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Partitioning of α-lactalbumin and β-lactoglobulin in aqueous two-phase systems of polyvinylpyrrolidone and potassium phosphate

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ABSTRACT

In the present study, the partitioning of α-lactalbumin, β-lactoglobulin, and cheese whey proteins in aqueous two-phase system of polyvinylpyrrolidone–potassium phosphate is investigated. The partitioning of proteins in this system depends on the polymer and salt weight percents in feed, temperature, and pH. The orthogonal central composite design is used to study the effects of different parameters on partitioning of α-lactalbumin and β-lactoglobulin. A second order model is proposed to determine the impact of these parameters. The results of the model show that the weight percent of the salt in feed has a large effect on the protein partitioning. The weight percent of polyvinylpyrrolidone in the feed increases the partitioning coefficients. By increasing the temperature, the viscosity of polyvinylpyrrolidone is reduced and the protein can easily be transferred from one phase to the other phase. The pH of the aqueous two phase system can alter the protein partitioning coefficient through the variation of the protein net charge.

1. Introduction

In recent years, separation of proteins and other biomolecules have been studied by many researchers as reported by Walter and Johansson [1]. Cheese whey has a highlighted importance among the bioproducts. The world production of cheese whey is estimated as 130 million tons per year, accounting for around 780,000 t of proteins [2]. More than 90% of the proteins in the cheese whey are α-lactalbumin (αla) and β-lactoglobulin (βlg) [3]. These proteins present high nutritional and biological values, as well as commercial importance. There are different methods for separation of the proteins from cheese whey such as ion exchange chromatography, membrane processes, and salt precipitation. Ion exchange chromatography is an expensive method, which provides low yields when applied for separation of a low-concentrated protein [3]. In separation by a membrane process, the residual fat in the cheese whey poses problems with irreversible fouling of the membranes [4]. On the other hand, precipitation of proteins by salts or organic solvents induces many difficulties in large scale applications.

Alternatively, aqueous two phase system (ATPS) can be applied for extraction of the proteins. ATPS is a simple, powerful, and highly efficient method. It is also an appropriate method for extraction of other proteins, enzymes, and biomolecules from fermentation media. ATPS have some advantages in comparison to the other commonly used separation and purification techniques. The advantages can be summarized as high water content of both phases (70–85%, w/w), high biocompatibility and low interfacial tension, low degradation of biomolecules, good resolution and high separation yield, relatively high capacity, ease of scale-up, low material costs, and the possibility of polymer and salt recycling [5].

This technique was introduced by Beijernick for the first time in 1896 [6]. The application of these systems for partitioning of cells and macromolecules was investigated by Albertson [7]. He found that some polymers and electrolytes form a two-phase system in a definite concentration. The ATPS consists of two immiscible aqueous solutions which may contain different polymers such as poly(ethyleneglycol) (PEG) and dextran, or one polymer and one salt (e.g., PEG and ammonium sulphate).

The isoelectric point of the αla and βlg are 4.7–5.1 and 5.2–5.4, respectively, depending on the different types of αla and βlg. Chen et al. studied the partitioning of αla and βlg in ATPS of polyethylene glycol (PEG)–potassium phosphate [8]. Coimbra et al. investigated the separation and purification of αla and βlg in a continuous ATPS of PEG–potassium phosphate [9]. They observed that αla and βlg prefer the polymeric and saline phases, respectively. Rito-Palomares and Hernandez studied the effect of process parameters on cheese whey protein partitioning in ATPS of PEG–potassium phosphate [10]. They showed that the cheese whey caused the binodal curve to move away from the origin at high concentrations of PEG that is attributed to the presence of residual fat in the whey. Rodrigues et al. partitioned αla and βlg in ATPS of PEG–ammonium sulphate [11]. They concluded that it is possible to purify βlg in

