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Redefinition of working aqueous two-phase systems: A generic description for prediction of the effective phase chemical composition for process control and biorecovery

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ABSTRACT

Aqueous two-phase systems (ATPS) have been widely adopted for the combined purpose of solid liquid separation, and recovery and purification of bioproducts such as proteins, viruses and organelles from biological feedstocks and fermentation broth. However, in spite of potential advantages over other techniques applied to concentrated biological feedstocks, ATPS have been applied at process scale only by a few industries and research establishments. ATPS are sensitive to loading with modest to extreme quantities of biological feedstock due to the contribution of that material to phase formation in combination with the conventional phase-forming chemicals. This causes problem associated with the definition and manipulation of loaded working systems, which may be addresses as in the present study with the aid of distribution analysis of radiolabel led analytes (DARA) in representative process samples. The present study focussed on establishing a generic description for characterising ATPS loaded with biological feedstocks and the redefinition of the biological feedstock loaded system composition in terms of phase forming chemical equivalents. This evaluation will be useful to achieve ATPS process implementation where phase recycle/reuse is adopted without compromise to process operations and consistent protein recovery performance.

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1. Introduction

Aqueous two-phase systems (ATPS) have been utilised for the recovery of value-added proteins from biological feedstock [1–5]. However, ATPS wider application in bioseparation has been restricted by (i) empirical protocols necessarily adopted to identify and exploit suitable processes [6], (ii) impact of biological feedstock upon system parameters, (iii) cost of phase forming chemicals [7,8] and (iv) environmental problem associated with the disposal of phase chemical after recovery processes. ATPS have thus not been widely adopted commercially for the large-scale recovery of biological products. The impact of the biological feedstock when loaded into ATPS upon the position of the binodal curve in the phase diagram is a major, and largely unquantified, consideration. The quantity and nature of the biomass load are increasingly recognised as strongly influencing phase separation. It is widely reported that the inherent incompatibility between phase forming chemicals in an ATPS increases in the presence of added biological feedstock, thus promoting phase separation at lower added chemical concentrations [9,10]. Well-characterised clean ATPS (e.g. PEG-phosphate) are thus immediately ill defined when loaded with complex feedstock. Such observations are generally reported to influence the position of the binodal curve in the phase diagram and variation in system parameters, such as volume ratio, partition co-efficient and tie-line length (TLL).

It has been reported that the binodal curve moves position under the influence of added biomass [7]. This conclusion was drawn from the observation that biphasic systems could be formed by the addition of biomass to systems selected just below the binodal curve (in the monophasic area) of the phase diagram. This encouraged an idea that the binodal curve displaced downwards upon biomass addition [9]. However, it has also been suggested that the binodal curve moves up in some systems where the biphasic system converts into a monophasic system upon addition of biomass typical of cheese whey which is salt-rich in content [11]. Both observations (downward or upward movement of binodal curve) indicate a need to redefine the binodal and the phase dia-

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gram in the presence of added biomass in order to characterise and quantitatively manipulate key features of a working separation system. Many research groups have characterised ATPS in the presence or absence of biomass [12,13]. However, certain studies have focussed upon developing a method that could be generically applied to representative *working* ATPS with biomass concentration up to 20–50% (wet w/w) [10,14]. The approach helped to identify the variation in biomass loaded ATPS with reference to a specific condition, but such data (i.e., obtained values of effective tie-line length (TLLe) could not be adopted due to insufficient information available to reposition biomass loaded system in the original phase diagram and/or to redefine phase chemical composition [9]. There was a clear need for further understanding on the variation of system parameters with varying biological feedstock addition. This information is an essential prerequisite for the maintenance of consistent partition performance of any selected ATPS or for the purpose of reuse/recycling of phase chemicals for economic advantage and to yield a process advantage, such as to predict the phase chemical composition or obtain requisite partition performances of candidate systems [14,15].

