

Sugaring-out: a Novel Phase Separation and Extraction System

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The addition of a mass-separating agent (MSA) into a homogeneous solution to create a second immiscible phase has been investigated as an alternative to the traditional liquid-liquid extraction method for the separation of chemical species and recovery of biological products. The concepts that have been exploited include “salting-out” and the aqueous two-phase separation method.

“Salting-out” refers to the separation of a miscible organic compound from its aqueous solution and the formation of a two-phase system caused by addition of a salt as a MSA (Frankforter and Temple, 1915; Kobe and Stone, 1940). The salting-out is attributed to the decreased solubility of organic solvents in water in the presence of electrolytes. Salting-out usually happens at high salt concentrations, which may induce some unwanted chemical reactions during extraction (Jones et al., 1993) and may not be good for the separation of labile biological components. In aqueous two-phase systems (ATPS), the phase separation is triggered by the addition of different polymers or a polymer and a salt into the aqueous solution. In comparison with the salting-out counterpart, the both phases in ATPS are water-based with water contents as high as 85-99%. The major limitations that have hampered the widespread industrial application of ATPS are the high costs associated with the consumption of polymers, the difficulties in predicting the partitioning behavior, and the lack of effective phase-recycling processes (Zijlstra et al., 1998).

Dr. Hao Feng’s lab in University of Illinois recently observed that acetonitrile (ACN) that is miscible with water in all proportions can form a two-phase system with water when glucose, xylose, arabinose, fructose, maltose, sucrose or their mixtures was added into the ACN-water solution (Wang et al. 2008). This new phenomena was named “sugaring-out”. The phase separation due to sugaring-out is shown in Figure 1, in which Sudan I was added (Fig 1-b) to visualize the phase formation in the colorless ACN and water mixture (Fig 1-a) when 50 g/l glucose or 50 g/l xylose was added. Only 20 seconds after the addition of the sugars, the mixtures were separated into two phases with the dye concentrated in the upper ACN-rich phase (Fig 1-d).

The ACN concentration in the upper phase increased when more sugar was added into the ACN-water mixture, and can reach up to 90% (w/v). The lower concentration limit for the

two-phase formation for glucose and xylose at 1°C was 15 g/L and 25 g/L, respectively. At higher temperatures, the concentration needed for phase separation increased. Addition of polysaccharides (starch and dextran) did not result in phase separation.

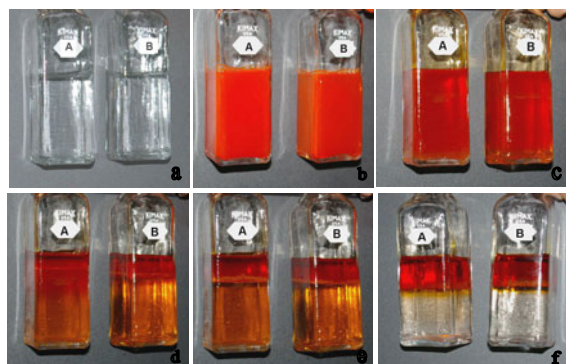


Figure 1. Sugaring-out phase separation process: *a.* Colorless ACN-water (v/v, 1:1) mixture at pH 6.5 and 1°C in both A and B; *b.* 0.05 g/L Sudan I was added into the mixture to visualize the subsequent two-phase formation; *c.* 5 seconds after 50 g/L glucose (A) and xylose (B) were added; *d.* 20 seconds later; *e.* 1 minute later; *f.* 2 hours later.

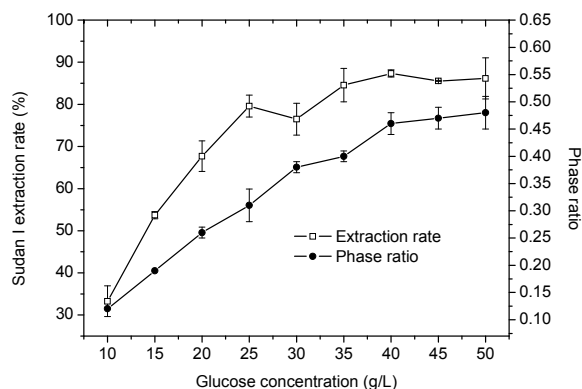


Figure 2. Sudan I extraction by a sugaring-out two-phase system (ACN: water, 1:1 v/v), equilibrated for 5 minutes at 1 °C.

In the Sudan I extraction test with a glucose-triggered ACN-water two-phase system, an extraction rate of > 80% was achieved when the glucose concentration was greater than 25 g/L (Figure 2). The distribution of syringic acid, furfural, para-coumaric acid, ferulic acid and 5-Hydroxymethyl furfural in the upper ACN phase and lower water phase was also examined (results not shown). The distribution coefficient of the five organic compounds in the ACN-water two-phase system was in the range of 1.7-8.9 when the corresponding sugar concentration was 15-50 g/L. The phase ratio of the five organic compounds in the two-phase system was in the range of 0.1-0.5.

The formation of an immiscible ACN phase by the addition of a monomeric sugar or disaccharide as a MSA into an ACN-water solution is a new phenomenon that has not been reported. Uncharged but polar biomolecules such as sugars dissolve readily in water because of the stabilizing effect of hydrogen bonds between the hydroxyl groups or the carbonyl oxygen of the sugars and the polar water molecules. This is also true for compounds containing N-H bonds like ACN (Lehninger et. al. 1993). It is likely that when adding sugars into an ACN and water mixture, some original hydrogen bonds in the mixture are replaced by hydrogen bonds formed between sugar and water molecules, which may force ACN molecules to separate from water molecules and form a new phase. As a result, the ACN volume increased when more sugars were

added into the ACN-water mixture. Since ACN is an excellent solvent for both inorganic and organic compounds due to its high dielectric strength and dipole moment (Kroschwitz and Howe-Grant, 1998), it is foreseeable that such an ACN–water two-phase system might find applications in the extraction of compounds such as metal-chelates, ion-pairs, and organic materials, or those that cannot be effectively extracted by conventional liquid-liquid extraction methods.

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