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Influence of system and process parameters on partitioning of cheese whey proteins in aqueous two-phase systems

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Abstract

A practical study is described to characterise some problems encountered in the application of aqueous two-phase systems (ATPS) to protein recovery. These factors include practical design of extraction stages and the impact of ATPS compounding methods and biological suspension upon process performance. They were addressed using the recovery of whey proteins as a model. The known effects of system parameters (i.e. tie-line length, volume ratio and system pH) were exploited to define the specific operating conditions of a two-stage ATPS process for the recovery of whey proteins. The partition of whey proteins in ATPS assembled using different methods resulted in changes in the partition coefficient of the proteins. Such changes were associated with the initial location of the proteins in the polymer or salt-rich solutions of the ATPS. Cheese whey loaded into the ATPS caused the displacement of the binodal curve from the origin. Such behaviour was attributed to the residual fat present in the whey. These findings highlight those factors perceived as negative constraints on the wider adoption of ATPS processes for protein recovery from complex biological systems.

Keywords: Aqueous two-phase systems; Partitioning; Cheese whey; Proteins

1. Introduction

Aqueous two-phase systems (ATPS) are formed by mixing aqueous solutions of hydrophilic polymers or polymer/salt combinations above a defined concentration [1]. The potential of ATPS involving poly(ethylene glycol) (PEG) and phosphate to be used for the recovery of macromolecules from fermentation broth and biological extracts has been demonstrated as an alternative to conventional processes for particle and solute handling [2]. However, the adoption and commercial application of extraction processes exploiting ATPS for the recovery of value products from biological suspensions require: (i) well characterised operating conditions that can be applied to a wide range of two-stage processes and (ii) an understanding of the process disadvantages attributed to two-phase partitioning [2].

A two-stage ATPS protein purification process is characterised by a first-extraction stage in which particles (cells or cell debris) and contaminants (e.g. RNA, carbohydrates, lipid) from the biological extract are eliminated in the bottom phase. This extraction stage generates a top phase enriched in the target soluble protein fraction. The high concentration of PEG in the top phase compromises the value of the product and presents practical problems in handling such a viscous phase. Consequently, in the second extraction stage (back extraction) the

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protein is partitioned to a more suitable environment (bottom salt-rich phase), which enables reuse of the polymer-rich top phase [3]. Unfortunately, to establish the partition conditions for each recovery process, time-consuming empirical experiments are required. However, by exploiting the known effect of system parameters (PEG and phosphate concentration, system pH, volume ratio, etc) on protein partitioning, two-stage ATPS processes can be designed and further implemented. In addition, the robustness of the process depends on the impact of process parameters (method of ATPS assembly, nature of the biological extract, etc.) on the ATPS. Therefore an understanding of such parameters may provide the basis for a well-characterised primary-stage process for the recovery of value-added products from biological suspensions.

In this research, the two-stage process for the recovery of proteins from cheese whey using PEG of nominal molecular mass 1000 Da and phosphate salt was selected as a practical model to achieve the objectives of this work. The selection of PEG 1000/phosphate was based on previous work [4]. We have identified a number of factors that are perceived as negative constraints on the wider adoption of this flexible separation technique. Thus this paper addresses some of these potentially negative factors including: (i) the practical design of an ATPS extraction process exploiting the known effect of systems parameters upon protein partition behaviour, (ii) the impact of the method of ATPS assembly upon protein partition and (iii) the impact of the biological suspension upon phase formation.

2. Experimental

2.1. Characterisation of PEG-phosphate two-phase systems

The binodal curves were estimated by the cloud-point method [5] using poly(ethylene glycol) (PEG, Sigma Chemicals, St. Louis, MO, USA) of nominal molecular mass, 1000 Da (50% w/w stock solution) and dipotassium hydrogen orthophosphate or potassium dihydrogen orthophosphate (Sigma) (30% w/w) according to required pH. Fine adjustment of pH was made by addition of orthophosphoric acid or sodium hydroxide. The systems tie-line length (TLL), which represents the length of the line that connects the composition of the top and bottom phase of a defined ATPS is calculated as described by Albertsson [1].