The impact of added biomass upon selected systems, and its influence has been addressed in the present study using as representative biological feedstock preparations of citrated bovine blood. Close monitoring of the variation in the system effective tie-line length and other parameters based upon the distribution analysis of radiolabelled analytes (DARA) in representative process samples was undertaken as previously described [9]. The mechanistic basis of biomass addition and its influence upon the system parameters were evaluated using different fractions (plasma, red blood cells (RBC), haemoglobin and RBC membrane protein) isolated from whole bovine blood to facilitate individual studies.. These observations were then compared with the behaviour of ATPS loaded with whole bovine blood. Comparison of these influences *in tandem* with variation in TLLe observed for each system (fraction loaded ATPS) provided an understanding of the molecular mechanistic basis of the impact of blood loading upon ATPS behaviour. Such information was used to redefine the properties of biomass loaded ATPS (in terms of equivalent phase chemical composition) previously reported as a restrictive bottleneck in many studies conducted with different biological feedstocks. The current study demonstrated the significance of DARA on establishing a generic description for characterising ATPS loaded with biological feedstock (citrated bovine blood) and the redefinition of the biological feedstock loaded system composition in terms of phase forming chemical equivalents [9].

2. Materials and methods

2.1. Choice of biological feedstock

Citrated whole bovine blood was selected as the biological feedstock being representative of a typical biological suspension without the hazard of pathogen contamination and characterised by a high solids concentration and a heterogeneous content of proteins and other macromolecules. The protein content present in bovine blood includes immunoglobins, haemoglobin, bovine serum albumin (BSA) and clotting factors which all have potential added value in the purified state [15]. The influence of citrate present in the feedstock (0.3%, w/v; up to 1.5 mg of citrate per g of whole bovine blood) was insignificant to the total mass of the phase forming chemicals adopted herein (250 mg/g system of PEG 1000 and potassium phosphates) as confirmed by Lebreton et al. [9,16,17]. For lysed blood experiments, whole blood was osmotically lysed with distilled water in the ratio 1:5 (blood:water). The efficiency of lysis was monitored by measuring haemoglobin concentration of the supernatant of lysed blood (centrifuged at 1200 × *g* for 5 min), which was estimated as 108 mg ml^{-1}, indicating a lysis efficiency of greater than 80%.

2.2. Construction of ATPS using solid phase forming chemicals

ATPS were constructed by successive addition of solid poly(ethylene glycol), di-hydrogen orthophosphate, di-potassium hydrogen orthophosphate, water and respective biomass fractions to yield the appropriate weight percent system at the desired pH. All batch experiments were conducted with 10 g system masses contained in a 14 ml centrifuge tube, and the system pH was maintained at 7.5 by the buffering action of a mixture of phosphates (mass ratio of 18:7; K_2HPO_4:KH_2PO_4). The adoption of solid phase forming chemicals in place of liquid phase additions had minimal impact upon partition performance. Centrifuge tubes were mixed for 30 min using a laboratory blood mixer to achieve effective equilibrium mixing and dissolution of phase forming chemicals and proteins, and then centrifuged (Jouan K442) at 1200 × *g* for 5 min to accelerate the phase separation. Samples were drawn from the appropriate phases and suitably diluted before estimation of protein content.

2.3. ATPS for distribution analysis of DARA

ATPS (representative of process samples) were constructed with the following radiolabelled amino acids: [2-^{3}H]glycine, L-[3-^{3}H]threonine, L-[methyl-^{3}H]methionine, L-[3,4(n)-^{3}H]valine, L-[4,5-^{3}H]leucine, L-[2,3,4,5,6-^{3}H]phenylalanine, L-[5-^{3}H]tryptophan in the absence and presence of bovine blood. Individual amino acids were partitioned in the ATPS. Each study involved the construction of eight ATPS (one for each amino acid and a blank) loaded with equal amounts of each solution of the titrated amino acids. All off the titrated amino acid solutions (at a final concentration of 1 μCi/μl) required dilution with non-radiolabelled amino acid (which served as a carrier) prior to loading in an ATPS. For the study of blank ATPS, 4 μl each of titrated amino acid solution was diluted in 6 ml of the appropriate non-radiolabelled amino acid (glycine, L-threonine, L-methionine, L-valine, L-leucine, L-phenylalanine, L-tryptophan) at a concentration of 27 mM. The selected ATPS of total weight 10 g was loaded with 200 μl of the resulting solution. The mixing and centrifugation procedures were followed as described above, and samples were drawn from top and bottom phase for scintillation counting.

2.4. Scintillation counting

Samples obtained from the top and bottom phases of the ATPS described above were diluted with scintillation fluid (High Safe 3, Fisons) in polypropylene tubes specially designed for scintillation counting in a Packard 2500 analyser (Hewlett Packard). Partition coefficients for each amino acid were calculated based on the scintillation count of top and bottom phase sample recovered from each system. The natural logarithmic functions of partition coefficients were plotted against the relative hydrophobicity (RH) where the slope of this linear plot represents the hydrophobic factor (HF) of that particular system and the TLLe for a system could be estimated using the correlation developed by Lebreton et al. [16].

