



## Characterization of liquid-liquid equilibria of aqueous two-phase systems containing a termosensible polymer (UCON) and sodium citrate

Gisela Tubío<sup>a,b</sup>, Armando Venâncio<sup>b</sup>, José A. Teixeira<sup>b</sup>, Bibiana B. Nerli<sup>a</sup>, **Guillermo A. Picó<sup>a</sup>**

<sup>a</sup>Physical Chemistry Department and CONICET, Faculty of Biochemistry and Pharmaceutical Sciences, National University of Rosario, Suipacha 570, S2002LRK Rosario, Argentina; <sup>b</sup>IBB-Institute for Biotechnology and Bioengineering, Center for Biological Engineering, Universidade do Minho, Campus de Gualtar 4710-057, Braga, Portugal.

E-mail: gpico@unr.edu.ar

Aqueous two-phase polymer systems (ATPS) have been studied in some detail over the past few decades for the separation of biological materials, as a result of their excellent compatibility with these materials [1]. These systems are attractive because of their low-cost, rapid phase disengagement, and availability of commercial separators, which allow a faster and continuous protein separation. Recently, ATPS combined with temperature-induced phase separation, which offer an effective solution to the problems of polymer removal and recycling [2], have been developed. Thermoseparating polymers have a decreased solubility in water solutions upon temperature increase. When a solution of these polymers in water is heated, the polymer separates out of the solution [3]. To aid the design and process optimization of the ATP extraction technique, more detailed information on the phase composition and the physicochemical properties of these systems is desirable. The dependence of phase composition on the polymer concentrations at a given temperature and pressure is often graphically displayed in a phase diagram. Present on the diagram is the binodal curve (BC), which delineates the potential working area for a particular two-phase system and is a fingerprint unique to that system under set conditions of, for example, pH and temperature. In this work, Ucon 50-HB-5100, a non-ionic random copolymer of ethylene oxide and propylene oxide (EPO), was selected as one of the phase-forming substances to form with sodium citrate (Cit) a novel ATPS. Cit are biodegradable and non-toxic and can be discharged into biological wastewater treatment plants. The determination of the BC was carried out by a turbidometric titration method at pH 5.2 and 8.2; and at three different temperatures 281, 293 and 313K. UCON and Cit equilibrium compositions in top and bottom phases were determined by a refractive index measurement and by an enzymatic assay, respectively. For all the assayed ATPSs, the increase in temperature causes an increase of the area of two-phase coexistence and an increase in the asymmetry. The binodal curves showed similar shape for the several pH values and tend to superimpose at high UCON or Cit concentrations, thus indicating that either the exclusion or the salting out effect, respectively prevail in phase separation. When UCON and Cit concentrations adopt intermediate values, a smaller concentration of Cit is needed for two-phase formation at basic pHs, with higher ratios between trivalent and divalent citrate ions. The trivalent citrate showed to be more effective in phase separation since, at higher pH values, smaller concentrations of Cit were needed for two phase formation. All the binodal curves were satisfactorily described using a four-parameter sigmoidal equation.

*G. Tubio acknowledges the financial support of CONICET and αLFA Valnatura from Programme European Commission*

[1] Albertsson, P. *Partition of Cell Particles and Macromolecules*, 2<sup>nd</sup> ed.; Wiley: NY, 1971

[2] P.A. Alred, F. Tjemeld, R.F. Modlin, *J. Chromatogr.* 628 (1993) 205–214

[3] Johansson, H.-O et al, *Biotech. Bioeng.* 66 (1999) 247-257