2.2. Influence of system parameters upon protein partition: design of an aqueous two-phase process

All experimental systems used to establish the operating conditions for the ATPS process were compounded for convenience on a fixed-mass basis on a top-loading balance. Predetermined quantities of solid PEG of a nominal molecular mass of 1000 Da, dipotassium hydrogen orthophosphate and potassium dihydrogen orthophosphate were mixed with cheese whey (obtained from a local dairy industry) to give a final weight of 15 g. Polymer and salt were dissolved and phases dispersed by gentle mixing for 30 min at 25°C. Complete phase separation was achieved by low-speed batch centrifugation at 1500 *g* for 20 min at 25°C. Visual estimates of the volumes of top and bottom phases and solids, were made in graduated centrifuge tubes. The volumes of the phases were then used to estimate the volume ratio (V_r=volume of the top phase/volume of the bottom phase). Samples were carefully extracted from the phases and diluted for biochemical analysis and subsequent estimation of the protein partition coefficient (K= concentration of solute in the top phase/concentration of solute in the bottom phase). Protein concentration in the samples was estimated by the method of Bradford [6].

2.3. Influence of process parameters on protein partition

2.3.1. Impact of the method of ATPS assembly on cheese whey protein partition coefficient

In order to examine the impact of ATPS assembly on observed protein partition, four ATPS of changing tie-line length (31.0 to 46.2%w/w) were selected. The four ATPS were assembled using cheese whey in three different studies (methods of assembly 1 to 3). The first set of experiments (method 1) was characterised by dissolving the appropriate amount of PEG and phosphate in the whey. The data from this set of experiments was used as a practical

reference. In the second set of experiments (method 2), solid PEG was mixed in whey and after dissolution to an appropriate concentration was placed in contact with a solution of phosphate of a fixed concentration. In the third set of experiments (method 3) solid phosphate was dissolved in whey and the phosphate-protein solution so generated was placed in contact with an appropriate PEG solution. Partition experiments were then completed by adjusting the pH to 7.0 with orthophosphoric acid. After mixing for 30 min, the phases were separated by batch centrifugation (25°C, for 20 min at 1500 *g*). Samples were taken for protein analysis and subsequent estimation of the protein partition coefficient.

2.4. Impact of cheese whey upon the position of the binodal curve

Six systems close to the binodal were selected (I to VI in Fig. 1) to study the impact of whey upon the position of the phase diagram. The systems were assembled as described above. Once the systems were carefully constructed, addition of whey was started. This was repeated for each system, until the existence of a biphasic system was not observed (monophasic region). The total amount of whey added for each system was recorded and used to estimate the composition (PEG and phosphate concentration) of the resulting system.

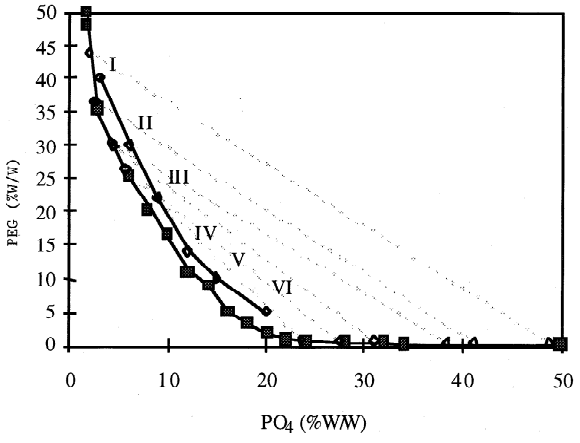

Fig. 1. ATPS selected for the study of the impact of cheese whey on the position of the binodal curve. The six systems (I to VI) were selected close to the binodal to evaluate the impact of the addition of cheese whey upon the position of the binodal. All systems were assembled as described in Section 2.

3. Results and discussion

3.1. Influence of system parameters on protein partition: design of an aqueous two-phase process

The predictive design of extraction processes using aqueous two-phase systems (ATPS) is limited by poor understanding of the molecular mechanism governing the behaviour of proteins in ATPS. Consequently, for each purification process, once general conditions have been selected on the basis of experience (polymer and salt type, molecular mass, etc.), more specific partition conditions (pH, salt and polymer concentration, etc.) need to be empirically established. In the latter study, the use of a practical approach which exploits the known effect of system parameters such as tie-line length (TLL), system pH and volume ratio (V_r) on the protein partition coefficient should reduce the number of empirical experiments. Correlation of the impact of increasing TLL on the protein partition coefficient with changes in the volume of the solvent of either phase available for protein and salt solubilisation has been attempted by several workers [7,8]. With TLL increasing, the free volume of the bottom phase decreases and promotes the partition of protein from the bottom phase to the top phase or to the interface. The impact of increasing V_r on the partition coefficient has also been previously reported [9,10].