2.5. Laser densitometry SDS-PAGE analysis

Protein concentrations of phase samples were routinely estimated from gel densitometric analysis with duplicate or triplicate experimentation. SDS-PAGE analysis of samples recovered from the phases was conducted according to the discontinuous method

of Laemmli [18]. Samples obtained from the phases were suitably diluted 20–40 times (to ensure the band intensity of stained protein fell within the standard limit of 2 mg/ml) before denaturation in reducing buffer (3.25% SDS, 20% sucrose and 5% β-mercaptoethanol in 62.5 mM Tris–HCl, pH 6.8). Mixtures were boiled at 100 °C for 10 min prior to loading into electrophoretic analysis on 12% T–2.65% C poly(acrylamide) separating gel and a 4% stacking gel using a Mini Protean vertical electrophoresis cell (Bio-Rad Labs.). The gel was stained with 0.1% Coomassie Brilliant Blue R-250, 40% methanol, 10% acetic acid. The gels were subsequently developed in a destaining solution (comprising 8%, v/v acetic acid and 25% methanol in deionised water), and dried using a gel drying kit (Promega). The proteins were quantified using Laser densitometry (Pharmacia LKB Ultrascan XL and BioSoft Quantiscan) based on the intensity of the stained protein bands relative to the known concentrations of standard haemoglobin and BSA electrophoresed in an identical manner.

2.6. Protein assay

The total protein content of samples taken from the ATPS was estimated using a Bicinchoninic Acid assay (BCA; Pierce, Rockford, IL, USA) and the results expressed relative to a calibration plot derived from similar assay of known concentrations of BSA. This assay was routinely performed to check the mass balance of haemoglobin and BSA relative to total protein mass (the total protein content of whole bovine blood was approximately 160 mg ml^{-1}, of which haemoglobin and BSA content was estimated as 118 mg ml^{-1} and 32 mg ml^{-1} respectively). The percentage deviation was within an average error of less than 5%.

2.7. Choice of system

The current investigations were focussed to assess the application of sensitive ATPS (systems close to the binodal curve and critical point) consisting of 12% PEG 1000 and 13% potassium phosphates having initial TLL 10%, w/w; pH 7.5; hereafter referred as System A_1. In addition, a monophasic system (12% PEG 1000: 12.5% phosphates) referred to as system A_2 was also investigated. Previous studies with system A_1 established that bovine blood has a strong influence upon system variables [9,17]. System A_2 was adopted because scouting experiments revealed that it was possible to construct ATPS with reduced quantities of phase forming chemicals in the presence of biomass to generate working biphasic systems. By choosing systems in the monophasic area, close to the binodal curve, the influence of different fractions was expected to highlight which of these fractions is responsible for the alteration of the system variables and its possible influence on the binodal curve within the phase diagram.

System A_1 was constructed with blood fractions (lysed bovine blood, whole bovine blood, plasma and haemoglobin) in the range 5–30% (w/w of system, in whole bovine blood equivalents). Blood fractions were included to *separately* monitor the influence of whole bovine blood and its fraction and thus determine which fraction contributes most to the overall influence of added biomass in selected ATPS. TLLe of assembled systems was estimated using DARA and revealed that plasma and haemoglobin fractions have significant influence on the system variables [9].

2.8. Blood fractions used in the present investigation

The fractions: plasma, red blood cells (RBC), haemoglobin and RBC membrane used in the experiments here were obtained from whole bovine blood as discussed in Blood Separation and Plasma Fractionation by Harris [19].

