Polymer induced multiphase generation in water/organic solvent mixtures. Strategies towards the design of triphasic and tetraphasic liquid systems

Luis H. M. da Silva† and Watson Loh*‡

Instituto de Química, Universidade Estadual de Campinas, Campinas, SP 13083-970, Brazil

The addition of polyethylene oxide to mixtures of CH_2Cl_2 and heptane caused the formation of a biphase liquid system, allowing the attainment of triphase and tetraphase liquid systems upon addition of aqueous salt solutions.

It has been known since the beginning of this century,[1] that ternary mixtures of water and certain pairs of polymers, such as polyethylene oxide (PEO) and Dextran, may form aqueous two-phase systems, each phase being rich in one of the polymers. The same phenomenon has been observed when some inorganic salts, like alkaline sulfates, carbonates or phosphates, are added to aqueous solutions of polymers like PEO.[2] Again, one of the phases is rich in the polymer and the other in the salt. Many theoretical models have been proposed to account for such phenomena,[3] but a comprehensive explanation is not yet available. In general, the current view assumes mutual segregation between the two polymers or the polymer and the inorganic anion, associated with their competition for the water molecules, leading to the exclusion of one of the components to another aqueous phase. Owing to the biocompatibility of their components and to the predominance of water as solvent, these systems have been extensively studied in bioseparation processes involving proteins, nucleic acids and even whole cells.[4]

CH_2Cl_2 and heptane are miscible over their whole composition range. However, upon addition of small amounts of polyethylene oxide (PEO 3350, Sigma) to a mixture of these solvents (CH_2Cl_2: Merck, p.a., previously distilled over CaH_2 and stored over molecular sieves; *n*-heptane: Merck, extra pure), we observed the formation of a stable two-phase liquid system. PEO is almost insoluble in alkanes,[5] but it is very soluble in polar organic solvents like CH_2Cl_2. Therefore, adding heptane to a CH_2Cl_2 solution of PEO should lead to polymer precipitation, as commonly performed in polymer fractionation. The occurrence of liquid–liquid demixing might be explained by the exclusion of PEO from the alkane solution associated with the carrying of CH_2Cl_2 molecules due to their strong interaction with the polymer, similar to what is observed for the aqueous two-phase systems. This view is in accordance with literature reports on solvent preferential adsorption onto polymers in ternary mixture.[6–9] The phase diagram for PEO, CH_2Cl_2 and heptane at 298 K is shown in Fig. 1.

Fig. 1 reveals that the biphase system forms even at low polymer fractions and is favoured by the addition of heptane, whereas an excess of CH_2Cl_2 (more than 60 mol%) leads to a homogeneous system. As PEO is strongly solvated by CH_2Cl_2, the bottom phase is expected to contain solvent molecules which are somewhat bound to the polymer, as they would otherwise mix with heptane. In fact, analysis of the phases' composition,§ performed for the systems represented in Fig. 1 as A and B, supported this view and the segregation between PEO and heptane. The upper phases of systems A and B were composed of 59.7 and 56.3% heptane, 40.3 and 43.7% CH_2Cl_2 and negligible amounts of PEO, respectively. The bottom phases contained 38.2 and 36.1% CH_2Cl_2, 61.8 and 63.9% PEO and no detectable heptane. As more polymer was added, more CH_2Cl_2 was extracted into the bottom phase. In addition, the volume of the bottom phase was observed to increase with the polymer content, agreeing with the proposed polymer solvation role of CH_2Cl_2.

Other polymers were investigated in order to verify the generality of this phenomenon. This behaviour was not observed with polypropylene oxide (1000, Aldrich), poly(tetrahydrofuran) (Terathane 1000, Aldrich) or poly(vinyl alcohol) PVA (13 000–23 000, 98% hydrolysed, Aldrich). For the first two only homogeneous systems were observed, probably because the polymers are less polar than PEO and do not interact so strongly with CH_2Cl_2, or are not so strongly segregated by heptane. For PVA, the addition of heptane led to polymer precipitation, probably due to its higher polarity. For poly(*N*-vinyl-2-pyrrolidone) (PVP, 10000, Aldrich), however, liquid–liquid demixing was observed, but the volume of the bottom phase (which contained PVP and CH_2Cl_2) was much smaller than the one formed with PEO. This suggests that, although PVP interacts sufficiently strongly with CH_2Cl_2 to carry some solvent molecules to another phase, these solvation molecules are fewer in number than those of PEO. Preliminary studies with PEO 10000 produced a phase diagram very similar to that of PEO 3350.