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the bottom phase and to concentrate αla in the upper phase of the system. However, the ammonium sulphate induces the aggregation of protein and causes its precipitation at the system interface [10]. Alves et al. analyzed the partitioning of whey proteins in ATPS of PEG–salt and PEG–maltodextrin mixtures [12]. Their results showed the feasible purification of αla and βlg by using ATPS. The purification of cheese whey proteins in polymeric and saline phases of ATPS of PEG–potassium phosphate using size exclusion chromatography was investigated by Rojas et al. [13]. They reported that the size exclusion chromatography is a suitable final purification step for cheese whey proteins present in the polymeric and saline phases of ATPS. Anandharamakrishnan et al. studied the isolation and concentration of whey proteins in ATPS of PEG–salt and PEG–maltodextrin [4]. They found that the ATPS is an effective method for removal of fat from the cheese whey. The study of Boaglio et al. revealed that the whey protein partitioning in ATPS of PEG–sodium citrate depends on several parameters such as ionic strength, pH, and type of the salt [3]. Recently, new polymer systems for separation of cheese whey proteins have been applied. The partitioning of whey protein in ATPS of polymer–polymer have been investigated by da Silva and Meirelles [14]. They reported the experimental data on partitioning of bovine serum albumin, αla and βlg in ATPS of PEG–maltodextrin. Monteiro et al. studied the partitioning behavior of αla and βlg in ATPS of triblock copolymers of ethylene oxide (EO) and propylene oxide (PO)–potassium phosphate by cloud point extraction [2].

Polyvinylpyrrolidone (PVP) is a water soluble and biocompatible polymer. Its aqueous solution with a suitable polymer or salt forms an ATPS. The liquid–liquid equilibria of ATPS of PVP–salt containing phosphate, sulphate or carbonate has been studied [15–20]. However, to our knowledge, there is no report on partitioning of proteins using ATPS of PVP–salt. In the present research the partitioning of pure αla, βlg and cheese whey proteins in ATPS of PVP–potassium phosphate is studied. The orthogonal central composite design (CCD) is used to study the effects of different parameters including salt and polymer weight percents in feed, temperature, and pH on partitioning of αla and βlg. Based on the results of the experimental design, a regression analysis is carried out and a model for protein partitioning is proposed.

2. Experiments

2.1. Materials

PVP with molar mass of 3500 was purchased from Acros. Dipotassium hydrogen phosphate (K_2HPO_4) and mono potassium hydrogen phosphate (KH_2PO_4) were obtained from Merck. αla and βlg were purchased from Sigma. The purities of the αla and βlg were more than 85 and 90%, respectively. The double distilled deionized water was used in the experiments.

2.2. Apparatus and procedure

The aqueous two phase experiments are carried out in a 25 cm^3 graduated cylinder. The ATPS are prepared by stock solutions of PVP, K_2HPO_4, and protein. The concentrations of PVP and K_2HPO_4 in stock solutions are 40 and 20% by mass, respectively. The protein concentration is 1 g/L. The stock solutions with certain weight are mixed and stirred by a magnetic stirrer for 5 min. The final mass of each ATPS is nearly 20 g. The pH of the systems is adjusted in the range of 6.5–8.5 by the mixture of KH_2PO_4 and K_2HPO_4. The cylinders are placed in a water bath at the specified temperature (283–313 K) for 24 h to reach into equilibrium. The temperature is controlled within ±0.1 K. The samples of the top phases of ATPS are carefully withdrawn at right above the interface. The samples of the bottom phase are withdrawn by using a plastic syringe with long needle. The samples are analyzed to determine the salt, the polymer, and the protein concentrations.

Table 1
The coefficients of Eq. (1) at 298 K.

α_0	α_1	α_2
1.333	K_2HPO_4, 1.517×10^{-3} KH_2PO_4, 1.166×10^{-3}	PVP (3500), 1.822×10^{-3}

The concentration of salt is determined using atomic absorption spectroscopy with a Perkin Elmer 1100B model. PVP concentration is determined by refractive index measurements at 298 K using a refractometer (Atago DR-A1, Japan). The refractive index of an aqueous phase depends on the concentration of PVP and the salt. According to Cheluget et al. [21], the influence of PVP and salt on the refractive index of an aqueous solution can be correlated by the following equation.

$$nD = \alpha_0 + \alpha_1 w_1 + \alpha_2 w_2 \quad (1)$$

where w_1 and w_2 are the mass fractions of the salt and PVP, respectively, and α_0 is the refractive index of water at 298 K (1.333). α_1 and α_2 are constant with the values as reported in Table 1.

The concentration of protein in each phase is determined by using UV (Agilent model) ultraviolet spectrometer at wavelength of 280 nm. As the salts, PVP and proteins have interferences on each other in the aqueous solution, the absorbance coefficients of salts and PVP are measured in a free protein solution. Then, the absorbance coefficient of protein in the sample is measured by comparing it with the blank solutions of salts and PVP. The pHs of the ATPS are measured with the pH meter Metrohm 627.

