Structural and Partitioning Studies of Bovine Serum Albumin in Mixture of (Poly(ethylene glycol) + K_2HPO_4 + H_2O)

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Secondary structural changes and partitioning process of bovine serum albumin (BSA) in the mixture of (poly(ethylene glycol 2000) + K_2HPO_4 + H_2O) have been studied at 23 °C. The effect of varying weight percents of poly(ethylene glycol) and K_2HPO_4 on the secondary structure of partitioned BSA and the partitioning process of BSA were examined. The equilibrium weight percents of the obtained aqueous two-phase system (ATPS) have been evaluated, and the phase diagrams including the tie lines are presented. It was found that the magnitude of the partition coefficient of BSA and the secondary structures of partitioned BSA depend on the length of obtained tie lines. Occurrences of changes on the secondary structure content of the partitioned BSA were observed by FTIR spectroscopy investigation. The amounts of the secondary structure content of partitioned BSA as α-helix, *β*-sheet, *β*-turn, and unordered structures of BSA were determined and reported.

Introduction

Aqueous two-phase systems (ATPSs) consist of two immiscible aqueous solutions that are in an equilibrium state. The ATPSs are divided into two main groups: (i) an aqueous mixture of two incompatible polymers such as poly(ethylene glycol) (PEG) and dextran and (ii) an aqueous mixture of one polymer and an appropriate salt such as PEG and sodium phosphate. In these mixtures, phase separation occurs at a specific concentration known as the ATPS critical concentration.[1,2] One of the most important features of an ATPS is that both phases are rich in water. Therefore, they have low interfacial tension [(0.0001 to 0.1) dyn·cm^{-1}] being less than that of conventional systems [(1 to 20) dyn·cm^{-1}].[1] The physical property of an ATPS provides suitable conditions for partitioning of biological particles with less harmful effects on their main structure and their activities. In addition to maintenance of biological properties, ease of scale up, high mass transfer rate, and selectivity are the advantages of applying these systems in the separation of biomaterials.[1,3]

The ATPSs have been widely used for extraction, separation, or purification of various microorganisms and biomolecules such as different types of bacteria,[4,5] viruses,[6] proteins,[1,7,8] nucleic acids,[9,10] antibiotics,[11] etc. Proteins are one of the most important biological substances used in the pharmaceutical and food industries. Many investigations have been conducted to find out the effects of various parameters on the partitioning of proteins. These parameters are concentration and molecular weight of the polymer; concentration and other characteristics of the salt; properties of biomaterials being partitioned such as size, molecular weight, and surface properties; and partitioning conditions such as pH, temperature, and ionic strength of mixtures. Each of these parameters could affect the secondary structure contents of partitioned protein. Secondary structure of proteins includes α-helix, *β*-sheet, *β*-turn, and unordered structures that are very important in the performance of each protein. But there is no comprehensive report of the effects of the partitioning process on the structure of partitioned biomolecules. So the effects of different parameters that are mentioned above on the secondary structure content of the partitioned protein must be considered.

The partitioning of proteins had its origin in the early 1950s when an attempt was made to find appropriate ATPSs to separate proteins from other biomaterials.[3] Partitioning of various proteins such as barley albumin and human serum albumin and its dependence on the concentration of potassium chloride have been investigated.[12] After these pioneering studies, various investigations have been made on the effects of different parameters on the partitioning of proteins.[13−15]

The partitioning of proteins such as α-amylase, bovine serum albumin (BSA), α-chymotrypsin, and lysozyme in a PEG + potassium phosphate ATPS have been studied in systems that were produced by manipulation of a monophasic mixture composition close to the critical point.[16] BSA has been used as a model protein to examine the effects of pH and concentration of NaCl on protein partitioning in aqueous PEG + dextran two-phase systems.[17] Balasubramaniam et al.[18] investigated the applicability of an ATPS to recombinant protein purification from tobacco with egg white lysozyme as the model protein. Partitioning of spleen proteinase from yellowfin tuna in an ATPS of PEG 1000 + magnesium sulfate was investigated to find the maximum partitioning of the proteinase.[19] Pico et al.[20] recently studied the partitioning of chymosin from *Aspergilus niger* and pepsin from bovine stomach in ATPSs formed by PEG + potassium phosphate. Chiang and Su[21] also studied the extraction of lysozyme from chicken egg white by PEG + salt, and they found the optimal conditions of the process using the Box−Wilson experimental method.

Very few observations have been made on changes of protein secondary structure content during partitioning. Pico et al.[22] studied the effect of high concentration monovalent cations such as chloride salts of Na^+, Rb^+, and Cs^+ on the partitioning of bovine albumin, trypsin, ovoalbumin, and lysozyme in an ATPS formed by PEG 1500 and potassium phosphate at pH 7.4. They observed the effect of those cations on the α-helix content of proteins.

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The main objective of this work is to investigate the effect of ATPSs on the secondary structural changes of partitioned BSA. In addition to the structural study of partitioned BSA, the partitioning process of BSA and the effect of PEG 2000 and potassium hydrogen phosphate (K_2HPO_4) weight percents on the BSA partition coefficient have been investigated.

Materials and Methods

Materials. The materials used in this study without further purification were as follows: BSA from Sigma USA (Lot No. 81K1815); PEG 2000, dipotassium hydrogen phosphate, sodium molybdate, and sulfuric acid from Merck Germany; and hydrazine sulfate from BDH UK.

