Chemical Engineering Thermodynamics

Recovery of Biomolecules and Pharmaceutical Principles using Green Solvents

KEYWORDS: Biomolecules; Active Pharmaceutical Ingredients; Liquid-Liquid Extraction; Green Solvents; ATPS

Aqueous Two-Phase Systems (ATPS) based on green solvents (e.g., ethyl lactate, deep eutectic solvents (DES) and choline-amino acid ionic liquids) and polymers (e.g., polyethylene glycol and polyvinylpyrrolidone) were found to be biocompatible and scalable alternatives for the sustainable extraction of biomolecules (e.g., antioxidants, vitamins and amino acids) and pharmaceutical principles (e.g., antibiotics, anti-inflammatories and antipyretics) at 298.15 K and 0.1 MPa.

Objectives

The extraction of biomolecules requires non-toxic and biocompatible media to preserve the activity of the species and to avoid denaturation. Aqueous Two-Phase Systems (ATPS), or Aqueous Biphasic Systems (ABS), are mainly composed of water and present low interfacial stress and ionic strength, for which they are promising alternatives for the extraction of biomolecules such as antioxidants, vitamins, and amino acids. These components can be extracted from abundant Portuguese biowaste such as vegetable peals and grape pomace and later included in high value-added products, reducing the environmental impact of the classical production methodologies, and contributing to a more circular economy. For example, the extracted vitamins can be included in food supplements to tackle malnutrition in developing countries, amino acids can be used in the synthesis of novel green solvents (such as choline-amino acid ionic liquids, CAAILs) and antioxidants can be applied in cosmetics and pharmaceuticals to reduce, for example, premature aging and hair loss.

On the other hand, given their high scalability and large capacity, ATPS can also be used to extract active pharmaceutical ingredients (APIs) from residual waters. These pollutants are not completely metabolised by the human body, and often contaminate water reservoirs due to inefficient treatment of hospital waste and improper disposal of medicines, endangering the aquatic ecosystems and contributing to water scarceness.

Current Development

So far, several vitamins (*e.g.*, B2, B9, B12, C), amino acids (*e.g.*, alanine, glycine, valine, serine, leucine) and antioxidants (*e.g.*, rutin, quercetin, epicatechin, resveratrol, ellagic acid, gallic acid, ferulic acid, nicotinic acid, syringic acid) were extracted using green ATPS to find the most suitable combinations of solute and ATPS components.

In these studies, it was found that organic salts (such as formates, tartrates and citrates) provided more biocompatible ATPS than inorganic salts and that ethyl lactate-based ATPS generally presented the most promising performance indicators (extraction efficiencies and partition coefficients). Moreover, a rigorous assessment of the influence of pH on the stability and intensity of the UV-Vis absorbance spectra and mean electrical charges of the biomolecules was carried out, which allowed to better characterise the extract and validate the performance indicators.

Regarding the application of eco-friendly polymers, polyethylene glycol (4000, 6000 and 8000 g·mol^{-1}) and polyvinylpyrrolidone (10 000 and 29 000 g·mol^{-1}) presented satisfactory results in the extraction of antioxidants and amino acids but were considered less feasible at large scale due to increased viscosity.

Concerning other green solvents, ionic liquids based on amino acids and choline (*e.g.*, cholinium L-alaninate, cholinium glycinate, cholinium leucinate and cholinium serinate) were synthesized (as shown in Fig. 1) and applied in the extraction of flavonoids (rutin and quercetin) and N-(2,4-dinitrophenyl)-amino acids (DNP-l-leucine, DNP-l-valine, DNP-l-alanine, and DNP-glycine). Even though the solutes were successfully extracted, the pH values of the ATPS were often larger than 13, for which these conditions were too harsh for some of the biomolecules.

