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An approach to determine the second virial coefficient for calculation of phase equilibria in water-protein-neutral polymer systems

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Summary

A method to determine the apparent coordinates of critical points and effective values of the second virial coefficients in water-protein-neutral polymer systems enabling one to obtain the above values from data on the phase analysis within a restricted region of two-phase compositions has been proposed. The data obtained by this method for the coordinates of the critical points and binodals in water-bovine serum albumin-dextran (W-BSA-D) and water-bovine serum albumin-polyethylene glycol (W-BSA-PEG) systems are compared with experimentally determinated. Several peculiarities of the mechanism of the phase separation in above systems have been outlined in the course of this study.

Introduction

The practical demand of food technology, biochemistry and medicine in highly purified proteins, polysaccharides and nucleic acids stimulates the investigations in the area of fractionation and isolation of above compounds. One of most effective methods of biopolymer fractionation, proteins in particular, is a drop-wise precipitation. It may involve the use of neutral high molecular weight water-soluble polymers (NP) as a precipitating or concentrating agents [1-5]. As it was shown elsewhere [6-8], in presence of NP the protein solubility in aqueous medium is usually an exponential function of the polymer concentration. As a rule this function plotted in logarithmic coordinates has a general character and is well known for various systems exhibiting phase separation. In case of systems with low molecular weight components their solubility in phases is controlled by Sechenov's law [9], whereas for systems composed of water, protein and low molecular weight component the protein solubility obeyes Cohn's law [10]. Vainerman and coworkers [11] showed that similar dependencies are also characteristic of system containing water, neutral polymer 1 and neutral polymer 2 (W-NP1-NP2).

From physico-chemical point of view the polymer's precipitation or - in more general case - concentration of one polymer in presence of another one can be considered as a consequence of their limited thermodynamic compatibility. The mechanism of phase separation in W-NP1-NP2 and W-P-NP systems is highly dependent on excluded volume effect [8]. Accounting for this feature Edmond and Ogston [12] developed a formal thermodynamic approach to describe above systems. Ability of W-NP1-NP2 system's discription in terms of second virial coefficients (SVC) independently determined (not from the data on a phase equilibrium) was shown [12,13]. At the same time applicability of such approach to systems of W-P-NP-type remained unclear. A tendency of protein macromolecules towards self-association growing with increasing concentration of neutral polymer in the system [14] might result in a considerable discrepancy between the SVC values determined separately in diluted solutions and those determined in concentrated systems under condition of phase separation.

In present study we propose a method of determining the apparent coordinates of the critical point and effective SVC values in W-P-NP systems using the data on the phase equilibrium obtained within a restricted two-phase composition range. The capacity of the method proposed is then validated in determination of coordinates of critical points and values in systems: I. W-BSA-D and II. W-BSA-PEG. The binodals of the systems computed using the obtained values of SVC are then compared with experimentally determined ones. We also examined several peculiarities of the mechanism of phase separation in above systems.

Experimental

Materials

We used the sedimentationally homogeneous BSA produced by Olaine Chemical Plant (USSR) with the content of dimers not exceeding 5% w/w, dextran 500.000 "Fluka", Switzerland (lot No 194046), and polyethylene glycol 20.000 "Loba", Austria (lot No 16578) without additional purification.

Preparation of initial solutions and systems I and II

The initial binary solutions of BSA and NP were obtained dissolving preparations in Na-acetate buffer (pH 5.0; I=0.2). The adjustment of concentrations was made diluting the initial solutions (25-30% w/w) with buffer. The three-component systems I and II were prepared by mixing various mass proportions of binary solutions of BSA (5-10% w/w) and NP (25-30% w/w). The obtained mixtures (3.0-3.5 g) were mixed for 20 min at a magnetic stirrer avoiding foaming and then transferred into the centrifuge test-tubes with subsequent incubation (from several hours to several days) in hermetically sealed test-tubes. Incubation stage having been completed, the systems were centrifuged at 20,000 g for 15 min (T-24, "MLW", GDR). After that both the protein phase (P-phase) and the neutral polymer phase (NP-phase) were subjected to further analysis.

Assessment of concentrations of BSA and NP in initial solutions and phase composition of systems I and II.

The BSA concentration in initial solutions and its content in phases of ternary W-P-NP systems was determined spectrophotometrically at 280 nm ("Specord UV VIS", GDR) whereas D and PEG concentrations were assessed refractometrically ("IRF-22", USSR) using calibration curves previously plotted for either compound. The concentration of BSA determined in NP-phases of systems I and II were corrected for absorption of NP at 280 nm. The refractometric data on NP concentration was also corrected on the effect of BSA. While determining the BSA concentration in P-phases of systems I and II the effect of NP at 280 nm was neglected since the NP content in P-phases was negligible. The concentration of NP in P-phases of systems I and II was estimated according to Edmond and Ogston [12] using the phase diagram technique. The position of binodals in systems I and II in the vicinity of critical point was assessed more precisely using the cloud point approach [17], while the position of rectilinear diameter was evaluated by the phase-volume-ratio method [18]. The experimental coordinates of critical point for each system were estimated using the intersection point of binodal with rectilinear diameter [17].