Preparation and Analysis of the Aqueous Two-Phase Systems. Two aqueous stock solutions were prepared: (a) PEG 2000 (40 % w/w) and (b) K_2HPO_4 (20 % w/w). A certain weight of solution (a) was put into a test tube. A solution (b) was then added dropwise to the test tube. First, a homogeneous mixture is obtained, but after a certain amount of solution (b) has been added, one further drop will cause cloudiness and turbidity. This cloud point is the indication of formation of an ATPS. The procedure described above is known as cloud point measurements,[1] and by using this procedure, 40 points were obtained and used to construct the binodal curve. In addition to this, five mixtures of (PEG 2000 + K_2HPO_4 + H_2O) with various compositions were prepared by weighing stock solutions (a) and (b) in a way that an aqueous two-phase mixture can be formed. The mixtures were vortex mixed for 2 min and centrifuged at 2000 rpm for 15 min to speed up phase separation; then, the obtained ATPSs were allowed to settle for 5 h to complete the two-phase equilibration.

The equilibrium weight percent of K_2HPO_4 was determined by the Vogel procedure[23] and by using the calibration curve. The PEG equilibrium weight percent was determined by refractive index measurements using a Kruss-Optronic-GmbH (Germany) refractometer. This method is the same as used by other researchers for determining PEG or PPG concentration in the aqueous mixture of different salts such as K_2HPO_4.[24−28]

The weight percents of PEG 2000 in two phases at equilibrium were calculated from refractive index measurements by using the following equation:

$$n_D = 1.33223 + 0.00140w_{salt} + 0.00150w_{PEG} \quad (1)$$

where n_D is the refractive index and w_{salt} and w_{PEG} are the weight percents of K_2HPO_4 and PEG 2000, respectively. The coefficients of eq 1 are evaluated by correlating the measured refractive indices for the mixtures (PEG 2000 + K_2HPO_4 + H_2O). The pH of all ATPSs was 9.3 ± 0.1 as was indicated by a digital pH meter (with a precision of ± 0.01) at 23 °C. All experiments were performed in at least triplicate.

Partitioning of Bovine Serum Albumin (BSA). An aqueous stock solution of BSA (5 g·L^{-1}) was prepared by weighing. All experiments were carried out at 23 °C. From the stock solution, samples containing (0.065 ± 0.001) g of BSA were taken and added to the five ATPSs previously prepared. The systems were vortex mixed for 2 min and centrifuged at 1500 rpm for 10 min to speed up the partitioning of BSA. Each ATPS was allowed to settle for 12 h to complete the equilibration. Samples were taken by weighing from each phase, and after dilution and weighing, the absorbance of each solution was measured at 280 nm by using a GBC-Cintra-5 spectrophotometer (Australia).

The partition coefficient of BSA (K_{BSA}) is defined as the ratio of equilibrium weight percent of BSA in the top phase $(w_{BSA})_T$ to the equilibrium weight percent of BSA in the bottom phase $(w_{BSA})_B$:

$$K_{BSA} = (w_{BSA})_T/(w_{BSA})_B \quad (2)$$

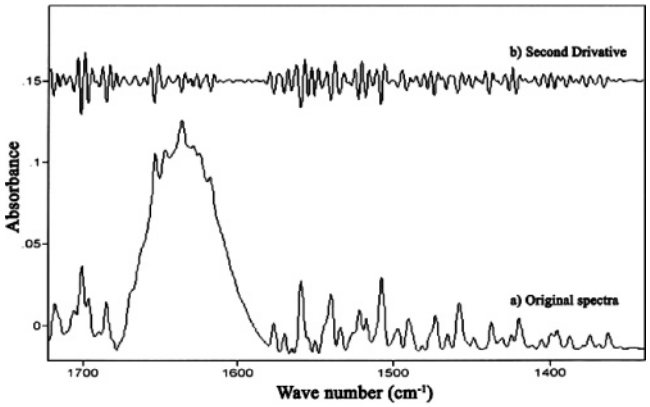

Figure 1. FTIR absorbance spectra of aqueous solution of BSA with concentration of 5 g·L^{-1} at 23 °C. (a) FTIR absorbance spectrum without any modification with software (original spectra) at (1720 to 1350) cm^{-1}. (b) Second derivative of BSA stock solution spectrum after baseline correction, auto-smoothing, and deconvolving at (1720 to 1350) cm^{-1}.

Secondary Structure Content Analysis of Partitioned BSA. Fourier transform infrared (FTIR) spectroscopy is a well-established technique for the analysis of protein secondary structure.[28−31] FTIR spectra of all samples were obtained after partitioning of BSA and compared with that of the BSA stock solution to find out the influence of the ATPS on the secondary structure of the partitioned BSA.

FTIR Spectroscopy. FTIR spectroscopy was done by a Boem−Hartman−Braun spectrophotometer (Canada) equipped with special cells for aqueous samples and a spectral resolution of 1 cm^{-1}. A total of 100 scans was taken to obtain the peak position and peak area with high accuracy. For each sample, the measurements were repeated at least three times.