Cheese whey is prepared in laboratory. The pasteurized milk is heated up to 60 °C, and then the rennet is added and the milk is allowed to cool to ambient temperature. The cheese whey is obtained by filtering the curd. In order to separate the fat, cheese whey is centrifuged for 30 min at 21,500 × g using a refrigerated Sigma 3K30 centrifuge. The protein content of the cheese whey is measured prior to the experiment.

3. Experimental results

The partitioning of proteins in ATPS depends on several factors such as polymer and salt concentrations, temperature, and pH. The experimental design is a useful tool to study the effect of these factors and their interactions on the protein partitioning. In this research, the orthogonal CCD is applied to analyze the partitioning of protein in ATPS of PVP–potassium phosphate [22]. To simplify the recording and processing of the experimental data, the factor levels have been selected so that the upper level, the lower level and the basic level correspond to +1.414, −1.414, and zero, respectively. Independent variables, experimental ranges and statistical levels for this system are given in Table 2. The parameters of the salt weight percent in feed (x_1), the PVP weight percent in feed (x_2), temperature (x_3) and pH (x_4) are chosen as independent variables. The weight percents of PVP, salt, and protein in the upper and lower phases are selected as dependent variables. The ranges of the independent variable are selected based on the

Table 2
The experimental range and level of independent variables.

Levels	−1.414	−1	0	1	1.414
x_1 (salt weight percent)	9	9.9	12	14.1	15
x_2 (PVP weight percent)	15	16.6	20.5	24.4	26
x_3(temperature)	10	14.4	25	35.6	40
x_4 (pH)	6.5	6.8	7.5	8.2	8.5

Table 3
The experimental design scheme for ATPS of PVP–K_2HPO_4–protein.

Exp. no.	x_1	x_2	x_3	x_4
1	1	1	1	−1
2	1	1	1	1
3	1	1	−1	1
4	1	1	−1	−1
5	1	−1	1	1
6	1	−1	1	−1
7	1	−1	−1	1
8	1	−1	−1	−1
9	−1	1	1	1
10	−1	1	1	−1
11	−1	1	−1	1
12	−1	1	−1	−1
13	−1	−1	1	1
14	−1	−1	1	−1
15	−1	−1	−1	1
16	−1	−1	−1	−1
17	0	0	0	0
18	−1.414	0	0	0
19	1.414	0	0	0
20	0	−1.414	0	0
21	0	1.414	0	0
22	0	0	−1.414	0
23	0	0	1.414	0
24	0	0	0	−1.414
25	0	0	0	1.414

preliminary experiments and in the region that the protein is not denatured.

The orthogonal CCD based on 2^3 full factorial experimental designs is performed for this system. The experimental scheme is shown in Table 3. The ATPS are prepared according to the conditions of Table 3 and the samples of top and bottom phases are withdrawn and analyzed. The results of the equilibrium compositions in upper and lower phases together with the real values of the independent variables are reported in Tables 4–6. In Tables 5 and 6, K is the partitioning coefficient of protein defined as the ratio of weight percent of protein in the top phase to that in the bottom phase and, Y is the recovery percent defined as follows:

$$Y = \frac{100}{1 + RK} \quad (2)$$

where R is the volume ratio of top phase to bottom phase, and K is the partitioning coefficient of the protein.

The results of protein partitioning for cheese whey in ATPS of PVP–K_2HPO_4 at pH 7.5 are reported in Table 7.

Table 4
The equilibrium composition as weight percent w in ATPS of PVP (1) K_2HPO_4 (2) H_2O (3) αla (4).

