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Kinetics of phase separation under different process and design parameters in aqueous two-phase systems

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Abstract

A practical study is presented of the effect of height/diameter (H/D) ratio of settlers on the kinetics of phase separation in aqueous two-phase systems (ATPSs). Poly(ethylene glycol) (PEG) 1000–phosphate systems with the presence of undiluted and diluted whey and disrupted yeast were used in batch studies. The influence of the biological suspensions on the rate of phase separation was investigated. It was observed that, phase separation time is much faster when disrupted suspension was used. The addition of undiluted suspensions to ATPSs slowed the process of phase separation. When the batch settler with a large cross section area (H/D ratio less than one) was used, the phases separated much faster than in a settler with a H/D ratio greater than one. Conclusions are drawn concerning the characterisation of the process and design parameters involved in the phase separation for the design of appropriate equipment.

Keywords: Aqueous two-phase systems; Phase separation kinetics

1. Introduction

The exploitation of aqueous two-phase systems (ATPSs) for the separation and recovery of desired biomolecules is gaining importance in biotechnology. Such a trend is justified in the relatively easy scale-up and the potential of continuous steady-state operation of the ATPS processes. However, most of the research in ATPSs have concentrated on the influence of factors affecting the partition behaviour of biomolecules. For industrial implementation of ATPSs it is necessary to study the aspects of these systems that provide insight into the kinetics of phase separation for the design of appropriate equipment. The separation of liquid phases can be performed using conventional chemical engineering unit operations, i.e., either in an unit gravitational field or by centrifugation. The physicochemical parameters, such as the density difference between the phases, the phase viscosities and the interfacial tension determine the choice and performance of an useful apparatus [1]. Passive settling to separate immiscible phases is simple in operation and incurs low cost.

In ATPSs, due to the physical properties of the aqueous phases, separation by sedimentation is possible in systems that exhibit a sufficiently high settling velocity {e.g., poly(ethylene glycol) (PEG)–salt systems; [1]}. This report also concluded that the settling velocity or phase separation time is strongly influenced by the height of the column. Batchwise settling in a simple tank has been described mainly for PEG–salt systems in the absence of solids [1]. The separation time for a model system (PEG,

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phosphate plus standard protein) using a spray column of 500 mm height and a inner diameter of 9.7 mm has been reported to be about 10 min [2]. This contrasts with the separation of glucose isomerase from cell debris within a PEG–phosphate system contained in a cylindrical glass vessel (33.7 l) including about 20% (wet w/v) cell mass of *Streptomyces* species. Here the required settling time was 15 h [1]. However, when the recovery of formate dehydrogenase from *Candida boidinii* was performed in a 120-l experiment in the absence of cell debris in a PEG–phosphate system, 99% of phase separation was achieved in 30 min [3]. A similar experiment for the separation of formate dehydrogenase from *C. boidinii* in a PEG–phosphate system containing 20% (w/v) cell homogenate has in contrast been reported to require between 10 and 20 h for settling and phase clarification when performed in an open glass vessel having a height/diameter (H/D) ratio of 2.5 [4].

In the context of kinetics of phase separations in ATPSs reports discussing the time of phase separation in terms of the physico–chemical properties of the phases (density, viscosity, interfacial tension) have been published [5,6]. These authors measured the dispersion height as a function of time and found that the kinetics behaviour depends greatly on which of the phases is continuous. Recently, the effect of the tie-line location on the kinetics of phase separation in batch PEG–salt ATPSs in the absence and presence of *Bacillus subtilis* extract has been published [7]. In this report, it was concluded that separation time is much more rapid when the bottom phase is continuous. In the research reported here, a number of process parameters and batch settlers have been identified to study their relationship with the phase separation in ATPSs. Two model biological suspensions (i.e., whey and disrupted yeast) undiluted and diluted were selected as process parameters to study their impact on the kinetics of phase separation. In addition, different batch settlers characterised by H/D ratio from 0.17 to 40.0 were used to examine the influence of this design parameter on the time of phase separation.

The present study is concerned with the influence of a defined design parameter on the kinetics of phase separation in PEG–phosphate ATPSs and the behaviour of batch systems in the presence of undiluted and diluted biological suspensions. This paper provides an insight into the kinetics of phase separation necessary to design of appropriate equipment for the commercial application of ATPS processes.