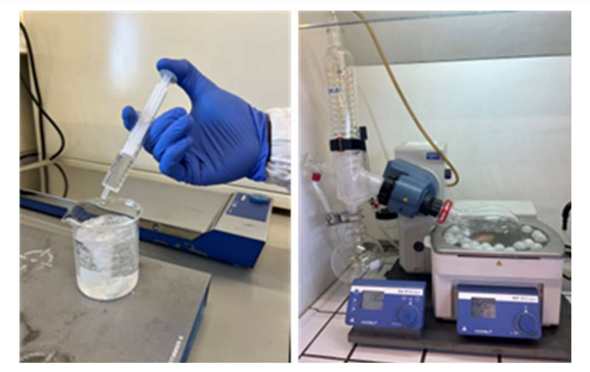

Fig 1. Synthesis and purification of cholinium L-alaninate ([Ch][Ala]).

Nevertheless, these conditions were favourable for the removal of active pharmaceutical ingredients (APIs) from water, so partition studies involving pollutants such as acetaminophen, salicylic acid and amoxicillin were carried out using the synthesized ionic liquids. These ATPS provided good performance indicators, with the largest partition coefficients being generally obtained in systems based on the ionic liquids cholinium leucinate and cholinium alaninate.

Furthermore, after having extracted the active pharmaceutical ingredients in green ATPS, these components were immobilised as cholinium-based ionic liquids, easing their storage and opening the door to future application as solvents and preservatives for food packaging. These experiments were performed at a small pilot-scale and the chemical structure of the synthesized ionic liquids was validated by density and infrared (IR) spectra measurements.

Collaboration with the Technical University of Dortmund

A collaboration with the Technical University of Dortmund involved two research stays of PhD students in Germany and Portugal. The project was mainly focused on the experimental determination and thermodynamic modelling of the partition of vitamins and amino acids in ATPS based on polymers. Moreover, it also included the experimental assessment and respective modelling of the solubility of antioxidants and vitamins (with and without covitamins) in aqueous media. For that purpose, the electrolyte Perturbed Chain-Statistical Associating Fluid Theory (ePC-SAFT) model, developed at the Technical University of Dortmund, was modified, yielding very satisfactory predictions of activity coefficients, partition coefficients and solubilities. In these studies, it was shown that the binary interaction parameters (BIPs) could be extrapolated from one system to another, increasing the predictiveness of the applied thermodynamic models.

Future Perspectives

To find the most favourable ATPS components for the extraction of each biomolecule or active pharmaceutical ingredient, it is still necessary to enlarge the number of assessed systems. For example, polymers besides polyethylene glycol and polyvinylpyrrolidone and green organic solvents besides ethyl lactate were not evaluated. As an example, the extraction of vitamin B12 in an ethyl lactate-based ATPS is shown in Fig. 2. These assays have been limited by the lack of liquid-liquid equilibria (LLE) data (binodal curves and tie-lines) concerning sustainable ATPS, so new liquid-liquid determinations will have to be undertaken to expand the number of studied systems.