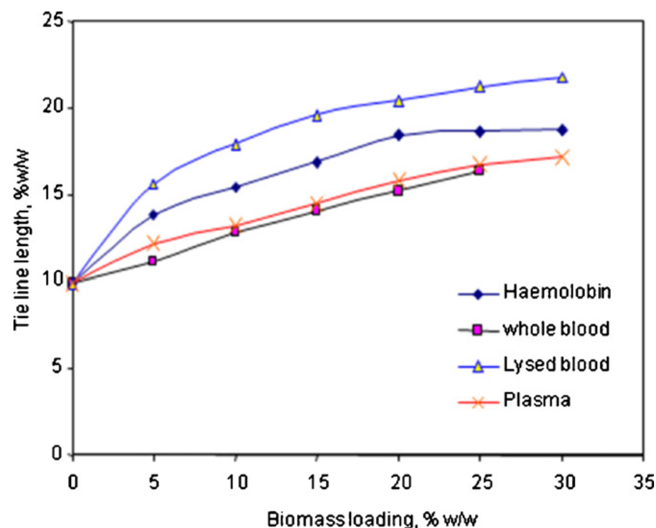

Fig. 1. Effect of biomass loading on change in TLLe for system A_1. TLLe was then measured using DARA based on the correlation developed by Lebreton et al. [9]. Refer to Fig. 2 for variation in partition coefficient and volume ratio.

3. Results and discussions

3.1. Impact of individual fractions of whole bovine blood in the selected systems

Fig. 1 shows the impact of biomass on the change in TLLe (expressed as % w/w unless otherwise specified) of loaded ATPS. It can be inferred that the TLLe gradually increased from 10 (for the blank system) to about 20 (for a 30% loaded system) in the handling of lysed blood. However, the *rate* of increase was found to be greatest between 0% and 15% blood loading, after which the rate decreased to approach a plateau beyond 20% (w/w) loading. The increase in TLLe may be attributed to the increase in haemoglobin mass partitioned to the bottom phase. When a certain value is achieved, the rate of TLLe increase slowed down and gradually approached a plateau beyond 20% loading. This was pronounced for haemoglobin (see Fig. 1) but less so for other fractions and whole blood. Effect of biomass loading upon volume ratio and partition coefficient of BSA and haemoglobin, is shown in Fig. 2. The volume ratio decreased with increase in bovine blood loading *in tandem* with partition co-efficient of BSA and haemoglobin. Although, the partition co-efficient of BSA shows a similar trend, haemoglobin has the greatest influence because of its higher concentration (about 240 mg in a 10 g system at 20% biomass loading compared to 70 mg BSA).

The observation made with system A_1 illustrates that an ATPS chosen in the sensitive area of the phase diagram could be stabilised by the utilisation of added biomass such as bovine blood. TLLe measurements based upon the DARA technique can effectively be used to characterise ATPS loaded with biomass in conjunction with recorded partitioning behaviour of major protein components and system volume ratio. However, this alone is not enough to redefine the position of loaded ATPS in the original phase diagram, which is an absolute prerequisite for estimation of phase chemical composition.

3.2. Influence of biomass addition in monophasic systems

A monophasic system consisting of 12% PEG 1000 and 12.5% phosphate (System A_2) was chosen to demonstrate the influence of biomass addition. When loaded with a 20% (w/w of system) equivalent of each blood fraction, biphasic systems were formed

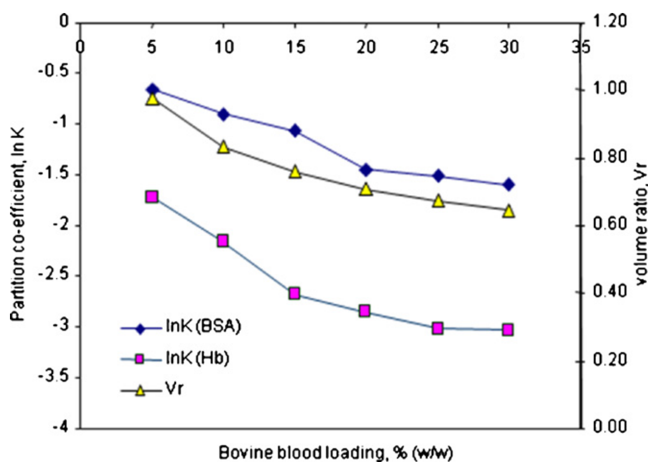

Fig. 2. Effect of biomass loading on the partition of haemoglobin and BSA and volume ratio. The partition coefficient ln *K* (*K* is the ratio of BSA or haemoglobin present between the top phase and bottom) of BSA and haemoglobin were calculated based on SDS-PAGE laser densitometry analysis of samples obtained from the phases. The volume of the phase thus formed was noted. The volume ratio was calculated from the ratio of the volume of top and bottom phase (volume ratio, $V_r = V_t/V_b$, where V_t – volume of the top phase and V_b – volume of the bottom phase).