2. Experimental

2.1. Biological suspensions and batch settlers

Two experimental models; (i) disrupted yeast and (ii) whey were selected for the present study. These experimental models are referred as ''biological suspensions'' throughout the paper. The nature of the biological suspensions used and the biomass concentration of such suspensions were considered as process parameters for this particular study. Baker yeast was slurried (30%, wet w/v) in 20 m*M* phosphate buffer, pH 7.0 and disrupted in a APV-Gaulin type homogeniser (APV, Crawley, UK) under operating conditions described previously [8]. Whey was obtained as described before [9]. Biomass concentration of whey and disrupted yeast was modified by diluting the original extract; two-, three- and four-fold using deionised water. The biological suspensions so generated were stored at 4°C until use. To study the effect of different H/D ratios (considered as the selected design parameter in this paper) on phase separation, five different glass batch settlers were selected (see Table 1). These batch settlers were characterised by a range of H/D ratio from 0.17 to 40.

2.2. Aqueous two-phase experiments and time of phase separation

In order to estimate the time of phase separation, for each set of experiments, two different ATPSs comprising 23% (w/w) of PEG 1000 and 18% (w/w) phosphate (Sigma, St. Louis, MO, USA) were mixed with whey (or disrupted yeast). The pH was adjusted to 8.5 using sodium hydroxide (1 *M*; BDH, Toronto, Canada) when needed. The ''*blank*'' system was separated by low-speed batch centrifugation at 1500 *g* for 20 min at 25°C, whilst the ''*experimental*'' was allowed to separate under gravity during which a record of the changing volume of the phases

Table 1
Settlers used to investigate the influence of the geometry on kinetics of the phase separation[a]

Geometry	Settler device description	H/D ratio (average)	Cross section area (cm^2)
1	Glass vessel	0.17	37.0
2	Glass vessel	0.25	24.0
3	Glass vessel	0.65	13.0
4	Glass column	4.0	3.0
5	Glass column	40.0	0.5

[a] The H/D ratio values are the result of the height (H) of the ATPS in the settler divided by the diameter of the settler (D). The cross section area was estimated with the diameters of glass vessel or column used.

formed with elapsed time was kept. The separation of PEG–phosphate systems under gravity was expressed as the relative phase volume ratio (volume ratio of the ''*experimental*'' system, divided by that from the ''*blank*'' system) relative to time. Results reported are the average of three independent experiments and errors were judged to be ±5% of the mean value.

3. Results and discussion

3.1. Effect of process parameters on the time of phase separation

Several parameters affect the phase separation velocity, such as composition of the ATPS and geometry of the settling tank or separation device. In order to estimate the effect of different biological suspensions and biomass concentration on the phase separation dynamics, bench scale experiments were carried out. It was decided to select the specific operating conditions for this work [23% (w/w) PEG, 18% (w/w) phosphate, pH 8.5, V_r=0.8] from a previous report [9] as those optimal for protein recovery from whey.

Fig. 1 shows the kinetics of phase separation for PEG 1000–phosphate systems at different concentrations of whey (Fig. 1a) and disrupted yeast (Fig. 1b) using a defined geometry (H/D=5.0). It is clear that the system loaded with the most diluted whey suspension (four-fold dilution) achieved total phase separation (relative V_r=1.0) in less time (less than 40 min; see Fig. 1a). The increase of the biomass concentration (as the dilution of the biological suspension decreased) of whey loaded onto the ATPSs had an important effect on the phase separation time. Time for the phases to separate increased as a consequence of the presence of solid from the whey. In this case, for the ATPSs loaded with the most concentrated whey used, approximately 90% of the total phase separation (as expressed by a relative V_r of 0.9) was achieved after more than 300 min. On comparison of Fig. 1a and b, it is evident that the presence of disrupted yeast (instead of whey) in the ATPS decreased the phase separation time. For the case of ATPSs loaded with the most diluted disrupted yeast suspension (four-fold dilution), approximately 40 s were necessary for maximum phase separation (relative V_r=0.92), in contrast for ATPSs loaded with four-fold diluted whey, the recorded separation time was 40 min (see Fig. 1). The changes in separation time observed by using different biological suspensions can be due to a number of factors. The different nature of the material added (i.e., whey and disrupted yeast), which includes properties and type of the proteins and other molecules presence in the biological suspensions have an effect on the surface properties of the ATPS.