Feed (wt%)			Top phase (wt%)			Botttom phase (wt%)		
w_1	w_2	w_4	w_1	w_2	w_4	w_1	w_2	w_4
24.3	14.0	0.01	46.07	1.74	0.019	0.57	26.13	0.000
24.2	13.9	0.01	48.14	1.83	0.019	0.94	26.29	0.000
24.4	13.9	0.01	47.11	1.04	0.020	0.76	27.43	0.000
24.3	13.9	0.01	44.88	1.78	0.016	0.96	26.39	0.004
16.5	14.0	0.01	46.62	3.21	0.015	1.43	19.97	0.004
16.4	14.0	0.01	39.80	1.76	0.015	0.95	21.41	0.002
16.6	14.0	0.01	39.64	1.53	0.013	0.65	22.57	0.009
16.5	13.9	0.01	37.39	2.34	0.018	0.81	24.72	0.000
24.4	9.8	0.01	44.09	1.86	0.019	0.81	19.66	0.002
24.4	9.7	0.01	40.38	4.40	0.022	2.92	16.86	0.004
24.4	9.8	0.01	39.72	1.85	0.013	1.05	21.42	0.008
24.4	9.8	0.01	34.98	2.72	0.017	0.40	25.15	0.005
16.7	9.8	0.01	36.13	3.55	0.015	3.42	14.62	0.003
16.6	9.8	0.01	32.09	3.82	0.010	6.02	13.62	0.010
16.7	9.8	0.01	30.71	3.06	0.011	3.78	16.14	0.009
16.7	9.8	0.01	28.63	4.04	0.015	6.65	14.15	0.002
20.4	11.8	0.01	46.28	1.68	0.014	1.92	20.45	0.007
20.5	8.8	0.01	37.91	2.78	0.019	5.15	13.78	0.004
20.5	14.8	0.01	52.09	1.20	0.018	1.05	24.44	0.003
15.0	11.8	0.01	39.58	2.76	0.015	3.88	15.75	0.005
26.0	11.9	0.01	48.21	1.53	0.021	1.39	23.87	0.004
20.5	12.0	0.01	45.57	2.26	0.032	2.20	20.00	0.014
20.5	12.0	0.01	50.00	2.55	0.047	1.86	18.61	0.003
20.5	11.8	0.01	41.57	2.86	0.000	3.25	19.45	0.000
20.5	11.8	0.01	42.78	1.66	0.019	2.22	19.81	0.002

Table 5
The weight percent of PVP (w_1) and salt (w_2) in feed, the partition coefficient and recovery percent of αla in ATPS of PVP (1) K_2HPO_4 (2) H_2O (3) αla (4).

w_1	w_2	T (°C)	pH	Y (%)	K
24.3	14.0	35.6	6.8	0.00	0.00
24.2	13.9	35.6	8.2	0.00	0.00
24.4	13.9	14.4	8.2	0.00	0.00
24.3	13.9	14.4	6.8	20.43	4.48
16.5	14.0	35.6	8.2	13.14	3.58
16.4	14.0	35.6	6.8	7.61	6.58
16.6	14.0	14.4	8.2	29.77	1.50
16.5	13.9	14.4	6.8	0.00	0.00
24.4	9.8	35.6	8.2	7.65	13.35
24.4	9.7	35.6	6.8	17.21	5.88
24.4	9.8	14.4	8.2	45.13	1.72
24.4	9.8	14.4	6.8	31.27	3.43
16.7	9.8	35.6	8.2	11.44	5.16
16.6	9.8	35.6	6.8	41.27	0.95
16.7	9.8	14.4	8.2	41.84	1.20
16.7	9.8	14.4	6.8	10.64	6.97
20.4	11.8	25	7.5	29.56	1.91
20.5	8.8	25	7.5	21.49	4.30
20.5	14.8	25	7.5	10.86	6.15
15.0	11.8	25	7.5	16.40	2.88
26.0	11.9	25	7.5	17.95	5.71
20.5	12.0	10	7.5	32.05	2.25
20.5	12.0	40	7.5	6.70	17.39
20.5	11.8	25	6.5	0.00	0.00
20.5	11.8	25	8.5	5.80	12.99

4. Results and discussion

The partitioning of αla and βlg depends on the salt weight percent in the feed, the PVP weight percent in the feed, temperature,

Table 6
The weight percent of PVP (w_1) and salt (w_2), βlg (w_5) in feed, the partition coefficient and recovery percent of βlg in ATPS of PVP (1) K_2HPO_4 (2) H_2O (3) βlg (5).