Data analysis was accomplished by using Bomem−Michelson 100 series FTIR software and GRAMS/386 software. The background of all spectra were corrected automatically, and subtraction of blank solutions of each sample (ATPSs without BSA) was performed.[30] Amide (I) region of BSA [(1620 to 1700) cm^{-1}] was selected to study the secondary structure content of the partitioned BSA. The second derivatives of all background-corrected spectra were analyzed after the correction of their base lines and deconvolution of the amide (I) band region. Changes in the amide (I) band (centered near 1650 cm^{-1}) were used to reveal the changes in the secondary structure content of the partitioned BSA.[32] Figure 1 shows the FTIR spectra of BSA before partitioning (BSA stock solution) and its second derivative at (1700 to 1400) cm^{-1}. In all samples, the secondary structure content was determined by the ratios of individual assigned bands areas to the total area in the amide (I) region.[30,33]

Results and Discussion

Binodal Curve of PEG2000 + K_2HPO_4 ATPS at 23 °C. By cloud point measurements, the data points for construction of the binodal curve for the mixture of (PEG 2000 + K_2HPO_4 + H_2O) were obtained at 23 °C. The points were fitted to a polynomial of the following form with the square of the correlation coefficient of 0.9981:

$$w_2 = 40.79 - 2.8327w_1 - 0.1873w_1^2 + 0.0193w_1^3 - 0.0004w_1^4 \quad (3)$$

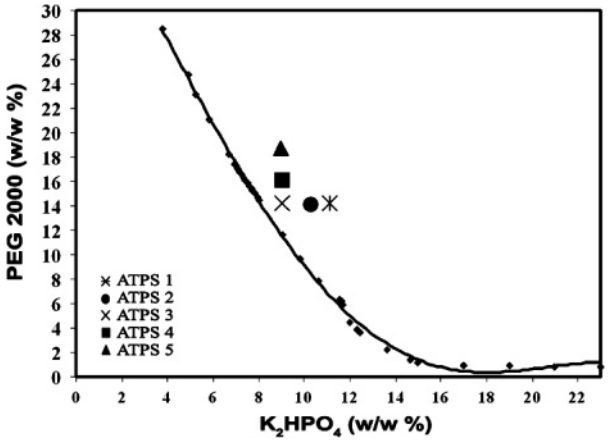

Figure 2. Binodal curve of the PEG 2000 + K_2HPO_4 ATPS at 23 °C and five prepared ATPSs for protein partitioning.

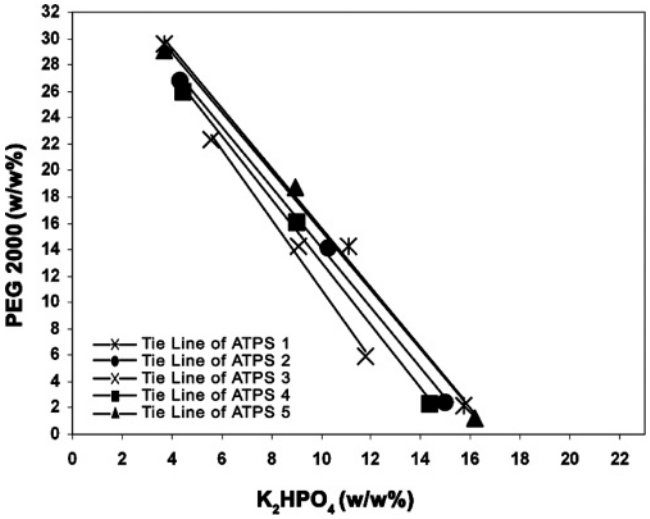

Figure 3. Five tie lines of (PEG 2000 + K_2HPO_4 + H_2O) systems at 23 °C. The point at the end of each tie line with higher weight percent of PEG 2000 indicates the top phase, and the point on other end indicates the bottom phase.

where w_1 and w_2 stand for K_2HPO_4 and PEG 2000 weight percents, respectively. The binodal curve is shown in Figure 2. Maderia et al.[34] obtained the binodal curve for the mixture (PEG 6000 + K_2HPO_4 + H_2O) at 25 °C. Although the molecular weight of PEG used by Maderia et al. is different, their binodal curve is similar to Figure 2. Snyder et al.[35] studied the mixture of (PEG + K_2HPO_4 + KH_2PO_4+ H_2O) at 25 °C using various molecular weights of PEG. Their binodal curves form at higher concentrations of PEG and salt.

ATPS Analysis. Figure 2 represents the five studied ATPSs. In this figure, three points show the systems with constant weight percents of PEG 2000, and three points show the systems with constant weight percents of K_2HPO_4. Total weight percents of the components in the ATPSs and their weight percents in the phases at equilibrium are reported in Table 1. As shown in this table, the weight percent of PEG in the top phase is higher than the bottom phase. The points with higher PEG weight percent at the end of each tie line in Figure 3 correspond to the top phase, and the points with lower PEG weight percent correspond to the bottom phase. Also, the points on the tie line correspond to the total weight percent in the ATPSs.

Each tie line length (TLL) was calculated by the following equation:[2,36]

$$\mathrm{TLL} = [\Delta(w_{\mathrm{PEG}})^2 + \Delta(w_{\mathrm{salt}})^2]^{0.5} \quad (4)$$

where $\Delta(w_{\mathrm{PEG}})$ and $\Delta(w_{\mathrm{salt}})$ represent the differences of PEG and salt (K_2HPO_4) weight percents in the top and the bottom phases at equilibrium respectively. Before considering the influences of TLL on the partitioning of BSA, it is important to find out the effects of PEG 2000 and K_2HPO_4 weight percents on BSA partitioning.

