Characterisation of new aqueous two-phase systems using thermoseparating polymers

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Abstract

Aqueous two-phase systems (ATPS) are increasingly being used for separation of proteins, cells and viruses and some small molecular weight substances since they offer a gentle and friendly environment. The use of new thermoseparating polymers opens new opportunities to develop more efficient aqueous two-phase systems as they make possible polymer recycling just by temperature control.

Phase diagrams for Ucon-$(NH_4)_2SO_4$ system at different temperatures shows that this system uses a smaller amount of salt and polymer than conventional systems, is thermoseparating and versatile, and particularly attractive as phase inversion is temperature dependent.

For the system PEG8000-PVA10000 the results show, as expected, that the polymer concentration for phase formation is higher than for systems using PEG with higher molecular weights.

I. Introduction

Aqueous two-phase extraction (ATPE) is an attractive separation method for enzymes and other biological substances [1-3]. The aqueous two-phase systems (ATPS) can provide a gentle and friendly environment to biological materials, and their performance can be controlled and optimized by varying solution conditions. In addition, being a liquid-liquid extraction type operation, these systems can also be readily scaled up. All these features make aqueous two-phase extraction a convenient and potentially useful method for the separation, purification and concentration of biomaterials.

The traditional aqueous two-phase systems have been the poly(ethyleneglycol) (PEG)/dextran and PEG/salt [4]. Novel aqueous two-phase systems for large-scale use, have been introduced where the systems are formed by random copolymers of ethylene oxide (EO) and propylene oxide (PO) [5-6] and starch derivatives [7-8]. The EOPO copolymers are thermoseparating, i.e., over the cloud point temperature the copolymer is not soluble in water and easy to recycle; starch derivatives are cheap and available in large quantities. Although PEG is also thermoseparating, the EOPO copolymers have the advantage of exhibiting lower cloud points (50°C) than PEG (above 100°C).

In this work, the system Ucon-$(NH_4)_2SO_4$ polymer was characterised at 22 °C, 30 °C and 40 °C, (Ucon 50-HB-5100 is random copolymer of 50% ethylene-oxide and 50% propylene-oxide with a molecular weight of 4000) and the corresponding phase diagram plotted. For the system PEG8000-PVA10000 (at 20 °C) the phase diagram is also presented.

II. Materials and Methods

For the system Ucon-$(NH_4)_2SO_4$ refractive index determination and titration were used. Titration allowed the accurate determination of salt concentration. The following reaction mechanism was applied to measure the salt concentration

$$2\ (NH_4)_2SO_4 + 6\ HCHO \rightarrow N_4(CH_2)_6 + 2\ H_2SO_4 + 6\ H_2O$$

0.1M NaOH solution (previously calibrated) was used to titrate H_2SO_4 using phenolphthalein as indicator. Standard calibration curve for the refractive index of salt solution was also previously made. Since the refractive index of Ucon and salt is additive the Ucon concentration was calculated from the refractive index values.

To determine the phase diagram for the PEG8000-PVA10000 refractive index and freeze-drying were used. These two methods were employed to determine the total concentrations of PEG-PVA in each phase. Standards calibration curves for all polymers were previously made using refractive index determination at 25 °C. By integrating these two methods, the concentration of each polymer in both phases was measured.

III. Results and discussion

III.1. System Ucon-$(NH_4)_2SO_4$

The results (binodals and tie-lines) obtained for the liquid-liquid equilibrium data of Ucon-$(NH_4)_2SO_4$ system at 22°C, 30°C and 40°C are present in Figs III.1.1 to III.1.4. The plait points were obtained by connecting the midpoints of the tie-lines and then extrapolating this midpoint curve to the binodal.

As can be seen, the higher the temperature, the lower polymer and salt concentrations needed for phase formation. As temperature increases, a more sharp decrease in salt concentration in the bottom phase occurs (0.3 M for the phase diagram at 40°C). This greatly reduces salt consumption and can be more easily adapted to be in accordance with the demands in environmental protection. In addition, Ucon as a random copolymer of ethylene-oxide and propylene-oxide, shows thermoseparating characteristics with water when temperature increases to 47°C. These characteristics can also be used for the recycling of this copolymer from the polymer-rich phase. In general, this new ATPS can be characterised as a low salt consumption and a thermoseparating system. The possibility of controlling system properties by choosing temperature (at 30°C and 40°C phase inversion occurs) makes it a flexible and promising phase system for separation of biomaterials.

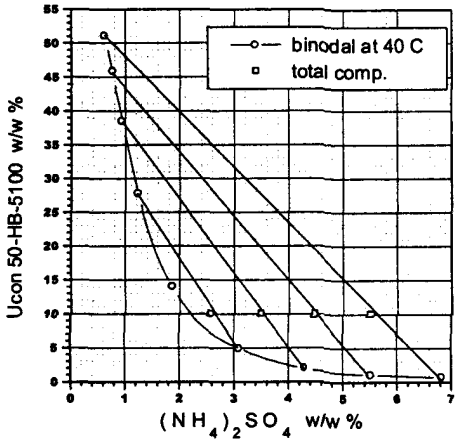

Fig.III.1.1. Phase diagram of Ucon-$(NH_4)_2SO_4$ ATPS at 40°C. Ucon is found in bottom phase

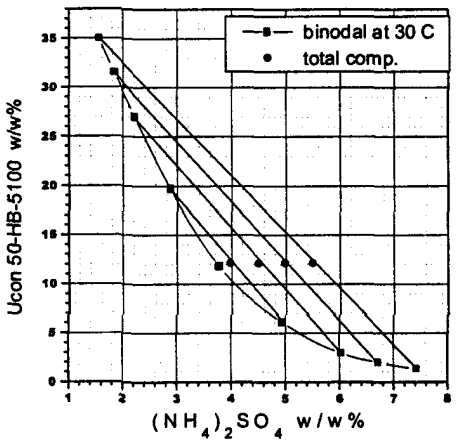

Fig.III.1.2. Phase diagram of Ucon-$(NH_4)_2SO_4$ ATPS at 30°C. Ucon is found in bottom phase.