In the present study, the recovery of protein from cheese whey was selected as a model process to establish the operating conditions of a two-stage ATPS processes using the approach described above. For this biological suspension or feedstock, it has been reported [11] that the major proteins, α-lactalbumin (α-La) and β-lactoglobulin (β-Lg), account for 50% (w/w) and 12% (w/w) respectively, of the proteins in cheese whey. Once the feedstock was selected, the impact of the selected system parameters (i.e. TLL, V_r and system pH) upon protein partition and protein recovery was investigated. The effect of TLL on partition coefficient was evaluated in six selected ATPS (Table 1). These systems characterised by increasing TLL (31.0 to 67.0%

Table 1
Effects of system tie-line lengths (TLL) on protein partition coefficient and estimated yield

System TLL (% w/w)	Concentration of PEG (% w/w)	Y_t (%)	Y_b (%)	Partition coefficient (ln K)
31.0	15.0	60.7	45.9	0.32
34.1	16.0	60.6	40.0	0.62
36.7	17.5	71.0	20.9	1.26
46.2	20.0	66.8	9.5	2.11
54.0	23.0	80.0	1.0	4.50
67.0	26.0	62.8	0.4	5.73

The tie-line lengths (TLL) of the systems were estimated from the composition of PEG and phosphate in the phases as described in Section 2. Y_t and Y_b represent the recovery of soluble protein in the top and bottom phases, respectively and are expressed relative to the original protein loaded to the systems. The partition coefficient represents the relative concentration of the protein between the phases. For all systems, volume ratio (estimated from nonbiological experimental systems) and the system pH were kept constant at 1.0 and 7.0, respectively.

w/w) and constant pH (pH 7.0) were located in a region of the phase diagram that promotes a volume ratio close to unity.

Table 1 illustrates the impact of TLL on the protein partition coefficient and phase recovery of whey proteins. The results show that increasing TLL caused both the partition coefficient of bulk protein (ln K) and the estimated protein yield (Y_t) from the top phase to rise. The increase of the partition coefficient can be correlated with the significantly negative impact of increasing TLL on the free volume of the bottom phase [7], thereby promoting protein partition to the top phase (Fig. 2). This, together with a constant volume ratio, produced an increasing trend in the estimated top-phase protein yield. However, the estimated yield from the upper phase exhibited a decrease for the system TLL of 67% w/w. This may be attributed to the fact that the free volume of the top phase is totally occupied by the partitioned protein (caused by the increased TLL) and once phase saturation is achieved, protein precipitation occurs. Such an explanation can be confirmed by a close examination of the estimated protein yield from the bottom phase (Y_b). For the systems close to the binodal curve (TLL of 31 to 36.7% w/w), Y_b decreased with the increase in TLL, which can be accounted for by the protein partitioned to the top phase. However, in the case of the systems distant from the binodal curve (TLL of 46 to 67% w/w), such reduction in Y_b was due to both the facts: (i) protein partitioned to the top phase and (ii) protein precipitation, as demonstrated by the protein mass balance (compare Y_t+Y_b for these systems in Table 1).

From the study of the influence of TLL on protein partition, the system comprising PEG 23% w/w, 54% w/w TLL, V_r of 1.0 and pH 7.0 provided the best conditions to satisfy the needs of protein yield (80%) and fine solid removal (data not shown). At this point, the influence of V_r upon protein partition and a further increase in process protein yield was examined. However, although changes in the parti-

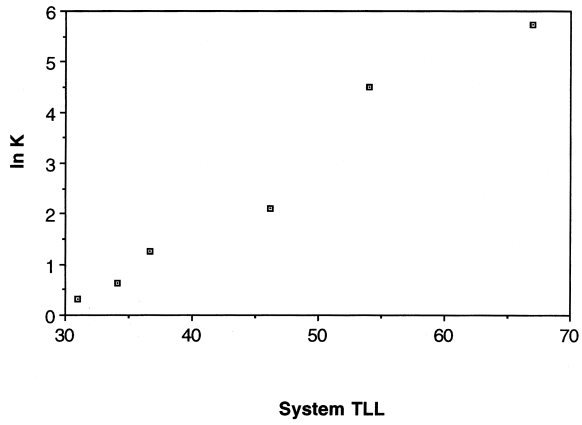

Fig. 2. Variation of partition coefficient with system tie-line lengths (TLL). The tie-line lengths (TLL) of the systems were estimated from the composition of PEG and phosphate in the phases as described in Section 2. The partition coefficient represents the relative concentration of the protein between the phases. For all systems, volume ratio (estimated from nonbiological experimental systems) and the system pH were kept constant at 1.0 and 7.0, respectively.