Effect of K_2HPO_4 Weight Percent on the Partitioning of BSA. Table 1 reports the partition coefficients of BSA in ATPSs. The first and second columns of Table 1 for systems 3 to 1 show that the total weight percent of PEG is almost constant (14.1 to 14.2) % as the weight percent of K_2HPO_4 increases from (9.6 to 11.1) %, and as a result, the partition coefficient in the last column of this table increases from 0.45 to 1.12. This is in agreement with the general conclusion on partitioning that the salt concentration increases the partitioning behavior of ATPSs.[2] Also an increase in total weight percent of K_2HPO_4 in the ATPS increases its weight percent in the bottom phase and transfers proteins from the bottom phase (phosphate-rich phase) to the top phase (PEG-rich phase).[37] Another explanation can be provided in terms of hydrophobic interactions, which cause an increase in weight percent of BSA in the top phase.[38] It should be noted that the ratio of K_2HPO_4 weight percent to PEG weight percent in both phases is a determining factor in partitioning of the BSA in the ATPS.

Effect of PEG 2000 Weight Percent on the Partitioning of BSA. The first and second columns of Table 1 for systems 3 to 5 show that the total weight percent of K_2HPO_4 is almost constant (9 %) as the weight percent of PEG increases from (14.2 to 18.6) %, and as a result, the partition coefficient in the last column of Table 1 increases from 0.45 to 1.2. This is the expected trend for the partition coefficient as observed by other researchers[2] and is due to an increase in the ratio of the K_2HPO_4 weight percent to PEG weight percent in the bottom phases of the ATPS.

Influences of TLL on the Partitioning of BSA. Figure 4 represents the effect of TLL on the partition coefficients of BSA. Figure 4 confirms the findings of other authors[1,16] in which the partition coefficient of BSA decreases as the TLL decreases. The minimum partition coefficient (0.451 ± 0.003) is obtained at the minimum TLL (= 17.65) and is the closest point to the binodal curve. Partition coefficient variation is due to many factors, such as protein interactions with the surrounding molecules (water, PEG 2000, and K_2HPO_4 molecules), for

Table 1. Total Weight Percent of the Components in ATPSs and Their Equilibrium Values in Each Phase at 23 °C[a]

	total (w/w %)		top phase (w/w %)		bottom phase (w/w %)		
system	K_2HPO_4	PEG 2000	K_2HPO_4	PEG 2000	K_2HPO_4	PEG 2000	partition coeff
1	11.100	14.230	3.694	29.626	15.757	2.114	1.128 ± 0.002
2	10.286	14.098	4.320	26.858	14.957	2.367	0.819 ± 0.002
3	9.067	14.190	5.584	22.382	11.807	5.860	0.451 ± 0.003
4	9.036	16.088	4.425	25.983	14.353	2.284	0.779 ± 0.003
5	8.982	18.664	3.720	29.058	16.186	1.253	1.203 ± 0.002

[a] Determined partitioned coefficients are reported in last column.

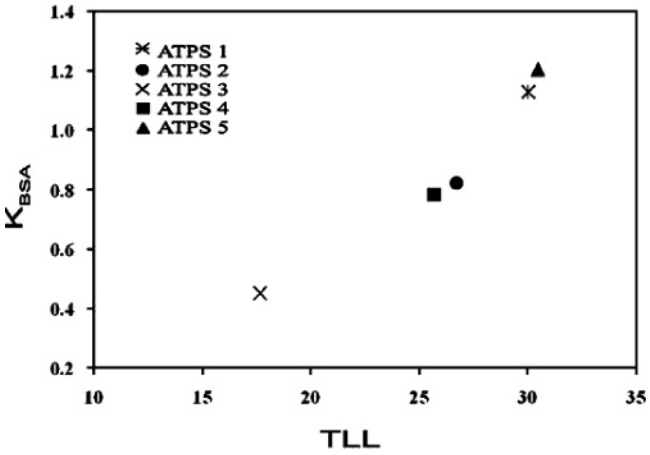

Figure 4. Effect of tie line length (TLL), as defined by the eq 4, on the partition coefficients of BSA (K_{BSA}). The K_{BSA} is defined as the ratio of equilibrium weight percent of BSA in the top phase to that of BSA in the bottom phase.

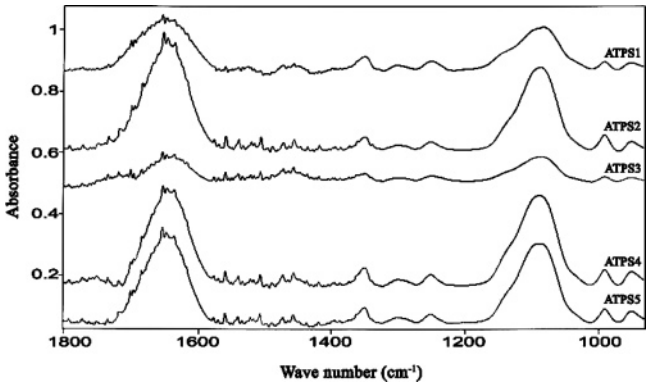

Figure 5. FTIR absorbance spectra of partitioned BSA within ATPSs (1 to 5) at (1800 to 900) cm^{-1}.

instance, hydrogen bonding, ionic and hydrophobic interactions, together with other weak forces can affect the partition coefficient,[39] and the net influence of these factors determines the protein partitioning in ATPS.

