Poly(ethylene glycol)–salt aqueous two-phase systems with easily recyclable volatile salts

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Abstract

Aqueous two-phase systems (ATPSs) have great potential in the downstream processing of fermentation products. However, the consumption of large amounts of auxiliary materials limits application in industrial practice. Promising alternatives to the salts used so far are volatile salts such as ammonium bicarbonate and ammonium carbamate, which can be recycled to the extraction system as gaseous carbon dioxide and ammonia. In this work, it is demonstrated that ammonium carbamate in combination with poly(ethylene glycol) (PEG, molecular masses of 2000, 4000 and 10 000) indeed produces aqueous two-phase systems (ATPSs) at a temperature of 25°C and atmospheric pressure. Ammonium bicarbonate is clearly not suitable as a phase-forming salt, because of its too-low solubility in water. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Aqueous two-phase systems; Volatile salts; Ammonium carbamate

1. Introduction

About a century ago, Beijerinck [1] discovered that addition of certain substances to water can create a system with two aqueous phases. While performing experiments in Delft on the structure and colloidal behaviour of starch particles, he found by accident that an aqueous solution of starch did not mix with an aqueous solution of gelatin. It lasted more than half a century, until the 1950's, before Albertsson [2] realized the potential value of these systems for the extraction of biomolecules. His pioneering work which he describes in the first book on aqueous two-phase systems [3], mainly focuses on systems containing two polymers as phase formers, but also gives some attention to aqueous systems with a polymer and a salt as phase formers. Since then, the attention for these systems has grown rapidly and especially in the last two decades a lot of work has been performed in order to acquire more fundamental knowledge about ATPSs and also to develop feasible separation processes for various bioproducts [4,5]. Recent overviews on ATPSs are given by Walter et al. [6] and Zaslavsky [7].

Both polymer-salt and two-polymer ATPSs have advantages over conventional extraction using organic solvents. Firstly, unlike organic solvents, water provides a very mild environment for large biomolecules such as proteins so that denaturation at room temperature is relatively limited. Secondly, a continuous extraction process with an ATPS is quite straightforward and efficient. It requires relatively simple equipment resulting in a process which is easy to operate. Thirdly, scaling up of extraction processes based on ATPSs is easy as well.

Polymer-salt ATPSs have additional advantages
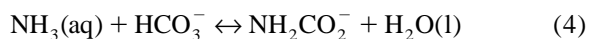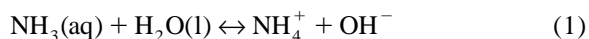
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over two-polymer ATPSs. The chemicals are lower priced (salt is much cheaper than dextran) and the phases have a lower viscosity so that a shorter time for phase separation is required. In polymer-salt ATPSs, poly(ethylene glycol) (PEG) is almost exclusively used as a polymeric phase former, although other polymers can also be applied. The phase behaviour of PEG–salt systems has been determined in detail for systems in which various inorganic salts have been considered as phase formers. Thus far, salts investigated include potassium phosphate, ammonium sulfate, magnesium sulfate, sodium sulfate, and sodium carbonate [3,8–11]. In addition to the compositions of both components in both phases, important other properties have also been reported, such as density (in most cases), sometimes viscosity [10,12], and very recently interfacial tension [13].

The major drawback of ATPSs based on the above-mentioned salts is the difficult regeneration and thus the large consumption of phase-forming chemicals. This gives rise to environmental as well as economical problems which force researchers to find a solution in order to make extraction with ATPSs feasible on a large scale. Studies on the recycling of PEG in two-stage extraction systems [14–17] show that PEG from the second stage can be reused in the first stage to an extent of 50 to 90%, depending on the nature of the feed. Recycling a higher percentage of PEG pollutes the process with undesired material originating from the fermentation broth.

