



Deep eutectic solvents in countercurrent and centrifugal partition chromatography



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ABSTRACT

Deep eutectic solvents (DESs) were evaluated as solvents in centrifugal partition chromatography, a liquid-liquid chromatography separation technology. To this end, the partition coefficients of ten natural compounds of different hydrophobicity were determined in non-aqueous biphasic systems containing DES. The influence of the composition of DESs and the presence of water in the biphasic system on the partition coefficient were also examined. In addition, several process relevant physical properties of the biphasic system, such as the density and viscosity of the phases, were measured. A mixture of three to four hydrophobic compounds was successfully separated in a centrifugal partition extractor using a heptane/ethanol/DES biphasic system.

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1. Introduction

The term deep eutectic solvent (DES) refers to a mixture of compounds capable of associating with each other to form a eutectic mixture with a melting point substantially lower than that of each individual compound [1]. The hydrogen-bonding interactions between the compounds of the mixture, a hydrogen-bond donor (HBD) and a hydrogen-bond acceptor (HBA), are suggested as the main cause for the depression of the freezing point [1]. In general, DESs have melting points lower than 100 °C [2].

Since 2003, when Abbott and his co-workers presented the first DES [1] obtained by mixing quaternary ammonium salts (e.g., choline chloride) and urea, this new solvent family rapidly emerged as a new generation of designer solvents. Similar to ionic liquids, the physical properties of DES, such as the freezing point, density, viscosity, surface tension, and conductivity, can be tailored by combining different HBAs and HBDs, and to some extent by varying their molar ratio in the mixture [3–7].

A summary of hundreds of DESs can be found in the literature [2,8–14]. Choline chloride (ChCl), a mass-produced non-toxic quaternary ammonium salt used as an additive to chicken feed, is the

most widely used HBA, and urea, carboxylic acids, and polyols such as glycerol are the most popular HBAs [2]. Due to their biocompatibility, renewability, and biodegradability, natural deep eutectic solvents (NADES) have recently received special attention. They consist of abundant primary metabolites such as sugars, amino acids, organic acids, and a small amount of water [14].

In the literature, DESs are often presented as a more economical and eco-friendly alternative to ionic liquids. One of the most beneficial characteristics of DESs is their easy preparation, as DES is commonly prepared by heating the solid mixture of its constituents at a temperature below 100 °C. Many DESs are made from cheap, readily available, and toxicologically well characterized starting materials.

The number of DES applications is growing rapidly [2,11,15]. Besides their use as solvents in chemical and biological synthesis, electrochemistry, and the preparation of inorganic materials, significant attention is now being paid to their application in separation processes [3,14]. The main area of research is the use of DESs as extraction solvents in solid-liquid and liquid-liquid extraction [3,8,12,14,16–19]. DESs have been used to extract flavonoids [20,21], phenolic compounds [12,15], and aromatics [18,40] from plants. Shahbaz et al. [22] used DESs to extract glycerol from biodiesel. This first application of DES as a solvent in liquid-liquid extraction was followed by studies on the separation of mixtures of alcohols and esters [8], benzene from a benzene/hexane mixture [17], and BTEX aromatics (benzene, toluene, ethylbenzene and *m*-xylene) from *n*-octane [18,23]. Recently, Zeng et al. [16] inves-

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tigated the partitioning of proteins in aqueous two-phase systems composed of DES (e.g. ChCl -urea) and K_2HPO_4 . Another approach for the extraction of compounds from mixtures is the in situ formation of DESs with the target compounds. This approach was used for the extraction of phenols from oils using quaternary ammonium salts [24,25].

To date, there is no publication on the use of DES in support-free liquid–liquid chromatography, also known as centrifugal partition chromatography (CPC) and countercurrent chromatography (CCC). A multi-solvent biphasic system is used in liquid–liquid chromatography, and the separation occurs as a result of the difference in the partitioning of the compounds of a mixture between the two liquid phases. One of the two phases is kept in place with the help of a centrifugal field, while the second phase is pumped through the column. The partition coefficient of the solutes to be separated is normally used as a screening parameter for the selection of a suitable biphasic system for the separation of a particular mixture. A system in which the partition coefficients range between 0.4 and 2.5 provides a good balance between the separation resolution and solvent consumption [26–28].