tion coefficient were associated with changes in the system V_r, an insignificant increase in the estimated top-phase protein yield was observed [12]. Consequently, the $V_r=1.0$ was kept constant.

The influence of system pH on protein partition behaviour was further examined to finally establish the specific operating conditions for the ATPS extraction. The influence of system pH on the partition of protein has been discussed by Walter et al. [13] and recently for bulk protein has been discussed by Flanagan [9] for milled brewers yeast. It was reported that increasing the pH in a range from 6.5 to 9.0 caused an increase in the protein concentration in the top phase and a decrease in the bottom phase. Such behaviour of proteins has been attributed to free-volume effects [8]. An alternative explanation may be associated to the speciation of the phosphate salts over this pH range and to conformational changes in the structural integrity of proteins [14]. Table 2 shows the influence of pH on partition coefficient and estimated protein yield of whey proteins. Partition coefficient increased with increasing pH (Fig. 3).

Some researchers suggested that since the repeating ether oxygen atoms of PEG are capable of forming metal ion associations, PEG behaves as if it were positively charged [15]. Polyions, such as proteins above their isoelectric point, will be accepted more favourably by the PEG-rich top phase as the system pH increases. The pH values used in these experiments were above the isoelectric points of whey proteins, (4.2–4.5 for α-La and 5.2 for β-Lg [11]), which agrees with this theory. Although, an increase in the estimated yield of top phase protein

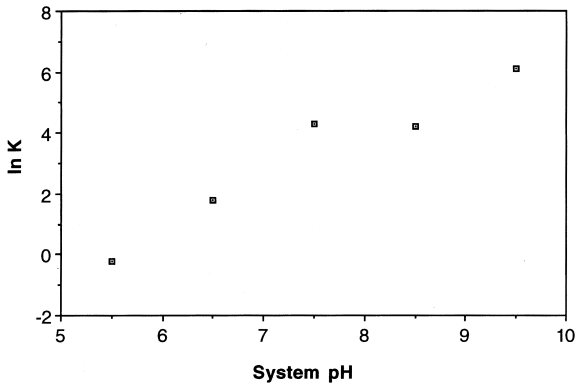

Fig. 3. Variation of partition coefficient with system pH. The partition coefficients represent the relative concentration of the protein between the phases and are expressed relative to system pH. System pH was adjusted as described in Section 2.

was possible by raising the system pH (from 5.5 to 9.5; see Table 2), it was beneficial to carry out the partition at neutral pH, to recover a fully functional protein since β-Lg was reported to denature at pH above 8.6 [16]. Thus, the specific operating conditions chosen for the first ATPS extraction stage were: PEG 23% w/w, TLL of 54% w/w, $V_r=1.0$ and pH 7.0.

In order to establish specific operating conditions for the back-extraction of the ATPS stage that promotes the partition of whey proteins to the phosphate-rich bottom phase (and remove the PEG from the proteins), the known effect of TLL, V_r and system pH on partition behaviour of such proteins was exploited. In order to avoid promoting proteins to the top phase, ATPS with a short TLL (i.e.30%

Table 2
Effects of system pH on protein partition coefficient and estimated yield

System pH	System V_r	Y_t (%)	Y_b (%)	Partition coefficient (ln K)
5.5	1.6	47.1	36.3	−0.21
6.5	1.2	64.1	8.8	1.80
7.5	1.0	77.6	1.0	4.3
8.5	0.9	83.0	1.6	4.2
9.5	0.8	80.6	0.2	6.1

System pH was adjusted as described in Section 2. The volume ratio (V_r) in nonbiological experimental systems along a single tie-line length (54% w/w) was estimated after phase separation in graduated centrifuge tubes. Y_t and Y_b represent the recovery of soluble protein in the top and bottom phase respectively and are expressed relative to the original protein loaded to the systems. The partition coefficient represents the relative concentration of the protein between the phases.