All the experiments in the studies of phase equilibria in systems I and II were carried out at temperatures 22±1 °C.

Results and discussion

According to Edmond and Ogston [12] the equivalence of chemical potentials of components in equilibrium phases of system W-NP1-NP2 implies that

$$\ln m'_1 = \ln m''_1 + a_{11}(m''_1 - m'_1) + a_{12}(m''_2 - m'_2) \qquad (1')$$

$$\ln m'_2 = \ln m''_2 + a_{22}(m''_2 - m'_2) + a_{12}(m''_1 - m'_1) \qquad (1'')$$

where m'_1, m''_1 and m'_2, m''_2 are molalities of polymers 1 and 2 in equilibrium phases (the phases are designated by superscripts ′ and ″); a_{11}, a_{22} and a_{12} are the second virial coefficients of polymers 1 and 2, and the cross virial coefficient characterizing the interaction of polymers 1 and 2. It can be concluded from (1′) and (1″) that decreasing the concentration of polymers 1 and/or 2 in the system should result in tendency towards zero of self-conjugated values of $(m''_1 - m'_1)$ and $(m''_2 - m'_2)$. For the critical point we may write $\ln m'_1 = \ln m''_1 = \ln m_1^{crit.}$ and $\ln m'_2 = \ln m''_2 = \ln m_2^{crit.}$. The interdependence of concentrations of polymers 1 and 2 in equilibrium phases enables us to express $\ln m'_1$ as a function of $(m''_j - m'_j)$ where i=1 or 2 and j=1 or 2. In general case the functions $\ln m_i = f(m''_j - m'_j)$ are non-linear [12] but as experimental data given in [7,8] and theoretical examinations of Friedman [19] indicate these functions are practically linear at small m_1 and m_2. Consequently, for liquid-liquid phase separation region with good approximation (1′) and (1″) may be written as follows

$$\ln m_1' = A_1(m_2'' - m_2') + B_1 \qquad (2')$$

$$\ln m_2' = A_2(m_2'' - m_2') + B_2 \qquad (2'')$$

where A_1, A_2 and B_1, B_2 are constants.
The intercepts (2′) and (2″) define the apparent values of logarithms of critical concentrations for components 1 and 2, i.e. $B_1 = \ln m_1^{crit.app.}$ and $B_2 = \ln m_2^{crit.app.}$

With increasing concentrations of P and/or NP the chemical potential of protein in system W-P-NP may attain the value compatible to that of the chemical potential of protein in solid residue. Assuming that the protein residue induced by neutral polymer with high molecular mass has a constant composition [7,8,20], we may write the relationship (1′) as follows

$$\ln m_1' = \ln m_{1s}'' + a_{11}(m_{1s}'' - m_1') + a_{12}(m_2'' - m_2') \qquad (3)$$

where $m_{1s}'' = const$ is the concentration of the protein in hydrated residue. As expression (3) suggests neglecting $a_{11}m_1'$ the tangent of the inclination angle of function $\ln m_1' = f(m_2'' - m_2')$ should be equal to an effective value of the cross virial coefficient ($a_{12}^{eff.}$) for the fixed composition of protein residue.

Thereby, the calculation of the coordinates of critical points and SVC values in system W-P-NP may be realized according to the following algorithm. Plotting the function $\ln m_1' = f(m_2'' - m_2')$ over the interval of NP concentrations sufficiently distant from critical point involving both the region of liquid-liquid phase decomposition and region of solid-liquid phase separation we can determine the values of $a_{12}^{eff.}$ and $m_1^{crit.app.}$ from (2′) and (3), whereas the function $\ln m_2' = f(m_1'' - m_1')$ can be used to estimate the value of $m_2^{crit.app.}$. Using the obtained coordinates of the critical point and value of cross SVC we can calculate the effective values of SVC for protein and NP (a_{11} and a_{22}) and compute the binodal of the system W-P-NP [12].

The method proposed was used to calculate the binodals of I and II systems. As Fig. 1 shows, the functions $\ln m_1' = f(m_2'' - m_2')$ and $\ln m_2' = f(m_1'' - m_1')$ are linear for the systems I and II in both the area of liquid-liquid and solid-liquid phase separation.