In addition to the water/organic solvent systems widely used for liquid extraction, these organic biphase systems provide two phases without the use of water, which might be harmful to some chemical compounds, and which do not have such extreme polarity differences and thus might overcome solubility problems for some extracts. Furthermore, the strong interaction between the polymer and CH_2Cl_2 could be used to extract this chlorinated solvent from mixtures with other less polar organic solvents, without the need for distillation.

This phenomenon also allows the generation of other multiphase systems. Addition of small amounts of water to this biphase system causes the appearance of a third phase, containing water and PEO. For this system, 77.0% of the

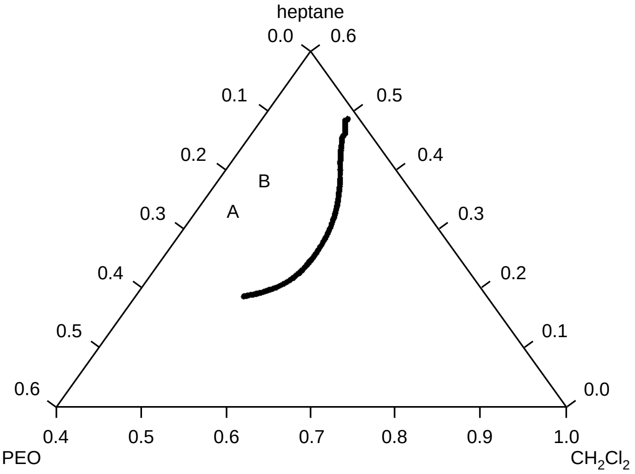

Fig. 1 Phase diagram for mixtures of PEO, CH_2Cl_2 and heptane; the polymer fraction is expressed in terms of monomer units. The two-phase region is the area containing points A and B (see text for the phase compositions).

polymer is in the aqueous phase. As more water is added, more PEO is transferred from the CH_2Cl_2 phase to the aqueous phase. At higher water contents (more than 74.0%), the system becomes biphasic again.

To avoid the destruction of the three-phase system due to polymer extraction, a salt solution may be used, taking advantage of its incompatibility with PEO. This has been observed for a three-phase system with an overall composition of 9.5% PEO, 21.7% CH_2Cl_2, 16.3% heptane, 0.1% sodium sulfate and 51.5% water. The upper phase contained heptane (58.5%) and CH_2Cl_2. The middle phase contained PEO (43%) and CH_2Cl_2. The bottom phase is constituted by the aqueous salt solution containing small amounts of CH_2Cl_2. No polymer was detected in this phase, confirming its segregation from the salt solution.

Other three-phase systems can be obtained by adding an organic solvent such as heptane to an aqueous biphase system, and might be useful in extracting apolar components in bioseparation processes designed for aqueous biphasic systems.

With the addition of larger amounts of salt, it is possible to obtain a four-phase liquid system, with an overall composition of 7.1% PEO, 15.5% CH_2Cl_2, 10.9% heptane and 66.5% sodium sulfate and water. In general, this system may be rationalised as a mixture of aqueous and organic biphasic systems. In fact, the upper phase of the above system contains only heptane (50.2%) and CH_2Cl_2. The second upper phase is composed predominantly of an aqueous solution of PEO (containing 66.3% of the total polymer). The third phase occurs as a sphere and contains PEO and CH_2Cl_2, with a little water. Addition of more salt turns this phase turbid and, by optical microscopy and addition of Methylene Blue, it was verified to consist of a water-in-oil emulsion, probably stabilised by PEO molecules. The spherical shape of this phase is determined by the attainment of the smallest area to volume ratio, in order to minimise the unfavourable interfacial energy due to the contact with the aqueous phase. Finally, the bottom phase of this four-phase system contains an aqueous salt solution, with no polymer due to its mutual incompatibility with sulfate.

These multiphasic liquid systems allow much higher selectivities than the usual water/oil or aqueous biphasic systems, providing phases with a wide range of polarities and chemical properties to suit different purposes. Furthermore, the presented general procedure may be extended to include other polymers or organic solvents and opens a variety of possibilities for improvement of liquid extraction processes.

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Notes and References

† On leave of absence from Departamento de Química, Universidade Federal de Viçosa, Viçosa, MG, Brazil.

‡ E-mail: wloh@iqm.unicamp.br

§ These compositions were determined by weighing the carefully separated phases and assaying heptane by gas chromatography (HP 5890 chromatograph, using N_2 as carrier, a FID detector and cyclooctane as internal standard) and PEO by dry weight. All compositions are expressed on the basis of mol% and, for PEO, mol refers to moles of monomer units.

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