Table 3
Effects of system volume ratio (V_r) on protein partition coefficient and estimated yield

System V_r	Concentration of PEG (% w/w)	Y_t (%)	Y_b (%)	Partition coefficient (ln K)
0.18	4.6	22.0	65.5	2.1
2.10	18.0	63.0	28.0	1.1
4.20	21.2	66.4	16.0	1.0
6.3	23.0	63.0	11.1	0.9

The volume ratio (V_r) in nonbiological experimental systems along a single tie-line length (30% w/w) was estimated after phase separation in graduated centrifuge tubes. Y_t and Y_b represent the recovery of soluble protein in the top and bottom phase respectively and are expressed relative to the original protein loaded to the systems. The partition coefficient represents the relative concentration of the protein between the phases. System pH was adjusted at 6.0 as described in Section 2.

w/w) and low pH (6.0) were selected. In the selected ATPS, an increase in the V_r caused the protein partition coefficient (ln K) to decline (see Table 3). These results appear to question the theoretical basis proposed by Hustedt et al. [17] for the estimation of protein yield in the phases, which assumes that the partition coefficient remains constant for systems along the same tie-line. Changes in the protein partition coefficient of whey proteins with V_r of ATPS along the same tie-line could be attributed to protein precipitation (see protein mass balance, $Y_t = Y_b$, for system V_r 4.2 and 6.3 in Table 3) due to phase saturation. Proteins in systems along an identical tie-line, exhibit phase preference (either top or bottom phase). Reduction of the volume of a phase preferred by proteins will cause them to concentrate and eventually precipitate when the limits of protein solubility are exceeded. As a result, the practical protein yield from that phase will decline. Protein precipitation may explain the decreasing trend of bottom phase protein yield observed in Table 3.

The system comprising $V_r = 0.18$, PEG 4.6% w/w, TLL of 30% w/w and pH 6.0 provided the best conditions to satisfy the needs of maximum protein yield (65.5%) for the back-extraction ATPS stage. The operating conditions for the two-stage process produced an overall whey protein recovery of 53%. Fractionation of sample phases (top and bottom phases from first and back-extraction ATPS stages) by SDS–PAGE (data not shown) suggested that the majority of protein present in the cheese whey (as represented by coomassie blue stained bands) partitioned initially to the top phase and subsequently to the bottom phase in the back-extraction [18]. As a result, a concentrate of α-La and β-Lg was obtained in the phosphate-rich bottom phase. Once the model process of the recovery of whey proteins by using ATPS was obtained, the impact of selected process parameters (i.e. the nature of the feedstock and the methods of ATPS assembly) on the performance of the established process were investigated.

3.2. Influence of process parameters on protein partition

3.2.1. Impact of the method of ATPS assembly on cheese whey protein partition coefficient

During the formulation of aqueous two-phase systems, it may be appropriate to consider that individual proteins may experience different effects based on the method of system assembly. ATPS are commonly made with stock solutions of PEG, phosphate and preparations of standard commercial proteins. However, an alternative practical approach is adopted when biological systems are handled (e.g. cheese whey) under normal processing conditions. This involves dissolving solid PEG and phosphate directly in the biological suspension, which apart from being practically convenient, serves to maintain a high throughput during the process. During mixing of the biological suspension and the solid PEG and phosphate, it is likely that the proteins are effectively in contact with a range of dynamically changing PEG and phosphate concentration (from low to high concentration values). Consequently, it can be assumed that proteins partition in different 'sub-ATPS', created during the mixing procedure, until the solid and phosphate are totally dissolved and well

mixed. The necessary proteins may cross and recross multiple interfaces to arrive at equilibrium, confronting the protein with different physical and chemical forces at the interface. This invites the assumption that such phenomena may have a significant impact on protein solubility and as a consequence on the recorded partition behaviour. In this context, reports which discuss the impact of the different methods of assembly upon the partition behaviour of protein are not common; but refer to Rito-Palomares and Lyddiatt, 1997 [4].