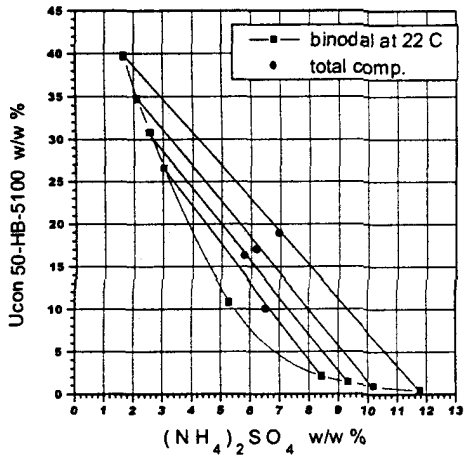

Fig.III.1.3. Phase diagram of Ucon-$(NH_4)_2SO_4$ ATPS at 22°C. Ucon is found in top phase.

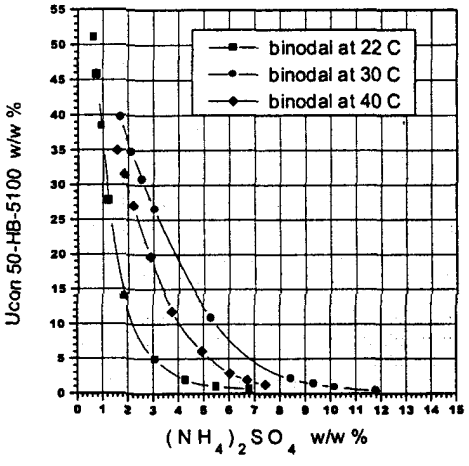

Fig.III.1.4. Binodals for the phase diagrams of Ucon - $(NH_4)_2SO_4$ system at 22°C, 30°C and 40°C.

III.2. System PEG8000-PVA10000

The liquid-liquid equilibrium data as well the binodal for PEG8000-PVA10000 system is shown in Fig.III.2.1.

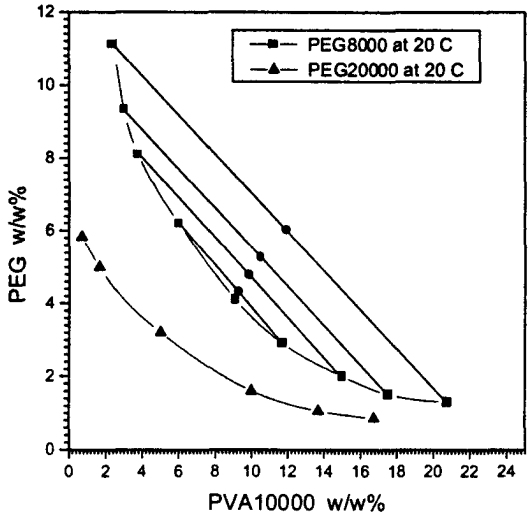

Fig.III.3.1.Phase diagram of PEG8000-PVA10000 ATPS at 20 C from this work and PEG20000-PVA10000 ATPS at 20 C from literature [2].

From phase diagram is possible to see, as expected [9], that by decreasing molecular weight of PEG, the polymers consumption increases, meaning that the binodal is moved far from the origin of the diagram.

IV. Conclusions

The phase diagrams obtained showed that the thermoseparating polymers are a good alternative to use in aqueous two-phase extraction, since it is possible to recycle the copolymer, heating the Ucon rich phase above the cloud point. Phase formation is also temperature dependent as phase inversion occurs by increasing the temperature (between 30°C and 40°C).

Although PEG and PVA are also thermoseparating polymers, in industrial scale they are not suitable for recycling since cloud points are high (above 100°C) which largely increases the process costs.

Acknowledgement

You-Ting Wu and Martinha Pereira were financially supported by the Praxis XXI program of FCT (Fundação para a Ciência e Tecnologia) of Portugal. The work was from project CEC (INCO-DC) with the contract number ERB IC18 CT97 0182.

Bibliography

[1] P. A. Albertsson, Partitioning of cell particles and macromolecules, 3rd ed, Wiley, New York, 1986.
[2] D. Fisher and I. A. Sutherland, Separations using aqueous phase systems: applications in cell biology and biotechnology, Plenum, New York, 1989.
[3] M. C. Almeida, A. Venâncio, J. A. Teixeira, M. R. Aires-Barros, J. Chromatogr. B., 711 *(1998) 151-159.*
[4] H. Walter and G. Johansson (eds) Methods in enzymology, 228 (1994) 451-469 Academic Press, London.
[5] P.A. Harris, G. Karlström, F. Tjerneld, Bioseparation 2 (1991) 237-246.
[6] J. Persson, L. Nyström, H. Ageland, F. Tjerneld, Journal Cromatography, B 711 (1998) 97-109.
[7] A. Venâncio, J. A. Teixeira, Bioprocess Engineering, 13 (1995) 251-255.
[8] A. Venâncio, J. A. Teixeira, M. Mota, Biotechnol. Prog., 9 (1993) 635-639.
[9] A.Venâncio, "Caracterização de novos sistemas para partição bifásica aquosa de biomoléculas", PhD thesis, Universidade do Minho, 1996.