Greve [18] comprehensively showed that recycling of salt in both stages (from stage 1 to stage 1 and from stage 2 to stage 2) can be performed by using various separation methods. However, only microfiltration and extraction with ethanol can be applied in practice without technical problems [18,19]. From these two methods, application of salt recycling by microfiltration leads to a more expensive process, while extraction with ethanol yields a process which is as expensive as a process without salt recycling [18,20]. A mathematical study by Fitzpatrick and Engler [21] shows the importance of either concentrating the recycle streams or applying bleeds in these streams. Otherwise, on one hand, no significant saving of both phase-forming chemicals is achieved, and on the other hand, the concentrations of undesired substances originating from the fermentation

broth quickly increase and the process streams contain an increasing amount of these undesired substances. In this work, we present a promising alternative approach using volatile salts, induced by combinations of NH_3 and CO_2, which can be easily recycled via transfer to the gas phase. The contaminants that are hardly or not volatile can be removed from the resulting aqueous solution by conventional means.

Volatile salts are created by dissolving NH_3 and CO_2 into an aqueous solution. Both dissolved species dissociate because of their respective basic and acidic nature. Eqs. (1)–(4) show these chemical equilibria that result in the formation of the ionic species that are called volatile salts.

$$NH_3(aq) + H_2O(l) \leftrightarrow NH_4^+ + OH^- \qquad (1)$$

$$CO_2(aq) + H_2O(l) \leftrightarrow HCO_3^- + H^+ \qquad (2)$$

$$HCO_3^- \leftrightarrow CO_3^{2-} + H^+ \qquad (3)$$

$$NH_3(aq) + HCO_3^- \leftrightarrow NH_2CO_2^- + H_2O(l) \qquad (4)$$

By adjusting the total pressure and the ratio of the two gases ('N/C ratio'), both the total ionic strength and the pH in aqueous solution can be controlled within certain limits. pH values can range from the pH of a solution created by dissolving CO_2 in water (pH values down to approximately 4) and the pH of a solution created by dissolving NH_3 in water (pH values up to approximately 12). The maximum total ionic strength is mainly limited by the solubility of ammonium bicarbonate. If molar N/C ratios close to two are applied, the total ionic concentration can amount up to 40% (w/w) which is more than sufficient for application in aqueous two-phase systems. Van Krevelen et al. [22] comprehensively showed that at a molar N/C ratio of 2, the concentration of carbamate reaches its maximum value. This results in a maximum ionic strength because of the salting-in effect on carbon dioxide (see Eqs. (2) and (4)), which by itself has a low solubility in water.

In this work, the phase behaviour of systems composed of water, ammonium carbamate, and either PEG 2000, or PEG 4000, or PEG 10 000 is determined at 25°C and at atmospheric pressure.

Ammonium bicarbonate is considered as a phase-forming salt as well. The location of the binodal and the tie-lines defining liquid–liquid equilibria are determined and attention is paid to the formation of solids. Along with the concentrations of polymer and salt, densities and viscosities of all phases are measured.

2. Experimental

2.1. Chemicals

Ammonium carbamate ($NH_4NH_2CO_2$, ACS grade, >99.5% purity) was obtained from Merck (Darmstadt, Germany) and ammonium bicarbonate (Baker analyzed, 99.4% purity) from Baker (Deventer, The Netherlands). PEG 2000, PEG 4000, and PEG 8000, all analytical grade, were purchased from Merck-Schuchardt (Hohenbrunn bei München, Germany). Water was distilled and deionized with a Milli-Q Water System (Millipore, Bedford, MA, USA).

2.2. Preparation of phase systems and samples

The experiments were performed in 100-ml glass bottles with a screw top and two side ports at different heights with septa allowing for sampling of both liquid phases. Either PEG 2000, or PEG 4000, or PEG 10 000, followed by water and at last, ammonium carbamate were added to these bottles by weight to a total amount of 80 g. A Mettler Toledo AB 204 balance (Mettler-Toledo, Greifensee, Switzerland) was used to determine the weights. The bottles were placed in a thermostated water bath and magnetically stirred for at least 24 h to ensure complete equilibration. The temperature of the bath was maintained at 25°C by a Julabo U3 Thermostate (Julabo Labortechnik, Seelbach, Germany). The temperature of the room in which the experiments were performed was sometimes higher than 25°C, and because the Julabo thermostate cannot subtract heat from the bath, a Lauda WK230 thermostate (Lauda, Lauda-Königshofen, Germany) was used for cooling. After equilibration of the systems, samples of approximately 20 ml of both phases were taken through

the septums with syringes in a gentle way to avoid remixing of the phases.