The full characterisation of an established ATPS process, demands the investigation of the physical consequences of the phenomenon of protein partition, especially those related to the protein crossing the interface. In order to investigate the impact of the methods of ATPS assembly on whey protein partition behaviour, four experimental ATPS were chosen of variable TLL but whose volume ratios were close to unity. The influence of increased TLL and the method of systems assembly upon the partition coefficient (ln K) is illustrated in Table 4. It is clear that the increased concentration of the phase components associated with increased TLL caused the partition behaviour of the whey proteins to rise in the three differently assembled systems. Such behaviour can be explained (as before in this paper) by changes in the free volume of the phases [8]. However, at long TLL (i.e. 46.2% w/w), the partition coefficient of the method of assembly 3 (in which the protein originated in the phosphate-rich solution) decreased. This behaviour can be attributed to the fact that the surface tension at the interface and the difference in the density of the phases increase with increasing TLL [5,19]. The free volume in the bottom phase decreases when the TLL is increased. As a result, the whey proteins in the lower phase may be promoted to partition to the top phase. However, the suggested increase of the surface tension limits protein migration across the interface from the bottom to the top phase. Consequently, the adverse conditions encountered by the proteins may cause precipitation at the interface (more than 35%, see P_{tot} value in Table 4). If protein precipitation occurred, this might account for the lower protein content in the top phase. Thus, the majority of the protein loaded remained in the bottom phase (compare Y_t and Y_b in Table 4 for this system).

It is evident from the results of Table 4 that changes in the partition coefficient of whey proteins occurred at short TLL for the different methods of systems assembly. The sensitivity of the ATPS close to the binodal to changes in system composition reported by Albertsson [1] can be used to explain

Table 4
Impact of the method of ATPS assembly on cheese whey protein partition coefficient

System TLL (% w/w)	Method of assembly	Partition coefficient (ln K)	Change in K (Δlog K)	Y_t (%)	Y_b (%)	P_{tot} (%)
31.0	1	0.34	0.0	56.3	40.0	96.3
	2	0.50	0.07	57.5	34.4	91.8
	3	0.00	−0.15	43.8	43.8	87.5
34.0	1	0.69	0.0	65.0	32.5	97.5
	2	0.83	0.06	57.5	25.0	82.5
	3	0.70	0.0	37.5	18.8	58.3
36.7	1	1.29	0.0	68.8	18.7	87.6
	2	1.28	0.0	45.0	12.5	57.5
	3	1.09	−0.08	37.5	12.5	50.0
46.2	1	2.30	0.0	62.5	6.25	68.8
	2	2.26	−0.02	60.0	6.25	66.3
	3	−0.70	−1.30	18.8	37.5	56.3

The different methods of ATPS assembly (1 to 3) are described in Section 2. Changes in K (Δlog K) are expressed relative to method 1. Y_t, Y_b and ln K are the same as in Table 3. P_{tot} represent the addition of Y_t and Y_b.

such differences between the partition behaviour of the proteins from the three different methods of assembly. Albertsson reported [1] that small changes in the composition of the PEG and phosphate is associated with great changes in the phase composition of the systems and, as a result, in the partition characteristics of the ATPS. An alternative explanation may be that associated with the different nature of the methods of systems assembly (e.g. proteins originated in different environments) or a combination of the two previous explanations. In this context, for systems located distant from the binodal curve (long TLL) no significant changes were observed between the partition coefficient of methods 1 and 2 (see Table 4). However, the partition coefficient of whey proteins from the assembly method 3, exhibited a particular behaviour. In these systems the decrease in the partition coefficient (compared with the other two methods; 1 and 2) may be attributed to protein precipitation at the interface caused by the increase in both the surface tension (resistance forces) and the preference of the proteins, initially loaded at the phosphate-rich solution, for the top phase, at conditions of long TLL.

Further analysis of the results from the alternative methods of ATPS assembly (methods 2 and 3; Table 4) revealed a significant increase in protein precipitation compared with the reference method as the TLL increased, particularly in the case of method 3. Such protein behaviour can be explained by the fact that proteins are initially loaded into extreme conditions (high concentration of PEG and phosphate) before the final partition conditions are achieved. This situation became more evident at long TLL in which both, the observed protein precipitation and the concentration of PEG and phosphate were higher compared with systems close to the binodal curve. From these results it can be concluded that for the processing of cheese whey by an ATPS process, the method of ATPS assembly does have an impact upon the partition coefficient of the whey protein, associated with the position of the ATPS with respect to the binodal curve (i.e. TLL). However, such changes are attributed to the sensitivity of the ATPS and an increase in protein precipitation from the alternative methods (2 and 3) compared with the reference method. It is clear that such a situation severely restricted the practical application of these two alternative methods to ATPS processes.