w_1	w_2	w_5	w_5 (Top)	w_5 (Bot.)	T (°C)	pH	Y (%)	K
24.3	14.0	0.01	0.009	0.009	35.6	6.8	50.66	1.02
24.2	13.9	0.01	0.009	0.010	35.6	8.2	52.19	0.92
24.4	13.9	0.01	0.003	0.014	14.4	8.2	85.11	0.21
24.3	13.9	0.01	0.000	0.017	14.4	6.8	0.00	0.00
16.5	14.0	0.01	0.001	0.029	35.6	8.2	94.26	0.03
16.4	14.0	0.01	0.008	0.016	35.6	6.8	50.35	0.53
16.6	14.0	0.01	0.000	0.029	14.4	8.2	0.00	0.00
16.5	13.9	0.01	0.018	0.006	14.4	6.8	18.65	3.17
24.4	9.8	0.01	0.008	0.012	35.6	8.2	62.54	0.66
24.4	9.7	0.01	0.000	0.018	35.6	6.8	0.00	0.00
24.4	9.8	0.01	0.000	0.017	14.4	8.2	0.00	0.00
24.4	9.8	0.01	0.011	0.009	14.4	6.8	55.05	1.28
16.7	9.8	0.01	0.004	0.019	35.6	8.2	74.08	0.23
16.6	9.8	0.01	0.007	0.015	35.6	6.8	60.90	0.43
16.7	9.8	0.01	0.000	0.021	14.4	8.2	0.00	0.00
16.7	9.8	0.01	0.017	0.010	14.4	6.8	32.44	1.73
20.4	11.8	0.01	0.014	0.008	25	7.5	32.56	1.66
20.5	8.8	0.01	0.014	0.008	25	7.5	38.89	1.85
20.5	14.8	0.01	0.005	0.020	25	7.5	75.24	0.25
15.0	11.8	0.01	0.001	0.029	25	7.5	94.27	0.03
26.0	11.9	0.01	0.000	0.020	25	7.5	0.00	0.00
20.5	12.0	0.01	0.025	0.021	10	7.5	46.93	1.20
20.5	12.0	0.01	0.011	0.031	40	7.5	78.54	0.34
20.5	11.8	0.01	0.000	0.000	25	6.5	0.00	0.00
20.5	11.8	0.01	0.009	0.013	25	8.5	53.71	0.69

Table 7
The equilibrium composition as weight percent w in ATPS of PVP (1) K_2HPO_4 (2) H_2O (3) cheese whey protein (4) at T = 298 K and pH 7.5.

Feed (wt%)			Top phase (wt%)			Botttom phase (wt%)		
w_1	w_2	w_4	w_1	w_2	w_4	w_1	w_2	w_4
12.1	20.5	1.31	2.11	46.60	0.25	19.02	2.65	0.32
9.1	20.5	1.31	3.61	37.20	0.32	15.69	3.82	0.24
15.0	20.6	1.31	1.41	50.60	0.14	22.82	2.21	0.45
12.0	15.0	1.31	3.04	44.23	0.40	16.28	3.28	0.22
11.9	25.9	1.31	1.50	51.19	0.34	22.30	2.23	0.26
12.1	16.6	1.31	2.62	41.59	0.49	17.54	3.03	0.17

and the pH. In order to investigate the effect of these parameters on protein partitioning, a model based on the results of experimental design is proposed. The behavior of the system is assumed to follow the quadratic equation as below:

$$w = A_0 + \sum_{i=1}^{k} A_i X_i + \sum_{i=1}^{k} A_{ii} X_i^2 + \sum_{i=1}^{k} \sum_{\substack{j=1 \\ i \neq j}}^{k} A_{ij} X_{ij} \quad (3)$$

where w stands for the dependent variables, X stands for the independent variables and A_0, A_i, A_{ii}, and A_{ij} are constant coefficients of different variables in the model. The experimental data (Tables 4 and 6) are interpreted by SPSS statistical software to estimate the dependent variables, and the coefficients of assumed quadratic equation are calculated. The independent variables and their interactions, which have been found to be insignificant are removed from the equation by a stepwise regression method, and hence, the Eqs. (4)–(11) are proposed for the weight percent of salt, PVP, αla, and βlg in the top and bottom phases of the ATPS.

$$w_1^{\mathrm{TOP}} = 63.60 - 2.578X_1 - 0.305X_3 - 10.216X_4 + 0.020X_2^2 + 0.003X_3^2 + 0.545X_4^2 + 0.303X_1X_4 - 0.123X_2X_4 + 0.020X_3X_4 \quad (4)$$

$$w_2^{\mathrm{TOP}} = -513.403 + 12.078X_1 + 7.584X_2 + 101.779X_4 - 0.425X_1^2 - 0.164X_2^2 - 6.690X_4^2 + 0.022X_3X_4 \quad (5)$$