It is clear that the kinetics of phase separation of ATPSs loaded with disrupted yeast differs from that of the ATPSs loaded with whey (Fig. 1b). It appears that the kinetics of phase separation of ATPSs loaded with disrupted yeast is composed of three stages. Stage 1 is the region characterised by no phase formation (relative V_r=0); identified between 0 and 80 s for all the systems studied here. In stage 2, maximum phase separation occurred, which is identified by peaks in Fig. 1b (relative V_r=0.92). Stage 3 is a region of particular phase separation behaviour. It seems that small particles produced by the disruption process (e.g., cell debris) do not settle and stay

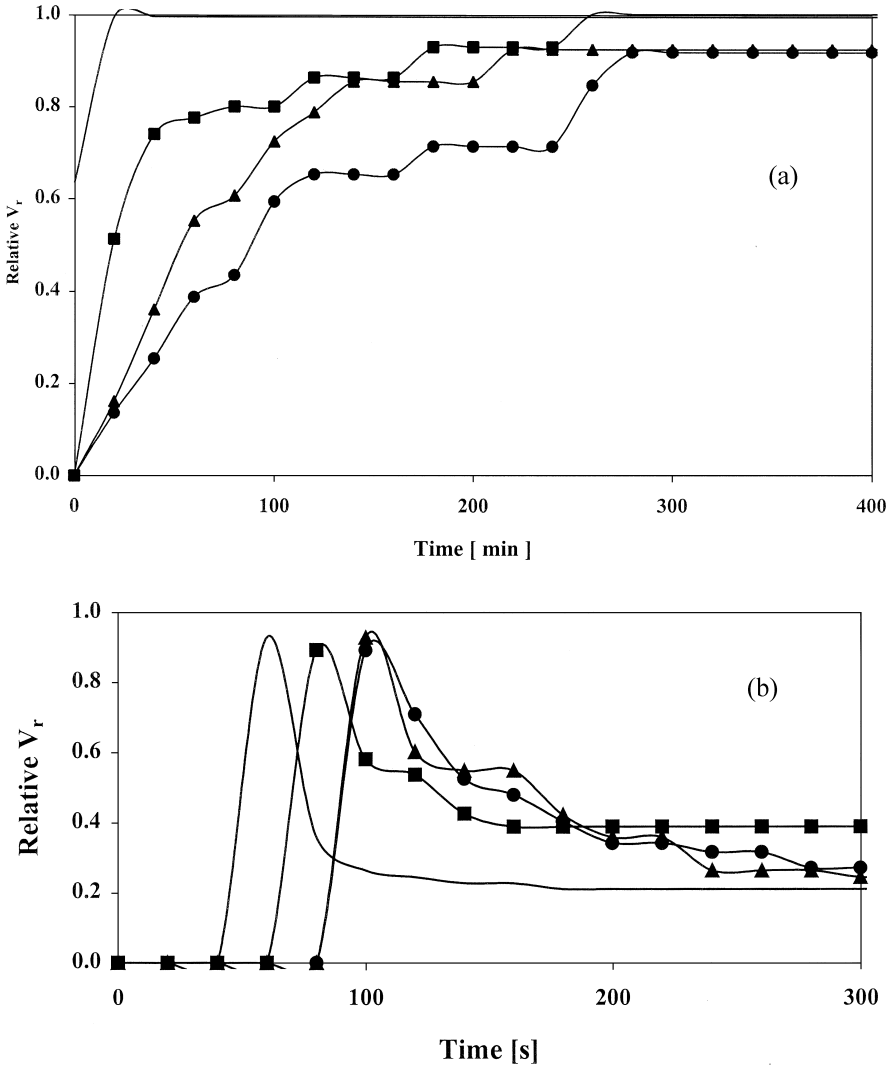

Fig. 1. Effect of whey (a) and disrupted yeast (b) on the kinetics of phase separation in batch ATPSs. The relative volume ratios from; undiluted (·) and two-fold (▲), three-fold (■) and four-fold (solid line) diluted whey or disrupted yeast are presented relative to settling time. ATPSs comprising 23% (w/w) of PEG 1000 and 18% (w/w) phosphate were mixed with whey (or disrupted yeast). The relative volume ratio with elapsed time was estimated as described in Section 2.

suspended at the phases (the majority in the bottom phase; data not shown) during the initial part of the process of phase separation (i.e., stages 1 and 2). Eventually they settled into the bottom phase and caused the volume of the phase to increase. As a consequence the resulting volume ratio (and relative V_r) decreases for all the ATPSs studied. It can be anticipated that such phenomena are not commonly observed when the systems are separated by centrifugation (as in the case of the ''*blank*'' systems used here).