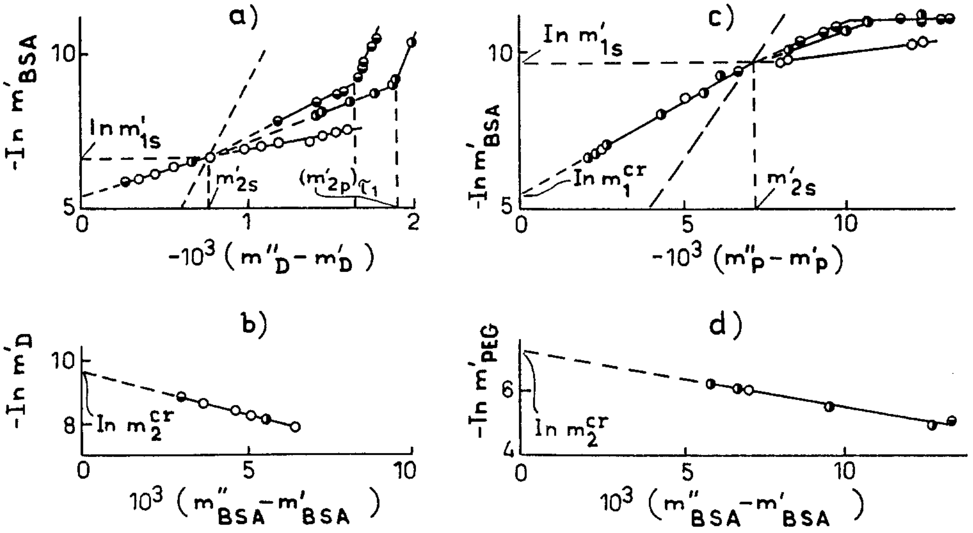

Fig. 1. Functions $\ln m'_1 = f(m''_2 - m'_2)$ and $\ln m'_2 = f(m''_1 - m'_1)$ obtained for the following incubation times: 4 (o), 24 (o) and 72 (o) hours. (While transferring to the molal concentration scale M_{BSA} assumed to be equal to 66,5 kD [16], whereas $\bar{M}_{n,D}$ and $\bar{M}_{n,PEG}$ were taken as 180 kD [15] and 17 kD [manufacturer's data], respectively).

This approach enabled us to evaluate intercepts of above functions extrapolating data lying sufficiently far from the critical point in liquid-phase region. The apparent critical concentrations of the components calculated in this way were close to those measured experimentally (i.e. determined from the intersection of binodal curve and rectilinear diameter) for both systems (Table 1).

Table 1. Coordinates of experimental and apparent critical points ($C^{cr.ex.}$ and $C^{cr.ap.}$) and precipitation thresholds ($C^{p.t.}$) in W-BSA-D and W-BSA-PEG systems (%,w/w).

System	$C^{cr.ex.}_{BSA}$	$C^{cr.ex.}_{NP}$	$C^{cr.ap.}_{BSA}$	$C^{cr.ap.}_{NP}$	$C^{t.p.}_{BSA}$	$C^{t.p.}_{NP}$
W-BSA-D	21.4	0.8	21.1	0.8	52.0	11.9
W-BSA-PEG	19.4	0.9	21.1	0.9	51.9	10.5

The evolution of systems I and II from liquid-liquid to solid-liquid region of phase separation occurs at certain m'_{2s} value corresponding to a precipitation threshold of BSA by a given polymer-precipitator. When $m'_2 >. m''_{2s}$ the whole solid-liquid region is divided in two linear parts in points $(m'_{2p})_{\tau 1}$, $(m'_{2p})_{\tau 2}, \ldots$ for any incubation time (τ_i).

System 1. When $m_2' > m_{2p}'$ the tangent of inclination angle function $\ln m_1' = f(m_2'' - m_2')$ is practically independent of τ_i, whereas when $m_{2s} < m_2 < m_{2p}$ the function exhibits an increasing tangent value caused by the rise in τ_i magnitude. Taking into account the constant value of above tangent for $m_2' > m_{2p}'$ the equilibrium position of function $\ln m_1' = f(m_2'' - m_2')$ for system I was determined by a parallel transposition functions obtained for different τ_i into the point with coordinates: m_{2s}', $\ln m_{1s}'$ (shown as a dashed line in Fig. 1a). The tangent of inclination angle of the stright line obtained was assumed to be equal the effective value of BSA-D cross SVC and the value of intercept - to a logarithm of the protein activity in saturated solution in absence of D [7].