$$w_{4-\alpha\mathrm{la}}^{\mathrm{TOP}} = -0.988 + 0.040X_1 + 0.020X_2 + 0.155X_4 - 0.002X_1^2 - 5 \times 10^{-4}X_2^2 - 0.010X_4^2 - 1 \times 10^{-4}X_1X_3 \quad (6)$$

$$w_{4-\beta\mathrm{lg}}^{\mathrm{TOP}} = -0.097 + 0.002X_1 + 0.015X_2 - 0.003X_3 - 4 \times 10^{-4}X_2^2 - 1 \times 10^{-4}X_3^2 - 3 \times 10^{-4}X_1X_4 - 1 \times 10^{-4}X_2X_3 \quad (7)$$

where w_1^{TOP}, w_2^{TOP}, $w_{4-\alpha\mathrm{la}}^{\mathrm{TOP}}$ and $w_{4-\beta\mathrm{lg}}^{\mathrm{TOP}}$ are the mass fractions of salt, PVP, αla and βlg in the top phase, respectively.

$$w_1^{\mathrm{BOT.}} = -11.931 + 4.949X_1 - 1.434X_2 - 0.421X_1X_4 + 0.274X_2X_4 - 0.009X_3X_4 \quad (8)$$

$$w_2^{\mathrm{BOT.}} = 81.563 - 5.859X_1 - 1.186X_2 - 6.333X_4 + 0.475X_1X_4 + 0.082X_1X_2 \quad (9)$$

Table 8
The R^2 values for each component in ATPS PVP–K_2HPO_4.

Equation	R^2
PVP in top phase	0.95
Salt in top phase	0.96
αla in top phase	0.91
βlg in top phase	0.93
PVP in bottom phase	0.96
Salt in bottom phase	0.97
αla in bottom phase	0.95
βlg in bottom phase	0.94

$$w_{4-\alpha\mathrm{la}}^{\mathrm{BOT.}} = -0.305 - 0.009X_1 + 0.002X_3 + 0.088X_4 - 3 \times 10^{-4}X_1^2 + 1 \times 10^{-4}X_2^2 + 1 \times 10^{-4}X_3^2 - 0.006X_4^2 - 2 \times 10^{-4}X_1X_2 + 1.4 \times 10^{-3}X_1X_4 + 1 \times 10^{-4}X_2X_3 - 4 \times 10^{-4}X_3X_4 \quad (10)$$

$$w_{4-\beta\mathrm{lg}}^{\mathrm{BOT.}} = -0.514 + 0.136X_4 - 5 \times 10^{-4}X_1^2 + 3 \times 10^{-4}X_2^2 + 1 \times 10^{-4}X_3^2 - 7.5 \times 10^{-3}X_4^2 - 4 \times 10^{-4}X_1X_2 + 2.7 \times 10^{-3}X_1X_4 - 1.5 \times 10^{-3}X_2X_4 - 7 \times 10^{-4}X_3X_4 + 1 \times 10^{-4}X_2X_3 \quad (11)$$

where $w_1^{\mathrm{BOT.}}$, $w_2^{\mathrm{BOT.}}$, $w_{4-\alpha\mathrm{la}}^{\mathrm{BOT.}}$ and $w_{4-\beta\mathrm{lg}}^{\mathrm{BOT.}}$ are the mass fractions of salt, PVP, αla and βlg in the bottom phase, respectively. In order to check the accuracy of the model, the results of each dependent variable with their coefficients are calculated to determine R^2. The R^2 values for mass percent of K_2HPO_4, PVP, αla, and βlg in the upper and lower phases are reported in Table 8. As the values of R^2 in the table shows, the model can well correlate the experimental data.

The experimental results for protein partitioning in Tables 5 and 6 indicate that αla prefers the PVP rich phase while βlg prefers the salt rich phase in most of the ATPS. These results are in agreement with the results of Coimbra et al. for the ATPS of PEG–potassium phosphate [9], and Rodrigues et al. for the ATPS of PEG–ammonium sulphate [11]. The results of the experimental design show that the weight percent of salt in the feed (X_1) has a great impact on the protein partitioning. The effect of salt weight percent in the feed is expressed as the interaction with the temperature and pH in the upper phase (Eqs. (6)–(7)), while the interaction with the PVP weight percent in the feed and pH are observed for the bottom phase (Eqs. (10)–(11)). By increasing the weight percent of salt in the feed, the αla partitioning coefficient is increased while the βlg partitioning coefficient is decreased. This behavior may be attributed to the hydrophobicity of the proteins. According to the Chen et al. the tryptophan dipeptide partitions strongly in favor of polymer rich phase, this dipeptide represents 5.2 mol% of total amino acid residues in αla, in contrast to 2 mol% in βlg [8]. The effects of the weight percent of salt in the feed on αla and βlg partitioning coefficients in ATPS of PVP–K_2HPO_4 are shown in Fig. 1.