Similar values of TLL were calculated for the systems (1 and 5); these values are 30.04 and 30.47. Also similar values of TLL were calculated for systems (2 and 4), these values are 26.70 and 25.69. The partition coefficients of systems (1 and 5) are 1.128 ± 0.002 and 1.203 ± 0.002, respectively. Systems (2 and 4) are 0.819 ± 0.002 and 0.779 ± 0.003, respectively. This indicates that systems having the same TLL have the same partition coefficient, and this can be used to obtain the optimum ATPS weight percent for an effective partitioning of proteins.

Secondary Structure Content of Partitioned BSA. Figure 5 represents the FTIR spectra of BSA in the ATPSs after partitioning, and Figure 6 shows the second derivative of the obtained spectra. No new peak for partitioned BSA was observed when the spectrum of the stock solution of BSA was compared with that of partitioned BSA. Moreover, the peaks in the spectra of partitioned BSA (Figures 5 and 6, ATPS 1 to 5) were located at wave numbers similar to those in the spectra of the BSA stock solution. So it can be concluded that the subtraction of the ATPS spectra did not create any spectral artifacts.[30]

The BSA molecule is made up of three homologous domains. Each domain has a specific structure. Relative amounts of secondary structure content of BSA includes α-helix, β-sheet, β-turn, and unordered structure, were determined by calculating the percentage area changes of the associated component band

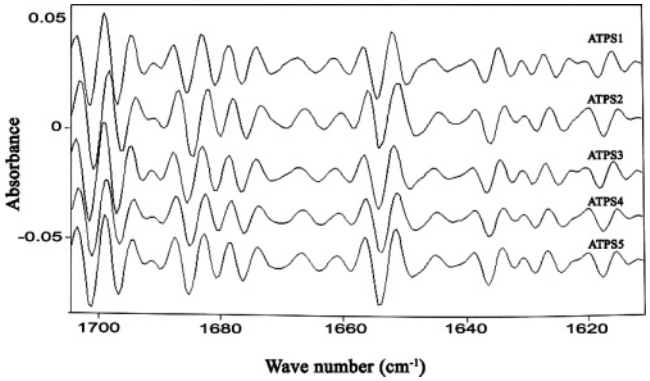

Figure 6. Second derivatives of ATPSs (1 to 5) spectra after baseline correction, auto-smoothing, and deconvolving in the region of amide (I) at (1720 to 1600) cm^{-1}.

under the amide (I) region. The amide (I) region was selected because it is well-understood and has been employed thus far in many IR investigations of protein secondary structure.[40] Many theoretical and experimental studies have been conducted to correlate IR absorption bands of proteins to their secondary structure content, based on the spectra of the proteins either in the solid state or in aqueous solution.[33,40−42]

Table 2 represents the peak position and analyzed data obtained from FTIR spectroscopy. The positions of peaks are in agreement with findings of other authors.[32,33,40−42] The obtained content of each secondary structure is represented in Table 2. Maximum structure content corresponds to β-structure (including β-sheet, β-turn, and dipole coupling in β-sheets) and was obtained as (44 to 49) %. From the results in Table 2, the total α-helix content is varied from (16 to 19) %. The amount of total unordered structure content varied from (34 to 37) %. It should be noted that the IR bands at 1695 cm^{-1} are due to the strong transition dipole coupling in β-sheets.[40] Therefore, the maximum structure content is obtained for the total amount of β-structure.

Total changes of α-helix, β-sheet, dipole coupling in β-sheets, β-turn, and unordered structures of BSA are shown in Figure 7. The change in structures is obvious during partitioning. It shows no loss in the β-sheet content of partitioned BSA. It would be concluded that the β-sheet structure is probably the most favorable structure in BSA and that other structures tend to form the β-sheet structure. This can be attributed to the minimum Gibbs free energy of the β-sheet structure among other secondary structures as explained by Sagawa et al.,[43] who stated that the β-sheet structure is thermodynamically more stable in BSA than the α-helix structure. On the other hand, the other main secondary structure of proteins (α-helix) had significant changes in ATPSs. The maximum change (10.40 %) corresponds to ATPS 1, which consists of the maximum amount of K_2HPO_4. The greater loss of α-helical structure content could be as a result of ionic or electrostatic interaction between some amino acids residues.[44] The higher amount of K_2HPO_4 can exert a greater electrostatic force on the functional groups of BSA. The greater loss of α-helix structure of BSA is in agreement with the finding of Lacefield et al.[32] The minimum change in the α-helical structure (−0.16 %) occurred in ATPS 3, which was the closest system to the binodal curve. The minimum change of the α-helix content could be as a result of the minimum ionic and electrostatic forces that exist in ATPS 1. Furthermore, it was observed that the α-helix content increased as a result of an increase in PEG weight percent. Another study[22] shows that the PEG 1500 decreased the α-helix content of BSA and increased the α-helix content of ovoalbumin and lysozyme.