A number of systems containing PEG 4000, ammonium bicarbonate, and water were prepared in the same way. However, in no system was a liquid–liquid phase split observed. Either a single liquid phase was formed or a liquid and a crystalline solid-phase. Table 1 gives the overall compositions of these systems. No samples were taken from these systems.

2.3. Analysis of samples

The density was determined using a Anton Paar DMA 48 density meter (Anton Paar, Graz, Austria) with an accuracy of at least 0.1 kg/m^3. The density meter was calibrated against air and water at 25°C before every set of measurements.

The viscosity was determined using a Haake VT550 coaxial cylinder viscometer (Haake Mess-Technik, Karlsruhe, Germany). The reproducibility of the viscosity measurements was approximately 0.1 mPa s^{-1}. The temperature was maintained at 25°C by a Lauda RM6 thermostate (Lauda, Lauda-Königshofen, Germany).

The pH was measured with a Metrohm 691 pH meter (Metrohm, Herisau, Switzerland). The pH meter was calibrated before each set of measurements and has an accuracy of ±0.05 pH-scale units.

The amounts of 'total ammonia' (NH_3, NH_4^+, and $NH_2CO_2^-$), total 'carbon dioxide' (CO_2, HCO_3^-, CO_3^{2-}, $NH_2CO_2^-$), and total water in both liquid phases were determined by GC-analysis with a Shimadzu GC-14B (Shimadzu Corporation, Kyoto, Japan). The GC was equipped with a 1.83 m×3.18 mm stainless steel HP Poropak-Q packed column (Hewlett–Packard, Amstelveen, The Netherlands) and a thermal conductivity detector (TCD) was used

Table 1
Overall compositions of PEG + ammonium bicarbonate + water systems and occurring phases

Water (wt%)	NH_4HCO_3 (wt%)	PEG 4000 (wt%)	Comments
74.98	15.03	9.99	one liquid phase
71.93	18.15	9.92	liquid + solid phase
64.69	15.40	19.91	liquid + solid phase

for detection of the three species. The column temperature was held constant at 70°C and helium was used as a carrier gas with a flow-rate of 50 ml min^{-1}.

The PEG-concentration was determined by measurement of total organic carbon (TOC) of the samples using a Dohrmann DC 190 high-temperature TOC analyzer (Dohrmann, Santa Clara, CA) with a stated precision of $\pm 2\%$. The samples were diluted by weight to approximately 0.05 g PEG/kg and then injected in the TOC analyzer. A part of the sample is automatically injected into the combustion tube and through catalytic oxidation at 680°C, this sample is completely oxidized to CO_2 and H_2O. The dried CO_2-containing gas is then passed through a CO_2 specific nondispersive infrared detector (NDIR) to quantify the total amount of CO_2, and consequently the total carbon (TC) amount. The amount of inorganic carbon (IC) is measured at room temperature in the same apparatus by passing another part of the sample through an acidic solution so that all IC reacts to CO_2 which is quantified by the same detector. The TOC is calculated by subtracting the IC value from the TC value.

3. Results

Table 1 gives the compositions prepared by weight of the three phase systems containing PEG 4000 and ammonium bicarbonate. It was not possible to accurately measure the densities, viscosities, or pH values because of the relatively large volatility of the salt components of the samples. Immediately after opening a bottle containing one of these systems, gas bubbles were formed in the liquid which indicates the evaporation of (mainly) carbon dioxide from the solution. Regarding the rather high concentrations of PEG and ammonium bicarbonate in the three solutions, it doesn't seem possible to create a liquid–liquid system with this salt under these conditions. The solubility of ammonium bicarbonate in aqueous solution at 25°C is 24.7 g/100 g water ($=19.8$ wt%). This value has been interpolated from solubility values given by Dean [23]. The ammonium bicarbonate content of the most concentrated system was 18.15 wt%. Under these conditions, a solid-phase

was present which was not analyzed. However, its presence indicates that no further concentration increase of either species could lead to the formation of an ATPS. When the PEG-content was increased and the ammonium bicarbonate content was decreased, again a solid-phase was formed, supporting the above conclusion.

