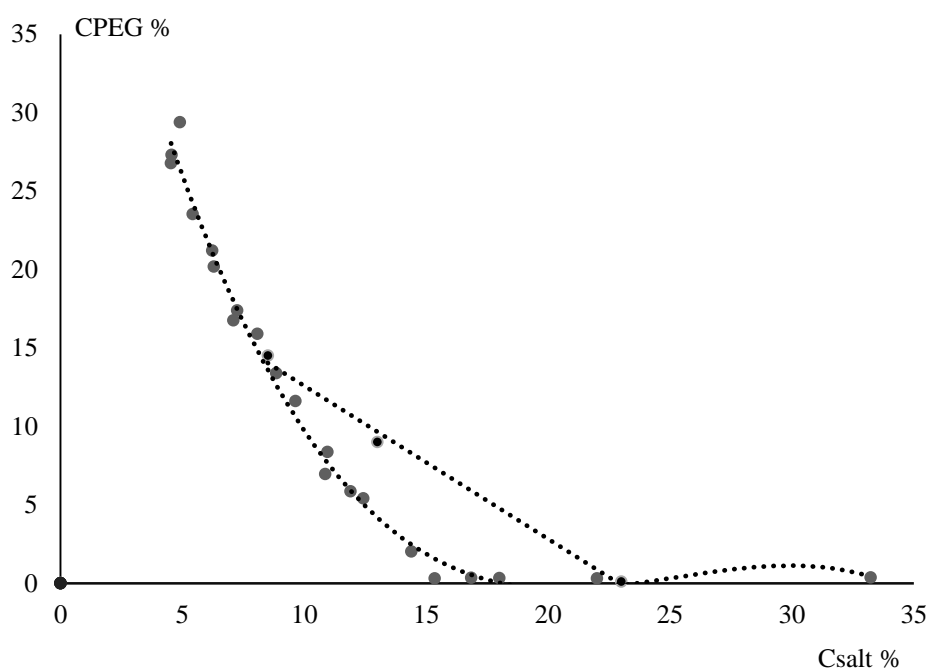
Figure 1. Binodal curve and tie lines of the two-phase system PEG/sodium citrate-H_2O

For a clear understanding of the meaning of parameters E and C in Equation (1), it should be noted that considering the smallness of the interfacial energy of the substance in a two-phase system (ΔG) relative to the energy of the substance molecules and the equality of the chemical potentials of each component in the phases of the system, for the distribution coefficient K, we can write: (2) from which we obtain (3) As follows from (3), the logarithm of the distribution coefficient of the substance in a two-phase system characterizes the free energy of interfacial substance transfer, in other words, the difference in the affinities of the substance to the aqueous environments of the system phases. Multiplying both sides of Equation (1) by RT and taking into account equation (3), we obtain (4). As follows from (4), the parameter E characterizes the difference in the degree of affinity of the aqueous environment of coexisting phases to the nonpolar CH2 group and in terms of transfer energy is described by the relationship: (5) Where - changes in the free energy of the system when transferring one CH2 group from one phase of the system to another. A similar equation can be written for parameter C (6) where $\Delta Gpol$ changes in the energy of the system when the polar group of the marker substance moves between phases. As follows from the above, parameters E and C characterize the ability of the aqueous environment of coexisting phases to participate in ionic (hydrophilic) and hydrophobic hydration processes. It is known from the literature (Zaslavsky *et al*., 1988) that as the composition of the system moves away from the critical point of distribution of a given substance in the system, it becomes more uneven and the parameters E and C depend on the composition of the system. An analysis of the magnitudes showed that there is a mutual relationship between these parameters, which is described by the relationship: (7) n* - coefficient of proportionality. An analysis of the obtained data showed that n* in equation (7) can be represented as a hypothetical number of methylene groups in the molecule of DNF amino acids, at which the substances in the phases are evenly distributed, regardless of the composition of the environment, since in

this case the conditions are met, lnK=0, K=1, CI=CII. In this case, there is a complete mutual compensation of the contributions to the distribution coefficient of the effects of ionic and hydrophobic hydration, etc. parameters E and C (Figure 2).
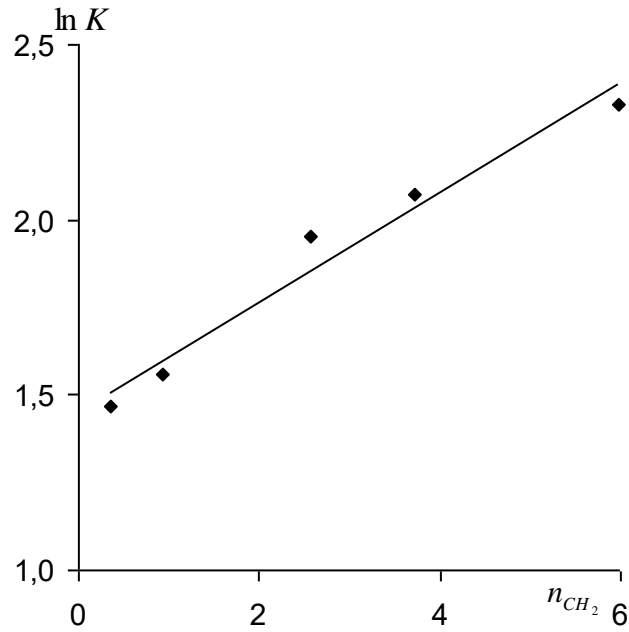
Figure 2. Dependency of the logarithm of the distribution coefficient of amino acids on the number of methylene groups in the side chain