except with RBC membrane loadings. Fig. 3 depicts the phase volume recorded for such system. It can be observed that the system A_2 loaded with 20% of fractions (plasma, RBC and haemoglobin) and whole blood resulted in biphasic systems, due to presence of substantial amount of respective component, whilst RBC membrane loaded system has not resulted in biphasic system. The major difference between systems A_1 and A_2 was the difference in the quantity of phosphate used. Biphasic system formation in the presence of biomass in system A_2 could occur in one of the following two ways: (i) the binodal curve moves down towards the origin (see Fig. 4a) or (ii) the system position moves along the coordinates (see Fig. 4b). Although the first option is widely assumed, information recorded from the present study supports the alternative possibility (ii) as more likely [11]. The components present in each fraction may have more chance to compensate for low PEG or phosphate concentrations to transform monophasic into biphasic systems. In such a situation, top or bottom phase volumes may increase depending upon whether the added biomass compensates for PEG or phosphate enabling the system to move to a new position in the phase diagram (see Fig. 4b) to accommodate the additional components. Fractions such as plasma had little influence (see Fig. 3) on volume ratio, whilst RBC membrane had insufficient mass to promote a biphasic system. The volume of the bottom phase increased in the case of systems loaded with whole bovine blood and haemoglobin. This is coincided with the observation made in system A_1 (see Fig. 2).

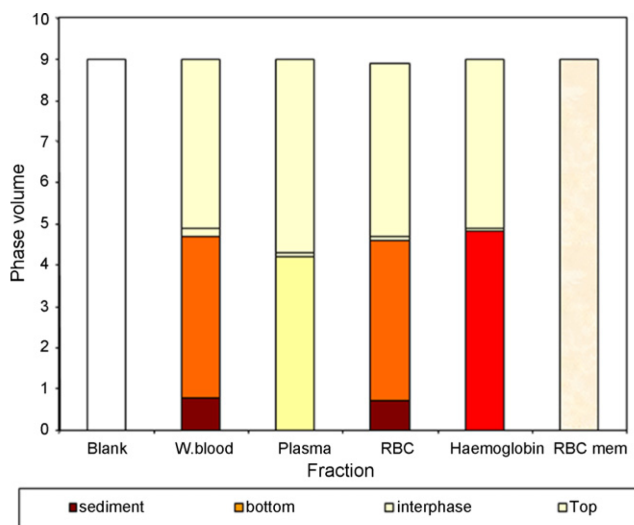

Fig. 3. Variation of phase volume in system 12% PEG 1000, 12.5% phosphate salt and 20% biomass. ATPS consisting of 12% PEG 1000, 12.5% potassium phosphates (System A_2), 20% citrated whole bovine blood or fractions (plasma, red blood cells (RBC), haemoglobin and RBC membrane proteins). The volumes of the ATPS thus constructed were recorded to identify how each fraction influenced the phases.

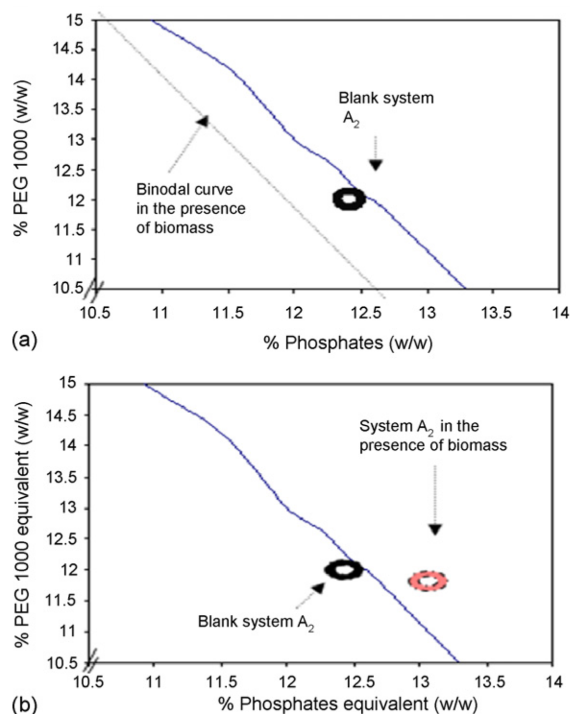

Fig. 4. Influence of added biomass upon phase diagram. (a) Influence of added biomass upon displacement of the binodal curve. (b) Influence of added biomass upon displacement of system position.