Ammonium carbamate shows more potential to function as a phase-forming salt. Table 2 gives experimental data on the composition of both phases of various ATPSs containing PEG with an average molecular mass of either 2000, or 4000, or 10 000 g mol^{-1}. For each phase system also, the composition of a single phase system is included that is very close to the two-phase region, in order to obtain a better insight in the location of the binodal close to the plait point. Densities, viscosities and pH values of all phases are displayed in Table 3. Not included in Tables 2 and 3 are systems with higher overall concentrations of PEG and/or ammonium carbamate, which exhibited formation of solids. pH values of all phases do not significantly differ from each other considering the accuracy of measurement. Figs. 1 and 2 show the density difference between the two phases and viscosity of the top phase, respectively, as a function of the tie-line length (TLL), that is a quantity often used to characterize the position of a certain two-phase system in an ATPS phase diagram. As already observed before for more conventional ATPSs [3], increasing the TLL results in an increase in both density difference between the two phases and viscosity of the top phase. Increasing the molecular weight of PEG results in an decrease in density difference between the two phases and an increase in viscosity of the top phase for the same PEG content. The trend in the density differences can be explained by the fact that less salt is required for phase separation in systems with high molecular mass PEG than in systems with low molecular mass PEG. Fig. 2 clearly shows that a solution of polymers with a higher molecular mass leads to a higher viscosity. The viscosity of the top phases from systems with PEG 10 000 is quite large and it is evident that these large viscosity values are not very beneficial for the mass transfer rate of biomolecules during extraction. Furthermore, the time needed for coalescence (phase separation) is approximately proportional to the viscosity [24]. Another aspect of these ATPSs with

Table 2
Phase compositions of PEG+ammonium carbamate+water systems

Top phase			Bottom phase		
Water (wt%)	$NH_4NH_2CO_2$ (wt%)	PEG (wt%)	Water (wt%)	$NH_4NH_2CO_2$ (wt%)	PEG (wt%)
PEG 2000					
67.33[a]	18.79[a]	13.88[a]			
63.95	17.82	18.23	69.42	22.92	7.66
55.97	13.70	30.33	69.18	27.12	3.70
52.16	12.64	35.20	67.58	29.98	2.44
47.59	11.53	40.88	64.47	33.28	2.25
43.08	11.29	45.63	61.77	36.21	2.02
PEG 4000					
74.51[a]	17.26[a]	8.23[a]			
67.02	13.01	19.97	76.21	19.32	4.47
64.74	12.26	23.00	76.14	20.31	3.55
62.72	11.56	25.72	75.82	21.99	2.19
58.40	10.98	30.62	73.42	24.80	1.78
53.15	10.41	36.44	71.72	26.93	1.35
PEG 10 000					
81.56[a]	13.99[a]	4.45[a]			
75.40	12.17	12.43	80.31	15.19	4.50
68.52	10.65	20.83	80.22	18.12	1.66
63.88	9.92	26.20	78.63	19.87	1.50
59.65	9.44	30.91	75.54	23.13	1.33
55.28	8.61	36.11	73.25	25.34	1.41

[a] These systems do not split into two phases; the composition given is the composition of the overall system, which is determined by weight.

PEG 10 000 which makes them less attractive to use than the systems with lower molecular mass PEG, is the very small density difference, because coalescence time is even more than inversely proportional to density difference [24].

Figs. 3–5 show the experimentally determined phase compositions of all top and bottom phases including overall compositions and tie-lines between corresponding phases. The down triangles denote overall compositions that did not result in a phase split. The binodal lines plotted in these figures were obtained by fitting the phase composition data to simple mathematical functions. The fact that the up triangles that indicate overall composition lie approximately on the tie-lines shows that the analysis methods used in this work to obtain these data give satisfactory results. Fig. 6 compares the binodals for all three systems. One can clearly observe that, as expected [3], a higher molecular mass of PEG causes the bimodal to shift to lower concentrations of both PEG and salt.

4. Conclusions

Ammonium carbamate can be used as a phase-forming salt in aqueous two-phase systems. Due to the presence of mainly monovalent ions, the concentrations of this salt needed for phase separation are somewhat higher than those of more conventional salts. Other properties of the phases are more or less similar to those of conventional systems based on nonvolatile salts such as phosphates or sulphates. PEG 2000 and PEG 4000 are suitable phase-forming polymers, but PEG 10 000 is less suitable for practical reasons. This polymer causes very small density differences between the two liquid phases and also causes high viscosities of the top phases.