Thus, the parameter n* can be considered as a characteristic of the aqueous two-phase system in terms of its separating ability, since an increase in n* indicates a greater difference in the structures of the aqueous phase media. It should be noted that the difference in the properties of the phases is described by parameters E and C, each of which takes into account a certain interaction.
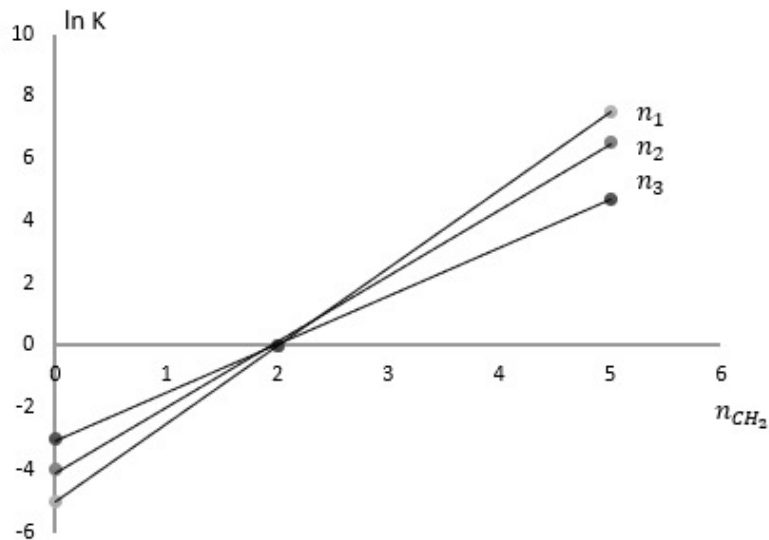
Figure 3. Dependency of the logarithm of the distribution coefficient of amino acids on the number of methylene groups in the side chain at various concentrations of components

However, since these parameters directly depend on the composition of the phase-forming components, it was not possible to compare systems formed by different pairs of components, as the dependencies of E and C on the composition for each system are specific (Figure 3). Thus, the introduction of the parameter n* removes this difficulty, since the stated does not depend on the composition of the phase-forming components of the two-phase system. Table 1 shows the obtained data for the parameter n* for the two-phase system PEG-sodium citrate-water and the influence of various additives on the values of this parameter.

Table 1. Separating capacity (n*) of the PEG-sodium citrate-water system under the influence of a series of salts, system distribution coefficient (K), logarithm of the distribution coefficient (lnK) depending on the number of methylene groups in the side chain of amino acid DNFA-α

n_{CH_2}	K	lnK	n*
without additives			
0.36	4.33366	1.466412	
0.94	4.74373	1.556824	
2.57	7.061611	1.954673	9.26
3.73	7.930884	2.070764	
5.98	10.25999	2.328252	
Na$_2$SO$_4$ (c=2.36 mol/L)			
0.36	7.942671	2.072250	
0.94	9.234088	2.222902	
2.57	12.79482	2.549041	14.69
3.73	16.18119	2.783850	
5.98	17.29530	2.850435	
K$_2$SO$_4$ (c=0.47 mol/L)			
0.36	5.355821	1.678184	
0.94	4.683842	1.544119	
2.57	5.190102	1.646753	14.35
3.73	6.484121	1.869356	
5.98	9.014178	2.198799	
Na$_2$CO$_3$ (c=3.76 mol/L)			
0.36	9.177056	2.216706	
0.94	10.78093	2.377779	
2.57	15.28370	2.726787	13.5
3.73	19.74630	2.982966	
5.98	22.75177	3.124643	
KCl (c=5.5 mol/L)			
0.36	4.200508	1.435206	
0.94	4.700985	1.547772	
2.57	7.306859	1.988814	7.19
3.73	10.02149	2.304732	
5.98	11.97123	2.482506	
KBr (c=3.53 mol/L)			
0.36	4.741111	1.556272	
0.94	3.945686	1.372623	
2.57	8.07225	2.088432	6.98
3.73	7.591427	2.02702	
5.98	13.11005	2.573379	
KJ (c=1.04 mol/L)			
0.36	4.310870	1.461140	10.61
0.94	3.426667	1.231588	

2.57	4.596301	1.525252	
3.73	6.135901	1.814157	
5.98	7.248731	1.980826	
NaNO$_3$ (c=4.67 mol/L)			
0.36	2.527881	0.927381	
0.94	2.535843	0.930526	
2.57	3.353309	1.209948	12.67
3.73	3.629305	1.289041	
5.98	3.625652	1.288034	

As can be seen from the table, the separating ability of the PEG-C$_6$H$_5$O$_7$Na$_3$ system is n*=9.2. When salts (Na$_2$SO$_4$, K$_2$SO$_4$, Na$_2$CO$_3$) are added to this system, the separating ability of the system increases, reaching n*=14.6, 13.5, 14.35. Therefore, adding these salts to the two-phase system leads to an increase in the difference in the affinities of the phase-forming components to water environments and as a result, to an increase in the separating ability of the system.

4. Conclusions

It can be considered that such two-phase systems with high separating ability are more suitable for "rough" separation of biological substances. The obtained data can be used for separation and purification of various biological materials in the future and the mechanisms of the influence of additives on the magnitude will be discussed.

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