Under normal circumstances, since, phosphate or PEG are different from the components of the added biomass fraction, thus the resulting system required a different phase diagram to fix its position. Such an approach, however, would be laborious and impractical (because there are possibilities of process and/or batch to batch variation requiring construction of many phase diagrams). Hence, a reference should always be made to a blank system (in the present case, A_2 a monophasic system). The added biomass components present in the dispersed phases should be considered as part of the phase forming chemicals when represented in the original binodal curve (constructed with only phase forming chemicals). This is evidenced by system A_2 constructed using the haemoglobin fraction (Fig. 3). In this particular case, the haemoglobin introduced had a strong influence, altering the volume of the bottom phase, and acting predominantly as a bottom phase chemical. It can be recalled, from the basis of ATPS formation that if more and more bottom phase chemical is introduced into an existing ATPS, the bottom phase volume increases in order to accommodate that individual species (provided it is dispersed within the medium) [1,7,11]. The possibility of the binodal curve moving downwards to enhance the formation of a biphasic system (in monophasic system loaded with biomass) is remote. In contrast, movement of the monophasic system position in the original phase diagram is more likely to occur. This is further evidenced by an increase in the TLLe estimated for biomass loaded systems and decrease in volume ratio as observed in Figs. 1 and 2.

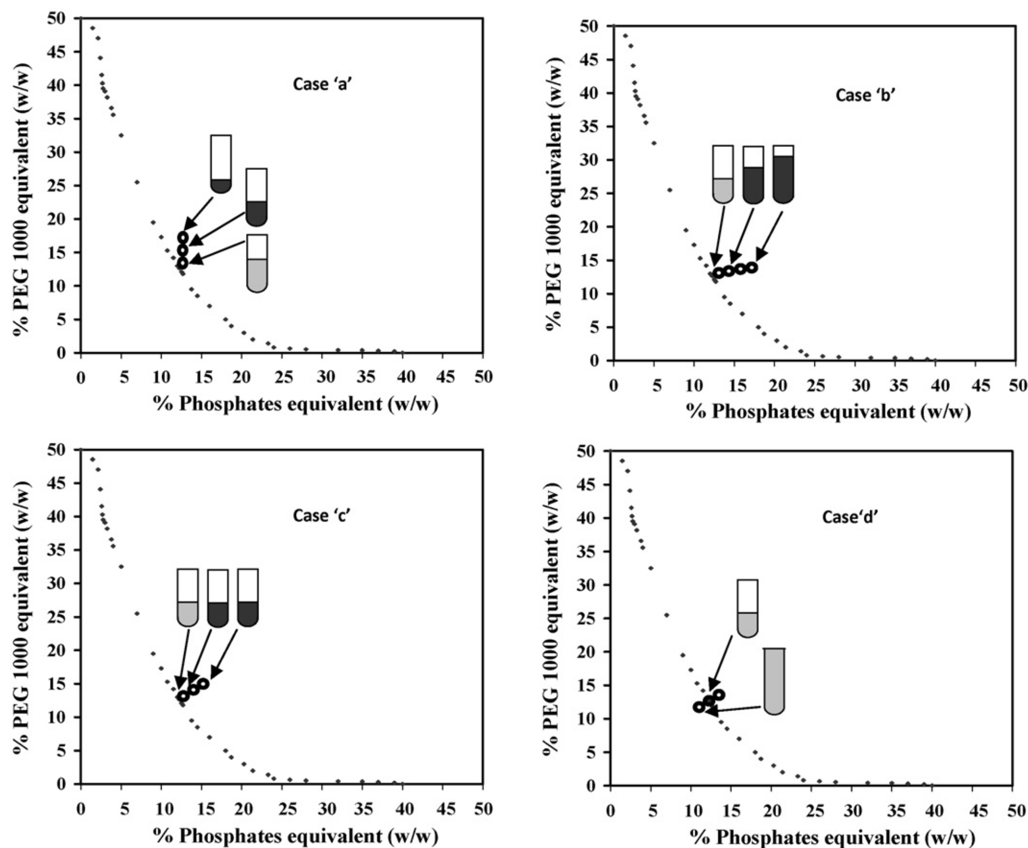

Fig. 5. Movement of selected sensitive ATPS on the binodal curve arising from PEG 1000 and potassium phosphates under the influence of added biomass. Case A: if added biomass has more influence on the top phase chemical (PEG) the chosen system moves in the direction indicated on y-coordinate. Case B: if added biomass has more influence on the bottom phase chemical (potassium phosphates) the chosen system moves in the direction indicated on x-coordinate. Case C: if added biomass has similar influence on both the top and bottom phase chemicals (PEG and potassium phosphates) the chosen system moves up to a higher position on x- and y-coordinates. Case D: if added biomass has equal negative influence on both the top and bottom phase chemicals (PEG and potassium phosphates) the chosen system moves down to a lower position on x- and y-coordinates.