3.2. Effect of design parameters on the time of phase separation

An important advantage of the exploitation of aqueous two-phase systems for the recovery of proteins from biological extracts is the potential use

of settling equipment. This requires the design or selection of appropriate geometry of the batch settler. For the present study five batch settlers characterised by different geometries (expressed as H/D ratios from 0.17 to 40; see Table 1) were selected. Fig. 2 illustrates the effect of different H/D ratios on the kinetics of phase separation when undiluted whey is added to ATPSs. It is evident that as the H/D ratio increases the time for phase separation rise. For batch settlers characterised by H/D ratios less than one (i.e., 0.17, 0.25 and 0.65) approximately 200 min was required to achieve 80% (expressed as relative $V_r=0.80$) of total phase separation. In contrast, to achieve a similar degree of phase separation, more than 400 min was needed when batch settlers with H/D ratios greater than one were used. It is clear that as the cross section area of the devices used increases, the time required for the phases to separate decreases (see Fig. 3). Such patterns can be attributed to the available cross section area of the settlers. Batch settlers with H/D ratios less than one, present sufficient available area for the solids (presence in the biological suspension) to distribute across the interface and minimise solids accumulation. In batch settlers were the cross section area is reduced (H/D ratio greater than one), solids accumulation at the interface rapidly and severely affected the efficient phase separation. As a consequence the time for total phase separation increased (Fig. 3).

On comparison of Figs. 2 and 4, it is evident that the kinetics of phase separation of ATPSs loaded with disrupted yeast, on selected settlers characterised by different H/D ratios, show differences in the pattern exhibited by ATPSs loaded with whey. Such a particular behaviour of the ATPSs added with disrupted yeast can be explained on the basis of the nature of the biological suspensions (see Section 3.1). The columns with H/D ratio of 40 exhibited a significant solids accumulation at the interface of the ATPSs (data not shown). Such behaviour can be attributed to the reduced cross section area (0.5 cm^2) of such settlers. In Fig. 4, the batch settler with a H/D ratio of 4.0 although presented approximately 90% phase separation efficiency (expressed as relative $V_r=0.90$) in less than 50 s (the minimum phase separation time obtained from all the batch settler used in this study), as the time proceeded the efficiency of phase separation decreased to 42% (relative $V_r=0.42$). Such behaviour can be associated with the effect of the solid accumulation at the

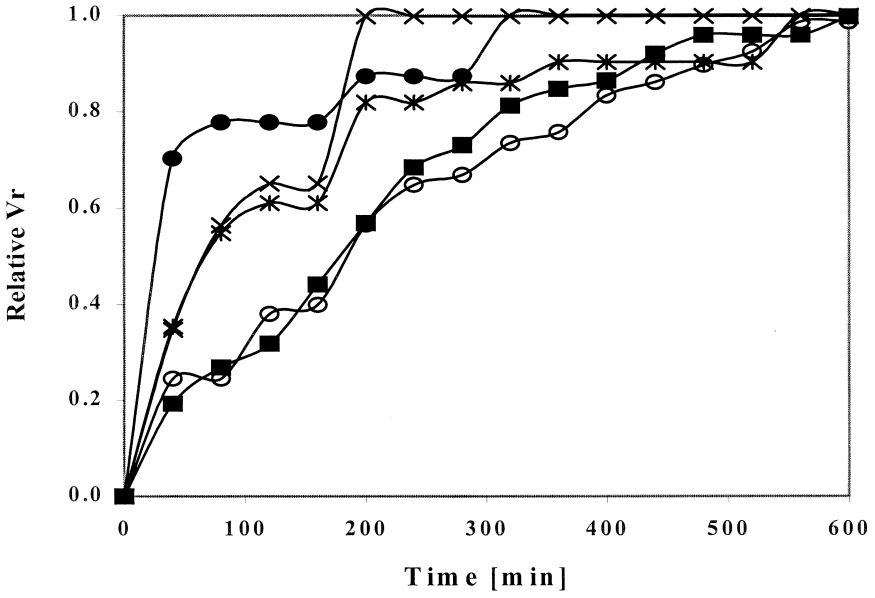

Fig. 2. Effect of H/D ratio of the settlers on the kinetics of phase separation in batch ATPSs loaded with whey. ATPSs comprising 23% (w/w) of PEG 1000 and 18% (w/w) phosphate were mixed with undiluted whey. The relative volume ratios with elapsed time were estimated as described in Section 2 in batch settlers of H/D ratio of 0.17 (·), 0.25 (×), 0.65 (*), 4.0 (■) and 40.0 (○).