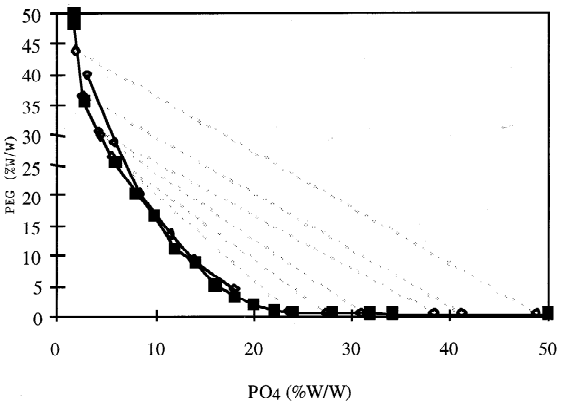

Fig. 4. Impact of cheese whey on the position of the binodal curve. The original position in the binodal of the six systems (I to VI) selected is depicted in Fig. 1. The new position of the binodal curve with the presence of cheese whey was estimated by careful addition of cheese whey to the selected ATPS until the monophasic region was achieved as described in Section 2.

3.3. Impact of cheese whey on the position of the binodal curve

It has been established that several system parameters (e.g. system pH, molecular mass of PEG, type of salt, etc.) have an impact on the position of the binodal curve [20]. The understanding of such effects has been exploited to select operating conditions for ATPS processes. However, reports which discuss the effect of the biological suspension on the position of the binodal curve are not common (see Rito-Palomares and Lyddiatt, [4] and Kohler, 1991 [20]). These authors addressed the impact of a yeast suspension on the binodal curve. In these cases, displacement towards the origin of the binodal curve was attributed to the presence of cell debris and intracellular biopolymers, which make a critical contribution to the systems and reduce the amount of phase chemicals required to form two phases. Such behaviour has been exploited to eliminate cell debris from a biological suspension during protein recovery. In the present research, six ATPS close to the binodal were selected to examine the behaviour of cheese whey on the position of the binodal curve.

The impact of cheese whey on the position of the binodal curve is illustrated in Fig. 4. In contrast to

what was expected, a slight displacement of the binodal away from the origin was observed at high concentration of PEG. Such behaviour of the binodal curve was confirmed by an unsuccessful attempt to construct ATPS with cheese whey using PEG and phosphate composition obtained directly from systems located at the binodal curve (data not shown). The addition of biological suspensions affects the phase formation in a manner that at present cannot be fully explained. For the case study here, it was suggested that the presence of residual fat in cheese whey caused the upwards shift of the binodal curve [21]. However, further characterisation of the biological suspension used (cheese whey), together with a more practical approach using simplified models, is required to untangle such a phenomenon. From the process point of view, the observed behaviour of the binodal curve with the presence of whey implies that more chemicals (i.e. PEG and phosphate) need to be used for the construction of ATPS.

4. Conclusions

Problems encountered in the practical application of ATPS to protein recovery (i.e. practical design of extraction stages, the impact of methods of ATPS assembly and biological suspension on process performance) were addressed using the process of recovery of whey protein as a model. The known effects of system parameters (i.e. tie-line length, volume ratio and system pH) were exploited to define the specific operating conditions of a two-stage ATPS process for the recovery of whey proteins. Fractionation of cheese whey was characterised by stages for the successive removal of fine-suspended solids and polymer. The successful development of a two-stage ATPS process, capable of operation under conditions of suspended solids and protein contaminants, demonstrates the potential for the application of a practical approach for the design of ATPS processes for primary recovery and partial purification of target solutes from complex biological suspensions.

In the study of the impact of three different methods of ATPS assembly upon the whey protein partition behaviour, it was concluded that protein originating in the phosphate-rich solution exhibited the lowest protein partition coefficient when compared with that from systems with proteins originally in the PEG-rich solution or loaded at PEG-phosphate dissolution. Such behaviour was associated with protein precipitation caused by increasing changes in the free-volume of the phases and the resistant forces at the interface in tandem with the initial location of the proteins in the ATPS.

It was shown that cheese whey caused the binodal curve to move away from the origin at high concentrations of PEG. Such behaviour was attributed to residual fat present in the whey. We conclude that studies of the type discussed here indicate that further work on the practical problems encountered in the generic implementation of ATPS processes is needed, in order to promote the commercial application of this technique.

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