Under the conditions applied in this work, ammonium bicarbonate is not suitable as a phase-forming salt. The solubility of ammonium bicarbonate in aqueous solutions in the presence of PEG is too low to induce liquid–liquid phase separation. When ammonium bicarbonate is added to a solution of PEG,

Table 3
Densities, viscosities and pH's of all phases of PEG + ammonium carbamate + water systems

Top phase					Bottom phase				
$NH_4NH_2CO_2$ (wt%)	PEG (wt%)	Density (kg m^{-3})	Viscosity (mPa s^{-1})	pH	$NH_4NH_2CO_2$ (wt%)	PEG (wt%)	Density (kg m^{-3})	Viscosity (mPa s^{-1})	pH
PEG 2000									
18.79[a]	13.88[a]	1096.5[a]	4.4[a]	9.52[a]					
17.82	18.23	1097.6	7.0	9.57	22.92	7.66	1103.3	3.5	9.59
13.70	30.33	1102.7	16.5	9.62	27.12	3.70	1114.1	2.5	9.57
12.64	35.20	1107.3	22.9	9.63	29.98	2.44	1123.0	2.2	9.58
11.53	40.88	1113.1	35.3	9.68	33.28	2.25	1135.3	2.5	9.58
11.29	45.63	1118.8	51.3	9.65	36.21	2.02	1147.1	2.7	9.57
PEG 4000									
17.26[a]	8.23[a]	1083.8[a]	3.5[a]	9.64[a]					
13.01	19.97	1084.9	10.9	9.61	19.32	4.47	1086.8	2.3	9.58
12.26	23.00	1086.1	13.1	9.70	20.31	3.55	1088.3	2.1	9.67
11.56	25.72	1090.2	19.9	9.68	21.99	2.19	1094.4	2.2	9.66
10.98	30.62	1095.3	31.0	9.71	24.80	1.78	1102.1	2.0	9.70
10.41	36.44	1102.5	47.7	9.62	26.93	1.35	1114.8	2.1	9.58
PEG 10 000									
13.99[a]	4.45[a]	1063.6[a]	2.5[a]	9.49[a]					
2.17	12.43	1069.5	[b]	9.49	15.19	4.50	1070.5	2.7	9.49
10.65	20.83	1075.5	[b]	9.55	18.12	1.66	1076.6	1.4	9.53
9.92	26.20	1082.8	[c]	9.54	19.87	1.50	1084.1	[b]	9.52
9.44	30.91	1091.9	140	9.57	23.13	1.33	1096.1	1.8	9.58
8.61	36.11	1098.1	228	9.60	25.34	1.41	1106.1	1.8	9.51

[a] These systems do not split into two phases; the composition given is the composition of the overall system, which is determined by weight.
[b] There was not enough sample available from these phases to measure the viscosity.
[c] Not measured.

formation of solids already occurs at a salt concentration that is too low to form an ATPS.

Future work will be aimed at developing a model for describing the phase behaviour of volatile salts in ATPSs [25]. Furthermore, experimental data will be generated using pressurized gases instead of solid
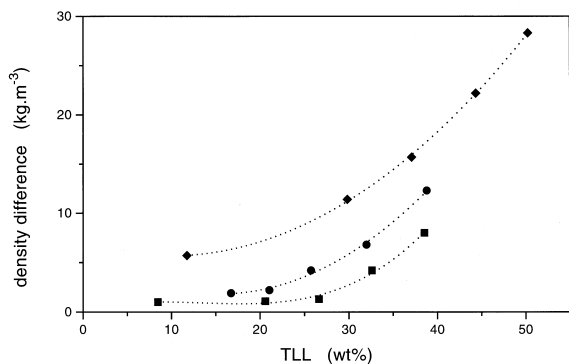