The effect of PVP weight percent in feed (X_2) on αla and βlg partitioning coefficients is shown in Fig. 2. The partitioning coefficients of both proteins are increased by increasing PVP weight percent. The effect of this parameter on partitioning coefficient of αla is more significant than the effect on βlg partitioning coefficient. This behavior is also observed by Alves et al. [12] and Rodrigues et al. [11] for the ATPS of PEG–salt. As the pH of the ATPS is greater than the isoelectric point of the proteins, the tendencies of αla and βlg to the PVP rich phase are increased by increasing the PVP weight percent.

As it can be seen in Fig. 3, the temperature of the ATPS has a great impact on the partitioning coefficient of αla but its effect on

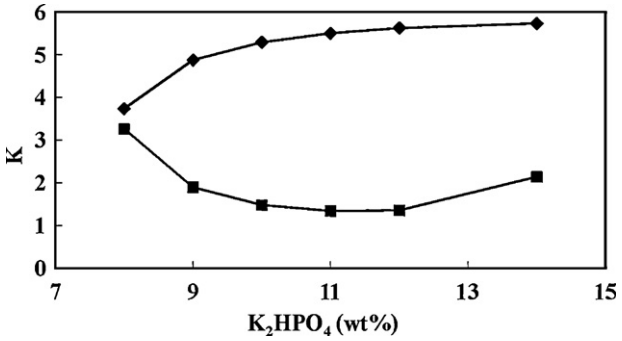

Fig. 1. The effect of K_2HPO_4 weight percent in feed on αla and βlg partitioning coefficients in ATPS of PVP (1) K_2HPO_4 (2) H_2O (3) αla or βlg (4) at temperature of T = 298 K, pH 7.5 and PVP weight percent in feed 20.4% (♦) αla, (■) βlg.

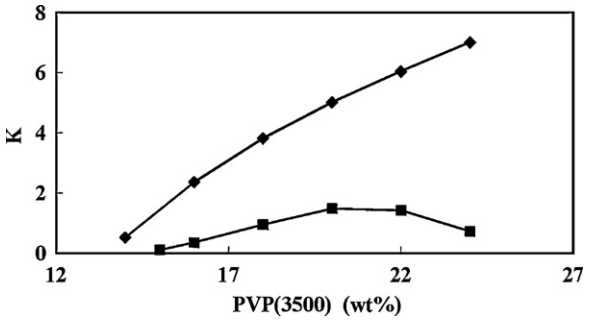

Fig. 2. The effect of PVP weight percent in feed on αla and βlg partitioning coefficients in ATPS of PVP (1) K_2HPO_4 (2) H_2O (3) αla or βlg (4) at temperature of T = 298 K, pH 7.5 and K_2HPO_4 weight percent in feed 9.8% (♦) αla, (■) βlg.

partitioning coefficient of βlg is small. This behavior may be due to the viscosity of the PVP. By increasing the temperature, the viscosity of PVP solution is reduced that facilitates the transfer of protein to the other phase.

The influence of the system pH on the protein partitioning is shown in Fig. 4. As the figure shows, the partitioning coefficients of proteins are decreased by increasing pH. This behavior can be due to the protein net charge. According to Albertsson equation, the value of the partitioning coefficient depends on the electrostatic and non-electrostatic contributions [7]:

$$\ln \quad K = \ln \quad K_0 + \frac{Z\Delta\Psi}{RT} \tag{12}$$

where Z is the protein net charge, $\Delta\Psi$ is the interfacial potential (difference between the electrical potential in the top and the bottom phases), and K_0 is the non-electrostatic term defined as the protein partitioning coefficient in the absence of electostatic effect. At pHs above the isoelectric point of proteins, the αla and