3.3. Generic applicability of the displacement of system position in the phase diagram due to added biological feedstock

The behaviour of ATPS studied with fractionated and whole bovine blood suggests that the introduction of a large quantity of biomass greatly influences the system parameters. This explains the formation of a biphasic system from monophasic systems loaded with biomass. In monophasic systems close to the binodal curve in the phase diagram, biphasic system formation may be possible in the absence of biomass if the system undergoes environmental changes, such as alteration in temperature or pH, etc. [6,8]. This is attributed to variation that promotes different orientations and associations among phase chemicals and water. In such instances, it is assumed that the binodal curve moves down to promote biphasic system formation, but in the case of biomass-loaded systems these changes are judged to be minimal. Hence, biphasic system formation could be attributed to any of the postulates proposed below, which have a generic applicability for the majority of ATPS loaded with biomass,

(a) Fig. 5: Case A, illustrates possible system adjustment where loaded biomass has a strong influence on the top phase composition as with fermentation broths rich in castor oil residues [14]. The oil will commonly partition to the top phase (polymer phase) and contribute to the top phase composition which promotes an increase in top phase volume and volume ratio such that the system position moves upwards in the direction of y-axis from its original position.

(b) Fig. 5: Case B, illustrates the movement of a selected system where added biomass has a strong influence on the composition of the bottom phase to promote increases in bottom phase volume and decreases in volume ratio. Under this condition, the systems position moves along the x-axis from its original position.

(c) Fig. 5: Case C, illustrates the possible movement of a system when loaded with biomass having equivalent influences upon both phases. The system position moves up either the x-axis or y-axis depending upon the extent of impact upon the top or bottom phase. Where the influence is constant, the volume ratio remains constant.

(d) Fig. 5, Case D, illustrates the possible movement of a selected system if loaded with a biomass characterised by solute absorption where the biomass sequesters phase chemicals or diminishes the interactions between them. In such conditions, the system position moves down towards the origin, and if the ATPS is positioned just above the binodal curve, the resultant biomass loaded system will become monophasic.

Systems loaded with complex biomass near the binodal curve undergo a complex composite of the changes described in Fig. 5. However, the realisation of this behaviour appears to be stronger in the case of systems selected close to the critical point (change over point, monophasic to biphasic, volume ratio is one). It has been reported that macromolecules such as proteins, nucleic acids and carbohydrates present in the biomass above critical concentrations

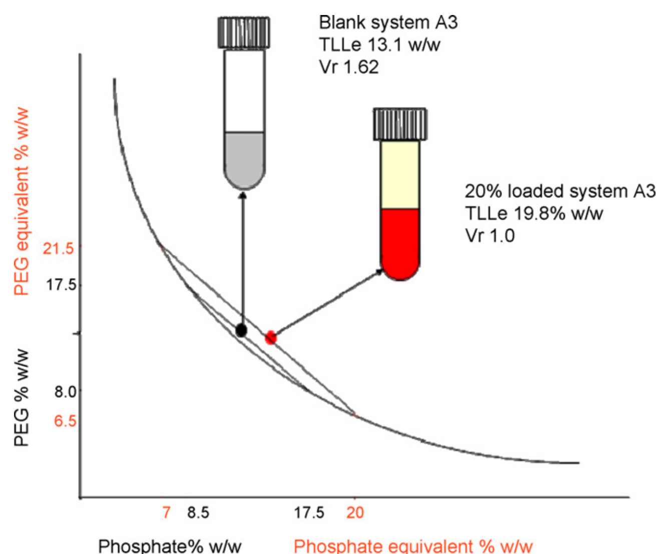

Fig. 6. Schematic representation of calculation of equivalent phase chemical composition of blank and loaded system A_3. The figure illustrates the position of blank and loaded system s and estimation s of phase chemical compositions in the respective phase based on the postulation.

influence and/or enhance system behaviour by contributing to the phase formation process [20].