Table 2. FTIR Band Positions of Stock Aqueous Solution of BSA and Partitioned BSA in ATPSs (1 to 5) Accompanied with Band Assignments in Amide (I) Region at (1700 to 1620) cm^{-1} [a]

		stock solution of BSA		ATPS 1		ATPS 2		ATPS 3		ATPS4		ATPS 5	
	assignment	wave no./cm^{-1}	secondary structure content/%	wave no./cm^{-1}	secondary structure content/%	wave no./cm^{-1}	secondary structure content/%	wave no. /cm^{-1}	secondary structure content/%	wave no. /cm^{-1}	secondary structure content/%	wave no./cm^{-1}	secondary structure content/%
1	dipole coupling in β-sheets	1696.81	14.87	1697.28	14.38	1696.56	11.47	1697.36	15.00	1696.60	12.01	1696.58	11.51
2	β-sheet	1692.36	1.61	1692.93	3.70	1692.10	0.91	1692.89	3.51	1692.21	1.33	1692.50	1.49
3	β-turn	1689.59	4.25	1689.95	1.71	1689.57	3.56	1690.15	2.83	1689.69	3.38	1689.67	3.06
4	unordered	1685.20	13.48	1685.83	10.19	1684.88	11.66	1685.57	10.77	1685.11	11.70	1685.15	11.59
5	β-turn	1680.43	6.82	1681.25	8.13	1680.54	6.70	1680.99	7.67	1680.42	7.15	1680.63	6.96
6	β-sheet	1676.50	3.91	1676.90	7.01	1676.07	6.35	1676.76	6.74	1676.30	6.68	1676.33	6.60
7	unordered	1670.13	5.33	1672.10	6.20	1669.54	6.40	1671.72	6.18	1669.77	6.76	1670.56	6.72
8		1663.65	4.10	1664.54	3.86	1663.48	4.32	1664.28	3.43	1663.82	3.97	1664.00	3.92
9	α-helix	1658.55	3.94	1659.27	4.48	1658.55	4.72	1659.13	4.46	1658.67	4.28	1658.80	4.67
10		1653.71	14.26	1654.81	11.83	1653.86	12.50	1654.43	13.72	1653.86	13.98	1654.16	14.18
11	unordered	1648.29	10.76	1649.89	10.84	1648.02	10.94	1649.28	10.29	1648.60	10.45	1648.96	10.70
12		1642.09	3.83	1643.13	3.28	1642.18	3.65	1642.98	3.47	1642.18	3.79	1642.63	3.88
13		1636.53	7.03	1636.95	5.37	1635.88	6.36	1636.80	5.59	1636.23	6.27	1636.29	6.34
14	β-sheet	1631.85	1.80	1632.60	3.63	1631.65	1.56	1632.56	2.70	1631.99	1.66	1632.00	2.23
15		1628.41	1.65	1629.17	2.58	1628.45	2.91	1629.13	2.65	1628.90	3.15	1628.72	3.03
16		1624.02	2.38	1624.25	2.80	1624.51	5.99	1624.42	1.00	1624.33	3.44	1624.26	3.13

[a] Percentage of structural content under relative peak position is determined as the ratio of relative peak area to the total area of amide (I) region under (1700 to 1620) cm^{-1}. Assignments are from refs 33 and 40−42.

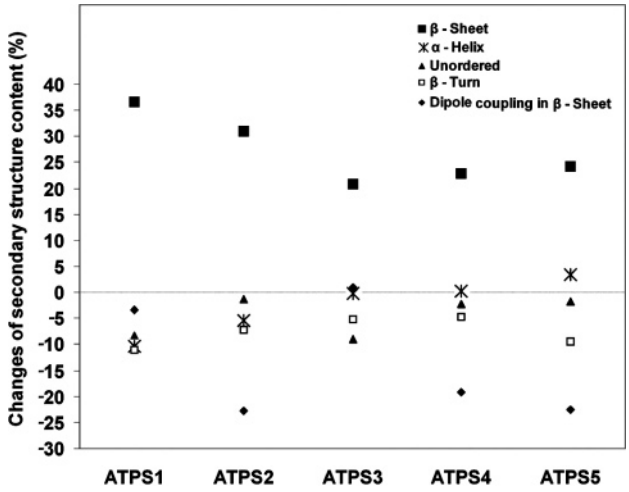

Figure 7. Changes of different structural contents of BSA after partitioning in ATPSs.

It can be concluded from Figure 7 that the weight percent of salt has a significant effect on the loss of secondary structure content in BSA partitioning as compared to the PEG weight percent. In addition to ionic strength and electrostatic forces, the secondary structure content changes of partitioned BSA probably depend upon the effects of surface tension. Moreover, the role of liquid−liquid interfaces should also be considered.

Conclusions

The influence of TLL on the partition coefficients of BSA and the effects of the equilibrium weight percent of ATPS components on the secondary structure content of the partitioned BSA were investigated in the mixture of (PEG 2000 + K_2HPO_4 + H_2O) at 23 °C. The results show that the partition coefficient of BSA increases (from 0.451 ± 0.003 to 1.203 ± 0.002) with an increase in the TLL. Negligible differences in the partition coefficients of systems with similar TLL indicated that the same partition coefficients can be obtained with different ATPS equilibrium weight percents. The secondary structure studies of partitioned BSA indicated that each component weight percent had a different effect on the secondary structure content of partitioned BSA. The α-helix content loss was (0.16 to 10.40) % as a result of an increasing K_2HPO_4 weight percent. Increasing the K_2HPO_4 weight percent also resulted in a decreasing of the β-sheet content of partitioned BSA. It changed from (36.64 to 20.74) %.

