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Application of Phase-Volume-Ratio Method for Determining the Phase Diagram of Water-Casein-Soybean Globulins System

V. I. Polyakov, V. Ya. Grinberg and V. B. Tolstoguzov

Institute of Organo-Element Compounds, USSR Academy of Sciences,
Vavilov St. 28, Moscow V-312, USSR

Summary

The possibility of using the phase-volume method for determining phase diagrams of polymer mixtures in a common solvent has been ascertained. The above method was used to determine a phase diagram of water-casein-soybean globulins system.

Introduction

It was established earlier that proteins of different classes distinguished by Osborn can reveal limited thermodynamic compatibility (LTC) in aqueous media[1].The quantitative description of protein LTC calls for determining phase diagrams. However, for water-protein A-protein B systems it entails certain difficulties The similarity of protein chemical composition hinders using the method of chemical analysis of conjugated phases. The similarity of physico-chemical characteristics (specific optical rotation, extinction coefficient, etc.) also hinders their using for quantitative analysis of conjugated phases. The method of cloud-point curves can't be used because of the high viscosity of the concentrated protein solutions. The colorimetrical method of quantitative analysis of the conjugated phases used by Polyakov et al.[1] is rather labour-consuming.

In this connection, for determining phase diagrams of water-protein A-protein B systems, of great interest is the elucidation of the possibility of using the phase-volume - ratio method developed by Koningsveld et al.[2,3] for exact determination of the coordinates of the critical points of quasi-binary solvent-polymer systems.

The purpose of this study is to ascertain the possibility of using the phase-volume -ratio method for determining phase diagrams of polymer mixtures in a common solvent.

Experimental

Materials: casein according to Hammersten (ash content-2.3%, lipides-1.35%,carbohydrates-0.97%, N-13.5%, "Olaine", USSR); soybean globulins (ash content-3.9%, lipides-1.79%,carbohydrates-5.81 %, N-14.5%, isolated according to Robbins et al.[4].

The ternary water-casein-soybean globulins systems with different compositions were prepared by mixing binary solutions of each protein preliminarily purified from insoluble impurities by

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centrifuging. After mixing for 2-3 hours the phase state of ternary systems was estimated using the NU-2 optical microscope ("Carl Zeiss", Jena). When heterogeneity was detected the systems were centrifuged in the K-70 centrifuge ("MLW", DDR) at 3300 g and 25°C. The centrifuging lasted 6-10 hours, that was sufficient for complete separation of phases.The volumes of conjugated phases were determined using a horizontal microscope ("LOMO", USSR) with an accuracy of ± 0.03 cm^3.

Results and Discussion

If the value $r^+ = V^+/(V^+ + V^-)$ is used for the characteristic of conjugated phase-volumes (V^+ and V^-) ratio, then undoubtedly $r^+ \longrightarrow 0$ or $r^+ \longrightarrow 1$ in the vicinity of binodal, and $r^+ = 0.5$ in the points of phase diagram lying on the rectilinear diameter. On the basis of these obvious principles, Koningsveld-Staverman's phase-volume - ratio method can be used for determining phase diagrams of solvent-polymer A-polymer B systems. Then the polymer A and polymer B solutions are prepared with weight concentrations C_A^o and C_B^o. These solutions are mixed at different ratios. The mixture compositions are expressed through values of apparent weight fractions $y = C_A/C_A^o$ and $1 - y = C_B/C_B^o$, where C_A and C_B are the concentrations of A and B components in mixture. The geometric place of figurative points corresponding to mixtures with different y is the secant $C_A^oC_B^o$ on phase diagram plane (Fig. 1 a).If the straight line $C_A^oC_B^o$ intersects the miscibility gap then the mixtures with some y values break up into two phases. In each case the volumes of the conjugated phases are measured and value of r^+ is determined. The relation $r^+ = f(y)$ is plotted. Some typical $r^+(y)$ curves are presented in Figure 1 b. The curve 1 corresponds to the case when the points of intersection of the secant $C_A^oC_B^o$ with binodal lie on one side from the rectilinear diameter, the curve II - the secant intersects the binodal in the critical point, the curve III - the points of intersection lie on different sides from the rectilinear diameter. The extrapolation of $r^+ = f(y)$ to $r^+ = 0$ and $r^+ = 1$ is used to determine the position of binodal's points on the secant $C_A^oC_B^o$. The value of y at $r^+ = 0.5$ accounts for the position of the point of the rectilinear diameter on this straight line. If the above procedure is repeated for other values of C_A^o and C_B^o a set of points of the binodal and the rectilinear diameter can be obtained. The critical point of the system can be determined according to the intersection of the binodal and the rectilinear diameter. Since any point of the rectilinear diameter belongs to the middle of the tie line, its position and, thus, the compositions of the conjugated phases can be found by the trial-and-error method.