Fig. 1. Density differences of PEG + ammonium carbamate + water systems as a function of tie-line length and for different PEG molecular masses. (◆) PEG 2000; (■) PEG 4000; (●) PEG 10 000.
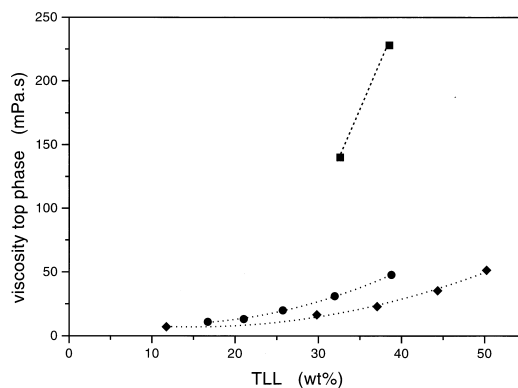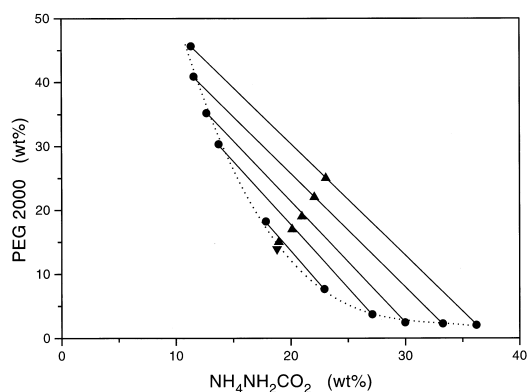

Fig. 2. Viscosity of the top phase of PEG + ammonium carbamate + water systems as a function of tie-line length for different PEG molecular masses. (◆) PEG 2000; (■) PEG 4000; (●) PEG 10 000.

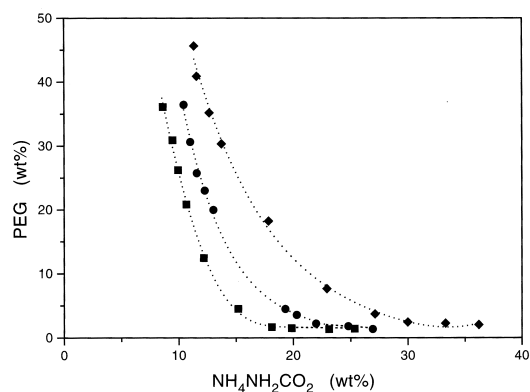Fig. 3. Phase behavior of PEG 2000 + ammonium carbamate + water systems.

Fig. 6. Binodals of PEG + ammonium carbamate + water systems for different PEG molecular masses. (◆) PEG 2000; (■) PEG 4000; (●) PEG 10 000.
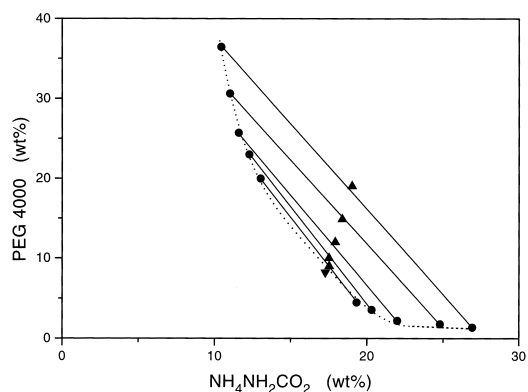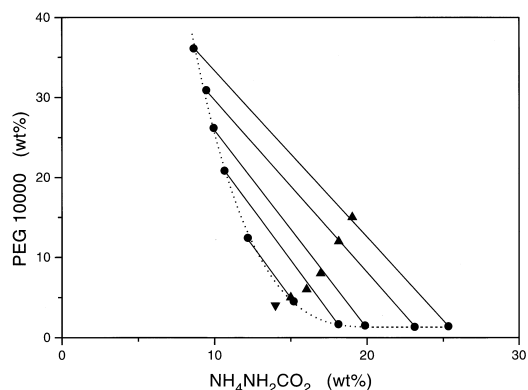
salts, which allows easy adjustment of the ratio of ammonia and carbon dioxide.

Acknowledgements

Fig. 4. Phase behavior of PEG 4000 + ammonium carbamate + water systems.

Fig. 5. Phase behavior of PEG 10 000 + ammonium carbamate + water systems.

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