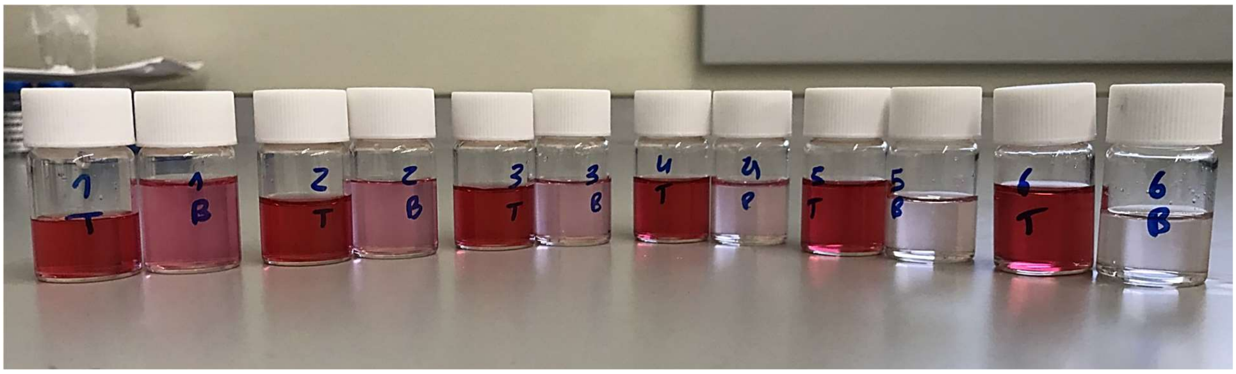

Fig 2. Top and bottom phases of the ATPS {ethyl lactate (1) + trisodium citrate (2) + water (3)} after the extraction of vitamin B12 at 298.15 K and 0.1 MPa.

Second, no solid-liquid equilibrium (SLE) was evaluated so far, which has been limiting the extraction of biomolecules from the raw materials (biowaste). Moreover, the current methodologies for partition determination involve a small scale (10 g), which, together with the lack of selectivity of the tested ternary systems, has also been delaying a direct solid-liquid extraction of the biomolecules. After having accomplished this step, studies concerning the activity of the biomolecules and the composition of the extract be performed. Then, these biomolecules could be microencapsulated or used to fortify foods, contributing to decrease malnutrition and the prevalence of certain diseases related to food inefficiencies (*e.g.*, premature aging and alopecia).

In the extraction of active pharmaceutical ingredients, a small pilot-scale was implemented (500 g) to allow the immobilisation of the pharmaceutical pollutants and of the salting out agents as ionic liquids. Nevertheless, only choline-based ionic liquids were synthesized and their application as ATPS components was not sufficiently evaluated. Therefore, liquid-liquid equilibria determinations involving these components should be determined to find a more specific application of the recovered chemicals. Furthermore, similarly to the extraction of biomolecules, no real samples of polluted water bodies were evaluated in the laboratory, even though real concentrations were used in the partition experiments.

Menthol and thymol-based hydrophobic deep eutectic solvents (DES) were used to extract nitrophenolic pollutants from water, but their ability to recover active pharmaceutical ingredients (APIs) or biomolecules was not tested. For that reason, and given the eco-friendly properties of these solvents, these may be carried out in future works. Finally, an industrial application of the most suitable extractive systems should be found by promoting collaborations with Portuguese companies. For example, the studied ATPS could be used to recover and recycle dyes in the textile industry, to extract valuable antioxidants from grape pomace and fruit peals and to reduce the consumption of water by purifying and refeeding industrial effluents. This way, it is believed that a more circular economy would be promoted by an increased valorisation of waste using ATPS, and that the cost and environmental impact of the current industrial processes could be significantly decreased.

Related Sustainable Development Goals

2 ZERO HUNGER
6 CLEAN WATER AND SANITATION
14 LIFE BELOW WATER

Outputs

PhD Theses

[1] Kamila Wysoczanska, Solubility and partitioning of biomolecules. Experimental studies and modeling, PDEQB, FEUP, 2020.

Master Dissertations

[1] C.S. Rebelo, Extraction of Antioxidants for Food Supplementation using Aqueous Two-Phase Systems with Low Environmental Impact, MEQ, FEUP, 2023.

[2] L.R. Barroca, Partition of Pharmaceutical Pollutants using Aqueous Two-Phase Systems based on Choline, MEQ, FEUP, 2023.

Selected Publications

[1] K. Wysoczanska et al., Fluid Phase Equilib. 456, 84-91 (2018)
[2] E. Gómez et al., Fluid Phase Equilib. 493, 1-9 (2019)
[3] P. Velho et al., Sep. Purif. Technol. 252, 117447 (2020)
[4] K. Wysoczanska et al., Fluid Phase Equilib. 527, 112830 (2021)
[5] P. Velho et al., J. Chem. Eng. Data 67, 1985-1993 (2022)
[6] P. Velho, E.A. Macedo, J. Chem. Eng. Data 68, 2385-2397 (2023)

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