For checking the above method, Albertson's data[5] on phase equilibrium in water-methylcellulose-dextran system were analyzed. In Figure 2 the binodal of this system obtained by the analysis of conjugated phase compositions is presented by the line 4, and the data on the phase-volume - ratio method by points 1,3. It is seen that the results of both methods practically coincide. It served as the reason for using the phase-volume - ratio method for determining the phase diagram of water-casein-soybean globulins system

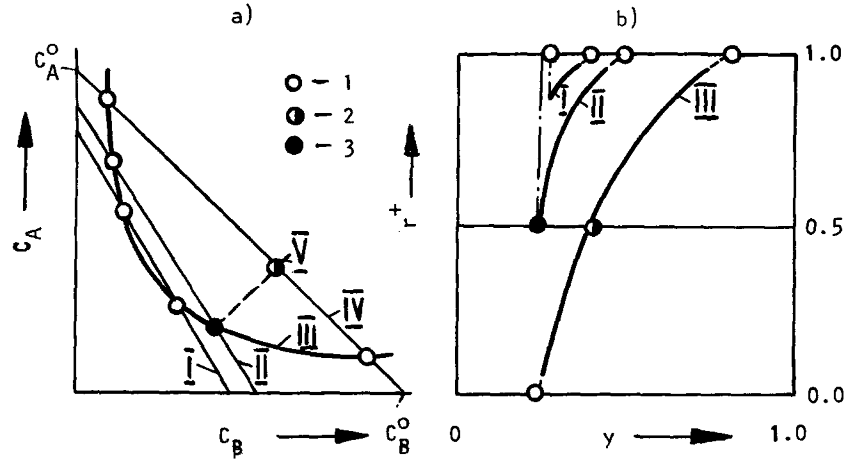

Figure 1. Schematic illustration of the phase-volume-ratio method: a) fragment of the phase diagram (I, II, III - secants with different values of C_A^0 and C_B^0, IV - binodal, V - rectilinear diameter); b) some typical dependencies of phase-volume ratio on mixture composition at different values of C_A^0 and C_B^0 (1 - points of the binodal, 2 - point of the rectilinear diameter, 3 - critical point)

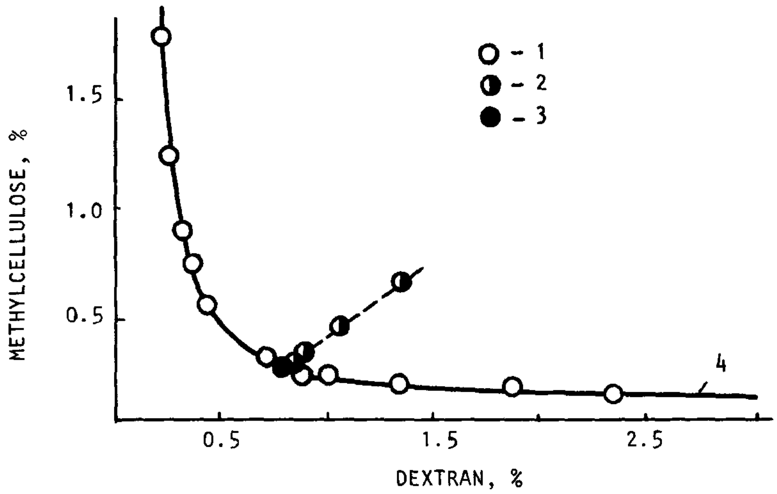

Figure 2. Phase diagram of water-methylcellulose-dextran system at 20° C. 1, 2, 3 - points obtained by the phase-volume-ratio method (the designations of the points correspond to that of Figure 1). 4 - binodal obtained by the analysis of conjugated phase compositions[5].

at 25°C and pH 6.9 . The phase diagram of this system is presented in Figure 3.

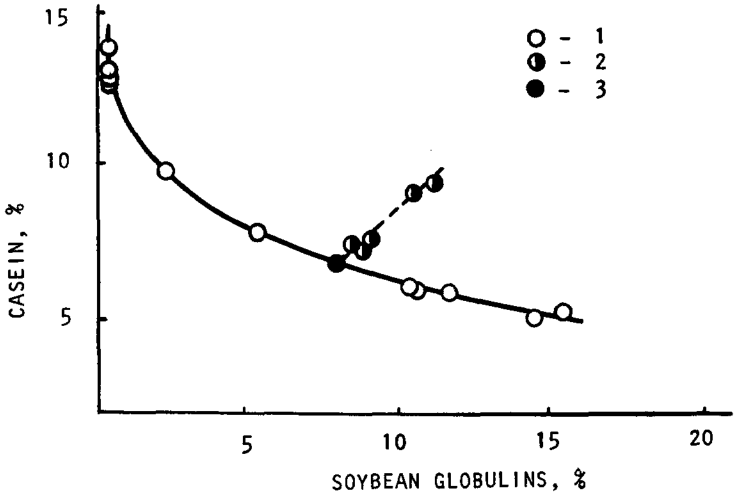

Figure 3. Phase diagram of water-casein-soybean globulins system at 25°C and pH 6.9 (designations of points correspond to those in Figure 1)

Under the above conditions this phase diagram has the critical point with the coordinates: casein concentration - 7.0%, concentration of soybean globulins - 8.0%. Its characteristics are the considerable difference in compatibility limits determined as asymptotes of both binodal branches and the rather large compatibility region. Water-casein-soybean globulins system considerably differs, in this respect, from water-protein-polysaccharide systems studied earlier[6,7].

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