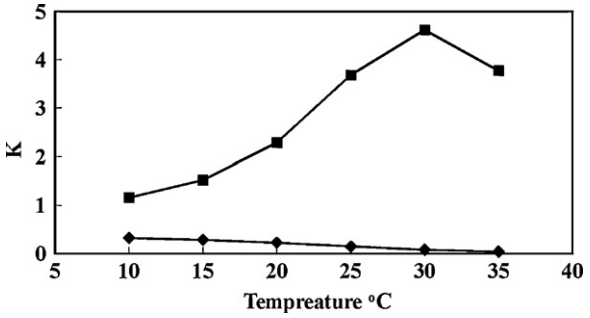

Fig. 3. The effect of temperature on αla and βlg partitioning coefficients in ATPS of PVP (1) K_2HPO_4 (2) H_2O (3) αla or βlg (4) at pH 8.2 and K_2HPO_4 and PVP weight percent in feed 14% and 16.5%, respectively (♦) αla, (■) βlg.

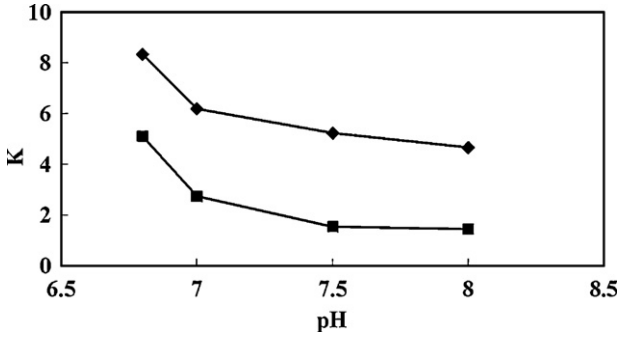

Fig. 4. The effect of pH on αla and βlg partitioning coefficients in ATPS of PVP (1) K_2HPO_4 (2) H_2O (3) αla or βlg (4) at temperature of T = 298 K, and K_2HPO_4 and PVP weight percent in feed 9.8% and 20.4%, respectively (♦) αla, (■) βlg.

βlg have both negative charges and the $\Delta\Psi$ provides positive values because the bottom phase is enriched by the phosphate anion. Therefore the electrostatic term of Albertsson equation will be negative ($Z\Delta\Psi/RT < 0$). The magnitude of increase of both the protein net charge and the interfacial potential at the medium pH is increased. Therefore the electrostatic term becomes more negative and the K value decreases.

5. Conclusion

αla and βlg constitute more than 90% of the cheese whey proteins. These proteins have a high nutritional value and can be used in food industries. Although ATPS is a powerful technique for separation of the proteins, the experimental data on partitioning of these proteins are not sufficient. In this research, new experimental data for partitioning of αla and βlg in ATPS of PVP–K_2HPO_4 have been presented. The experimental data show that the partitioning of the proteins in this system depends on the weight percent of salt in the feed, the weight percent of PVP in the feed, temperature, and pH. In order to study the effect of these parameters on protein partitioning, the orthogonal CCD based on the full factorial experimental design is applied to fit a polynomial model for the ATPS. The proposed model has a good agreement with the experimental data. The results of the model show that increase of the weight percent of salt in feed increases the αla partitioning coefficient. This behavior is reversed for βlg partitioning coefficient. The weight percent of PVP in the feed enhance the partitioning coefficients of both proteins while its influence on the αla partitioning coefficient is more significant. The effect of temperature on the partitioning coefficients of proteins is attributed to the variation of the viscosity of PVP solution. By increasing the temperature, the viscosity of PVP is reduced and the protein can easily transfer from one phase to the other phase. Reduction of αla and βlg partitioning coefficients by increasing pH may be attributed to the protein net charge. The experimental results shows the partitioning coefficients of αla and βlg are decreased by increasing pH.

In conclusion, the ATPS process of PVP–K_2HPO_4 described in this work, is shown to be an efficient and economical process for separation of cheese whey proteins. The experimental data are assisted to design an appropriate process for large scale applications.

Nomenclature

nD	refractive index
α_0	refractive index of water
α_1	refractive index coefficient for salt
α_2	refractive index coefficient for PVP
$x1$	salt weight percent in feed

$x2$	polymer weight percent in feed
$x3$	temperature
$x4$	pH
Wi	weight percent of species
R	the ratio of the volume of top phase per bottom phase
Y	recovery percent
A_i	linear coefficient in the model
A_{ii}	squared coefficient in the model
A_{ij}	interaction coefficient in the model
A_0	constant coefficients in the model
K	the partitioning coefficients of protein
R	the model accuracy or the gas constant

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