3.4. *Re-definition of biomass loaded ATPS – a generic description for prediction of the phase chemical composition*

Studies reported above provided vital information regarding the variation in TLLe, volume ratio and quantity of protein partitioned to the top and bottom phases. The four cases defined in Fig. 5 suggested that at least four different possibilities exist for the relocation of an ATPS loaded with biomass on the original phase diagram. Under normal circumstances, in a blank system, any increase in TLLe may attributed to a higher concentration of phase chemical composition. However, in systems loaded with biomass, the increase observed in terms of TLLe is primary due to movement of system from its original position in a phase diagram constructed using phase forming chemicals alone (PEG and phosphate). This was achieved using the following assumptions:

1. the binodal curve is stationary (based on the explanation and study reported in the previous section).
2. the loaded ATPS moves from its original position under the influence of added biomass and the movement is *in tandem* with an increase in TLLe and variation in the volume ratio.
3. the increase in TLLe obtained from DARA, is considered as an increase in concentration of phase chemical equivalents.
4. the direction and the extent of system movement due to addition of biological feedstock is associated with an increase or decrease in the volume ratio (refer to Fig. 5).
5. The partition behaviour of any ATPS chosen at any particular TLL is constant, and TLL run in parallel in the phase diagram.

3.5. *Demonstration of reconstruction of ATPS by utilising the top phase of another ATPS*

In order to test the above assumptions, a sensitive system consisting of 14% PEG 1000 and 12% phosphate was constructed (system A_3) with and without biomass (20%, w/w) to yield a total system mass of 50 g. The volume ratio and TLLe values were 1.62 and 13.1 respectively for the blank system as compared to 1.01 and 19.8 for the loaded systems. The composition of the phase chemicals in the top and bottom phase was estimated using DARA from the TLLe value as illustrated in Fig. 6.

The aim of the subsequent experiment was to construct an ATPS (consisting of 12% PEG and 13% phosphates; system A_1) by recycling the entire top phase of system A_3. The biomass loaded system A_3, yielded 22.7 ml of top phase, from which 22 ml was used (PEG equivalent 5.167 g and phosphate equivalent 1.6780 g; refer to Fig. 1) for construction of system A_1 with fresh additions of only 0.838 g (i.e., 6.000–5.167 g) PEG 1000 and 4.822 g (i.e., 6.500–1.678 g) potassium phosphate together with water to yield the final system mass of 50 g. Such estimations predicted an ATPS with a volume ratio of approximately 1.0 and TLLe values between 11 and 12. However, the actual experiment yielded a system having a volume ratio of 1.06 and TLLe of 13.8 (measured using DARA). The difference in TLLe of 2–3% from the expected value was more accurate than values recoverable from any other available determination. The quantitative evaluation was judged to be an accurate measure for the prediction of the phase chemical composition and has potential advantages in process implementation of ATPS as discussed [20].

4. Conclusions

Studies carried out with whole bovine blood and its component fractions have facilitated a better understanding of the mechanistic basis for the behaviour of loaded ATPS, and provided information concerning system variables in terms of TLLe estimated using DARA. The study revealed that a selected system adjusts its apparent position in the phase diagram by virtue of contributing phase chemical equivalents to the phase formation process. Such an understanding helped to formulate realistic postulations concerning the movement of system position within the phase diagram under the influence of added biological feedstock. This system movement primarily depends upon the initial location of the selected system within the phase diagram and the contribution of added biological feedstock. Systems identified in the sensitive area of the phase diagram (ATPS close to binodal curve) were strongly influenced by added biological feedstock. Added feedstock could be utilised to stabilise the system, as reflected in an increase in estimated TLLe, and this value helped to identify the degree of system movement and could be used to redefine the loaded ATPS in terms of phase chemical equivalents of loaded ATPS. This information has proved to be an invaluable tool for the monitoring and control of working ATPS loaded with complex biomass (e.g. microbial feedstocks) and could be harnessed for the recovery of specific protein fractions, and where recycle of phase forming chemicals. The current observations provide an ideal opportunity to increase the throughput and productivity of ATPS, thus a way forward to promote the industrial adaptation of ATPS characterised by improved system definition and better mechanisms of process control.

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