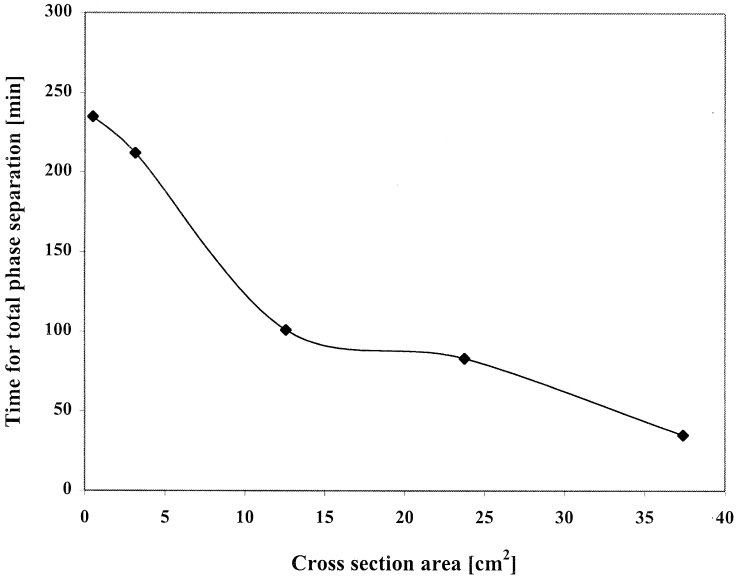

Fig. 3. Effect of cross section area of the settlers on the time of phase separation in batch ATPSs loaded with whey.

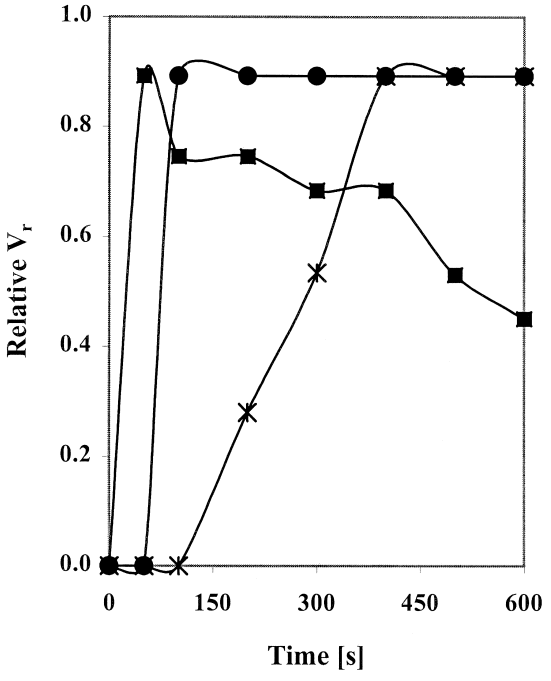

Fig. 4. Effect of H/D ratio of the settlers on the kinetics of phase separation in batch ATPSs loaded with disrupted yeast. ATPSs comprising 23% (w/w) of PEG 1000 and 18% (w/w) phosphate were mixed with undiluted yeast. The relative volume ratios with elapsed time were estimated as described in Section 2 in batch settlers of H/D of 0.17 (·), 0.65 (*) and 4.0 (■).

interface (due to the reduced cross section area available) on the kinetics of phase separation. The batch settler with a H/D ratio of 0.17 (maximum cross section area of the devices used here; i.e., 37 cm^2) exhibited the best phase separation performance. The findings reported here question the use of columns (H/D ratios equal or greater than 4.0) as batch settler for ATPS processes characterised by the presence of complex biological suspensions and suggest the use of settlers with H/D ratios less than one to reduce the necessary time for the phases to separate.

4. Conclusion

The effect of biological suspension and H/D ratio of the settlers on the kinetics of phase separation in batch PEG–phosphate ATPSs has been investigated. Phase separation time is much faster when disrupted yeast suspension is used. The concentration of the biological suspension has an important effect on the time for the phases to separate. The addition of undiluted suspensions to ATPSs slows the process of phase separation. In the particular case of the kinetics of phase separation of ATPSs loaded with disrupted yeast, three different stages were identified.

A stage of no separation phase (stage 1); a region of maximum phase separation (stage 2); and a region where the debris settle an affect the efficiency of phase separation (expressed as the relative V_r; stage 3).

The kinetics of phase separation of PEG–phosphate ATPSs in different batch settlers (characterised by different H/D ratios) was also studied. Time for the phases to separate is much faster in batch settlers with a large cross section area (H/D ratio less than one). It was concluded that the geometry of the separation device (expressed as H/D ratio) has a significant effect on the separation time. It was shown that for batch ATPS processes columns are not particular suitable to achieve a rapid phase separation and that tanks with reduced height should be used. We concluded that investigations, of the type reported here, to study the aspects of ATPSs that provide insight into the kinetics of phase separation are necessary to design of appropriate equipment for the commercial application of ATPS processes.

Acknowledgements

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