System II. In contrast to system 1 above a certain value of m_2' the concentration of BSA in NP-phase of system II is practically constant (about 0.07 % w/w). This result is in agreement with data published by Hasko et. al. [3] for BSA precipitation caused by PEG, which presumably suggests that applicability of relationship (3) may be limited from below on BSA concentration scale. The latter may introduce an uncertainty in direct determination of the effective value of BSA-PEG cross SVC from precipitation data. However, assuming that the values of BSA activity in saturated solution determined for this protein using various neutral polymeric precipitators should coincide, the equilibrium position of linear function $\ln m_1' = f(m_2'' - m_2')$ for $m_2' > m_{2s}'$ was obtained graphically. Plotting a straight line going through the intercept point determined for system I and the point corresponding to BSA precipitation threshold in system II - m_{2s}' and $\ln m_{1s}'$ (shown as a dashed line in Fig. 1c) we assessed the value of tangent of inclination angle of this line, the latter (according to Eq. (3)) being assumed to coincide with the effective value of BSA-PEG cross SVC. It is interesting to note that threshold concentrations of D and PEG in both systems differ, whereas equilibrium concentrations of BSA in P-phases determined from the phase diagrams of the systems practically coincide (Table 1).

Using the effective values of cross SVC for systems I and II as well as both the apparent and experimentally determined coordinates of critical points we calculated the effective values of SVC for BSA, D and PEG (Table 2). The values of cross SVC for systems I and II obtained in this study are close to the values of mutual exclusion volumes derived within the approximation of solid spheres [8,12]. Similar comparison drawn for calculated and experimental SVC values of BSA shows that the apparent magnitudes of SVC are considerably lower then the calculated ones. This may indicate at a certain tendency of BSA to self-association. On the contrary, the apparent values of SVC of both D and PEG are substantially superior to the theoretical ones, which may suggest a trend of individual macromoleculs of NP to repulsion [21].

Table 2. The values of the second virial coefficients determined for W-BSA-D and W-BSA-PEG systems using the data on precipitation and coordinates of critical points (kg/mole)

System	a_{12}^{*}	a_{11}^{**}	a_{22}^{**}	a_{11}^{***}	a_{22}^{***}
W-BSA-D	9500	310	147000	330	140000
W-BSA-PEG	1600	230	3400	230	3700

* - are derived from data on protein precipitation by neutral polymer;

** - are derived from data on $m_{BSA}^{crit.exp.}$ and $m_{NP}^{crit.exp.}$;

*** - are derived from data on $m_{BSA}^{crit.app.}$ and $m_{NP}^{crit.app.}$.

In order to evaluate the reliability of the method proposed we made use of the literature data on SVC values determined for BSA, D an their mixtures published by King et. al. [13]. The above values were obtained by the light-scattering technique. They were a_{11} = 2590; a_{22} = 6970 and a_{12} = 4510 kg/mole, respectively. We used these data to calculate the binodal according to Edmond and Ogston [12]. The obtained binodal was drastically inconsistent with our experimental results. This means probably that the data on the SVC values obtained by light-scattering method is incompatible with their apparent magnitudes in concentrated systems W-BSA-D. At the same time, the binodals calculated for systems I and II using the data on SVC obtained by the method proposed show a reasonable consistency with experimental results (Fig.2).

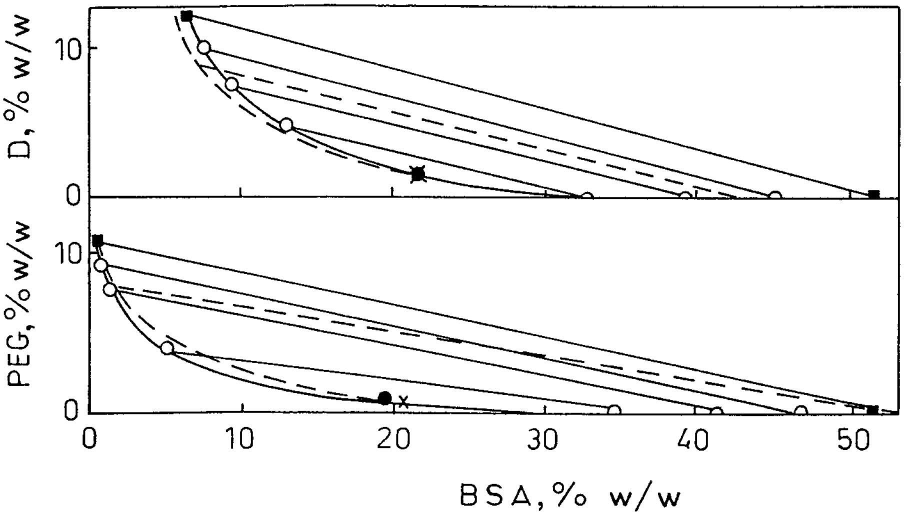

Fig.2. Phase diagrams plotted for systems W-BSA-D and w-BSA-PEG (the experimentally obtained binodal is shown as a solid line, dashed line indicates the calculated binodal, o - binodal points, ● - experimental critical points , x - apparent critical points, ■ - precipitation threshould points).

Thereby, the proposed approach is applicable for semiquantitative estimation of phase equilibria in W-P-NP systems.

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