Literature Cited

(1) Albertsson, P. A. *Partition of Cell Particles and Macromolecules*, 3rd ed.; John Wiley and Sons: New York, 1986.
(2) Zaslavsky, B. Y. *Aqueous Two-Phase Partitioning*: *Physical Chemistry and Biotechnology Applications*; Marcel Dekker: New York, 1994.
(3) Walter, H.; Brooks, D. E.; Fisher, D. *Partitioning in Aqueous Two-Phase Systems*; Academic Press: Orlando, FL, 1985.
(4) Albertsson, P. A.; Baird, G. D. Countercurrent distribution of cells. *Exp. Cell Res.* **1962**, *28*, 296−322.
(5) Von Hofsten, B.; Baird, G. D. Fractionation of cell constituents of Bacillus megaterium in a polymer two-phase system. *Biotechnol. Bioeng.* **1962**, *4*, 403−410.
(6) Blankschtein, D.; Liu, C.; Kamei, D. T.; King, J. A.; Wang, D. I. C. Separation of proteins and viruses using two-phase aqueous micellar systems. *J. Chromatogr. B* **1998**, *11*, 127−138.
(7) Diamond, A. D.; Hsu, J. T; Aqueous two-phase systems for biomolecules separation. *Adv. Biochem. Eng. Biotechnol.* **1992**, *47*, 89−135.
(8) Johnsson, G. Studies on aqueous dextran-PEG two-phase systems containing charged poly ethylene glycol. *Biochim. Biophys. Acta* **1970**, *222*, 381−389.
(9) Ohelsson, R.; Hentschel, C. C.; Williams, J. G. A rapid method for the isolation of circular DNA using an aqueous two-phase partition system. *Nucleic Acids Res.* **1978**, *5*, 583−590.
(10) Rudin, L.; Albertsson, P. A. A new method for the isolation of deoxyribonucleic acids from microorganisms. *Biochim. Biophys. Acta* **1967**, *134*, 37−44.
(11) Dutta, N. N.; Bora, M. M. Borthakur, S.; Rao, P.C. Aqueous two-phase partitioning of cephalosporin antibiotics: effect of solute chemical nature. *Sep. Purif. Technol.* **2005**, *45*, 153−156.
(12) Albertsson, P. A.; Nynce E. J. Counter current distribution of proteins in aqueous polymer phase systems. *Nature* (*London*) **1959**, *184*, 1465−1468.
(13) Johansson, G. Comparison of two aqueous biphasic systems used for partition of biological materials. *J. Chromatogr.* **1978**, *150*, 63−67.
(14) Kroner, K. H.; Cordes, A.; Schelper, A.; Morr, M.; Buckmann, A. F.; Kula, M. R. Affinity partitioning studied with glucose-6-phosphate dehydrogenase in aqueous two-phase systems in response to triazine dyes. In *Affinity Chromatography and Related Techniques*; Elsevier: Amsterdam. The Netherlands, 1982; pp 491−501.
(15) Birkenmeier, G.; Usbek, E.; Kopperschlager, G. Affinity partitioning of albumin and alpha-fetoprotein in an aqueous two-phase system using poly ethylene glycol-bound triazine dyes. *Anal. Biochem.* **1984**, *136*, 264−271.
(16) Belval, S.; Breton, B.; Huddleston, J.; Lyddiatt, A. Influence of temperature upon protein partitioning in poly(ethylene glycol)−salt aqueous two-phase systems close to the critical point 1 with some observations relevant to the partitioning of particles. *J. Chromatogr. B* **1998**, *711*, 19−29.

(17) Gunduz, U.; Korkmaz, K. Bovine serum albumin partitioning in an aqueous two-phase system: Effect of pH and sodium chloride concentration. *J. Chromatogr. B* **2000**, *743*, 255−258.
(18) Balasubramaniam, D.; Wilkinson, C.; Van Cott K.; Zhang, C. Tobacco protein separation by aqueous two-phase extraction *J. Chromatogr. A* **2003**, *989*, 119−129.
(19) Benjakul, S.; Klomklao, S.; Visessanguan, W.; Simpson, B. K.; Kishimura H. Partitioning and recovery of proteinase from tuna spleen by aqueous two-phase systems. *Process Biochem.* **2005**, *40*, 3061−3067.
(20) Pico, G.; Spelzini, D.; Farruggia, B. Features of the acid protease partition in aqueous two-phase systems of polyethylene glycol−phosphate: chymosin and pepsin. *J. Chromatogr. B* **2005**, *821*, 60−66.
(21) Chiang, B. H.; Su, C. Partitioning and purification of lysozyme from chicken egg white using aqueous two-phase system. *Process Biochem.* **2006**, *41*, 257−263.
(22) Pico, G.; Farruggia, B.; Rigatuso, R.; Capezio, L.; Diez, V. Influence of high concentration monovalent cations on the protein partitioning in polyethyleneglycol 1500-phosphate aqueous two-phase systems. *J. Chromatogr. B* **2004**, *809*, 301−306.
(23) Vogel, A. I. *Textbook of Quantitative Inorganic Analysis*, 3rd ed.; Longmans Publications: London, 1961; 810 pp.
(24) Cheluget, E. L.; Gelnas, S.; Vera, J. H.; Weber, M. Liquid−liquid equilibrium of aqueous mixtures of PPG with NaCl. *J. Chem. Eng. Data* **1994**, *32*, 127−136.
(25) Haghtalab, A.; Mokhtarani, B. The new experimental data and a new thermodynamic model based on group contribution for correlation liquid−liquid equilibria in aqueous two-phase systems of PEG and (K_2HPO_4 or Na_2SO_4). *Fluid Phase Equilib.* **2004**, *215*, 151−161.
(26) Zafarani-Moattar, M. T.; Hamzehzadeh, Sh. Liquid−liquid equilibria of aqueous two-phase systems containing polyethylene glycol and sodium succinate or sodium formate. *Comput. Coupling Phase Diagrams Thermochem.* **2005**, *29*, 1−6.
(27) Shang, Q. K.; Li, W.; Jia, Q.; Li, D. Q. Partitioning behavior of amino acids in aqueous two-phase systems containing polyethylene glycol and phosphate buffer. *Fluid Phase Equilib.* **2004**, *219*, 195−203.
(28) Zafarani-Moattar, M. T.; Sadeghi, R. Liquid−liquid equilibria of aqueous two-phase systems containing polyethylene glycol and sodium dihydrogen phosphate or disodium hydrogen phosphate Experiment and correlation. *Fluid Phase Equilib.* **2001**, *181*, 95−112.
(29) Haris, P. I.; Chapman, D. Does Fourier-transform infrared spectroscopy provide useful information on protein structure? *Trends Biochem. Sci.* **1992**, *17*, 238−333.
(30) Langer, R.; Fu, K.; Griebenow, K.; Hsieh, L.; Klibanov, A. M. FTIR characterization of the secondary structure of proteins encapsulated within PLGA microspheres. *J. Controlled Release* **1999**, *58*, 357−366.
(31) Gaber, M. H. Effect of γ-irradiation on the molecular properties of bovine serum albumin. *J. Biosci. Bioeng.* **2005**, *100*, 203−206.
(32) Lacefield, R. W.; Zeng, H.; Chittur, K. K. Analysis of bovine serum albumin adsorption on calcium phosphate and titanium surfaces. *Biomaterials* **1999**, *20*, 377−384.
(33) Riley, C.; Xie, J.; Kumar, M.; Chittur, K. FTIR/ATR study of protein adsorption and brushite transformation to hydroxyapatite. *Biomaterials* **2002**, *23*, 3609−3616.
(34) Maderia, P. P.; Xu, X.; Teixeira, J. A.; Macedo, E. A. Prediction of protein partition in polymer/salt aqueous two-phase systems using the modified Wilson model. *Biochem. Eng. J.* **2005**, *24*, 147−155.
(35) Snyder, S. M.; Cole, K. D.; Szlag, D. C. Phase compositions, viscosities, and densities for aqueous two-phase systems composed of polyethylene glycol and various salts at 25 °C. *Chem. Eng. Data* **1992**, *37*, 268−274.
(36) Haynes, C. A.; Johansson, H. O.; Karlstorm, G.; Tjerneld, T.; Driving, forces for phase separation and partitioning in aqueous two-phase systems. *J. Chromatogr. B* **1998**, *711*, 3−17.
(37) Videira, M.; Aires-Barros, M. R. Liquid−liquid extraction of clavulanic acid using an aqueous two-phase system of polyethylene glycol and potassium phosphate. *J. Chromatogr. A* **1994**, *668*, 237−240.
(38) Rees, A.; Sternberg, M. J. E.; Wetzel, R. *Protein Engineering*, 1st ed.: Oxford University Press: New York, 1994.
(39) Blazquez, C.; Camacho, F.; Calero, M.; Alarcon, F. J. Interaction probabilities in a four components aqueous two-phase system: polymer + salt + water + protein. *Chem. Eng. Sci.* **2001**, *56*, 4451−4456.
(40) Griebenow, K.; Santos, A. M.; Carrasquillo, K. G. Secondary structure of proteins in the amorphous dehydrated state probed by FTIR spectroscopy. *Internet J. Vib. Spectrosc.* **1999**, *3*, edition 1 (available at http://www.ijvs.com/volume3/edition1/section3.html).
(41) Wang, Y. G.; Xue, J. M.; Du, G. H. Effect of MeV proton irradiation on bovine serum albumin molecule. *Nucl. Instrum. Methods Phys. Res., Sect. B* **2006**, *245*, 318−321.
(42) Cui, F. Z.; Lin, Y. B.; Zhang, D. M.; Tian, M. B. Irradiation effects on secondary structure of protein induced by keV ions. *Radiat. Phys. Chem.* **2001**, *60*, 35−38.
(43) Sagawa, J.; Nagare, S.; Senna, M. Preparation and properties of bovine serum albumin thin films by pulsed laser deposition. *Appl. Surf. Sci.* **2005**, *244*, 611−614.
(44) Cox, M. M.; Nelson, D. L. *Lehninger Principles of Biochemistry*, 3rd ed.; Worth Publisher: New York, 2000.

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