The most frequently used biphasic systems in CCC and CPC are composed of three to four solvents of different polarity [26,29–32]. Such systems are very practical, since the composition and the polarity of the phases can be tuned by varying the ratio of the solvents. For example, numerous biphasic systems with overall compositions between 1/0/1/0 and 0/1/0/1 (v/v/v/v) can be created using the solvent system heptane/ethyl acetate/methanol/water [30]. Even though these systems cover a wide range of polarities, they are often unsuitable for the separation of highly hydrophobic (lipophilic) and hydrophilic compounds. The partition coefficients are very high or very low for such compounds because they preferentially dissolve in one of the two phases [33].

With the increasing popularity of CCC and CPC as a powerful method for the purification of active compounds from plant extracts and biotechnological products, it is necessary to develop biphasic solvent systems for the separation of both hydrophobic and organic solvent sensitive biomolecules such as proteins and nucleic acids [34–36]. The DESs might be good solvent candidates for both types of systems, namely non-aqueous systems and organic-solvent-free aqueous two phase systems (ATPS).

In this study, we focus on non-aqueous solvent systems and explore the possibility of using DESs as a substitute for water in biphasic systems composed of water and organic solvents for liquid–liquid chromatographic applications. Recently published data about the solubility of several poorly water-soluble drugs and metabolites has clearly demonstrated the solvation power of DESs. In most cases the solubility of the biomolecules is considerably higher than in pure water [37,38].

To the best of the authors' knowledge, this is the first publication on the applicability of DESs in liquid–liquid chromatography. One of the objectives of this study was to identify DESs that would form biphasic liquid systems with commonly used solvents in CCC and CPC such as heptane and ethanol. To assess the applicability of DES-based biphasic systems in CCC and CPC, the partition coefficients of several natural compounds were evaluated. The selected compounds had octanol-water partition coefficients ($\log P_{O/W}$) ranging between -5.10 and 12.00 and thus covered a wide range of hydrophobicity.

In addition, the influence of water absorption on the partition coefficient, which resulted from the exposure of the DES to air, was determined. Physical properties, including the density and viscosity of both of the phases of the DES-based system, were measured and compared to standard CCC or CPC biphasic systems. Finally, a centrifugal partition extractor with a twin-cell design was used to separate mixtures of selected compounds.

2. Materials and methods

2.1. Chemicals

For the preparation of the DESs, choline chloride (purity $\geq 98\%$, Alfa Aesar, Germany) and betaine (purity $\geq 98.0\%$, Fluka/Sigma–Aldrich, Germany) were used as HBAs. The HBDs, including their purities and producer, are listed in Table 1. The abbreviation of the different DESs used in this study, the molar ratio of their corresponding HBD and HBA and the melting points of the DESs are also provided in this table. The melting points of the individual HBDs and HBAs are: 305°C (choline chloride), 293°C (betaine), 33°C (levulinic acid), 135°C (malonic acid), -13°C (ethylene glycol), 18°C (glycerol), 20°C (1,4-butanediol) [47].

The solvents heptane (EMSURE, purity 99%) and ethanol (LiChrosolv, purity 99.9%) were purchased from Merck KGaA (Darmstadt, Germany). A list of solutes, including their molecular formula, molecular weight, detection wavelength, octanol-water partition coefficient, purity, and producer, is provided in Table 2.

2.2. Equipment

2.2.1. UV-Vis spectrometer

The concentration of the solute in the two phases of the biphasic systems was determined by ultraviolet-visible (UV-Vis) spectroscopy using a Specord 50 Plus (Analytic Jena AG, Germany) at 20°C .

2.2.2. Density meter

The density of the phases of the solvent systems was measured with an oscillating U-tube density meter DMA 5000 from Anton Paar GmbH (Ostfildern-Scharnhausen, Germany) at 20°C .

2.2.3. Rheometer

A MCR-102 rheometer (Anton Paar GmbH, Ostfildern-Scharnhausen, Germany) was used for the determination of the dynamic viscosity. The samples were measured at 20°C .

2.2.4. Centrifugal partition extractor

The liquid–liquid chromatography experiments were carried out at room temperature in a centrifugal partition extractor (CPE) model SCPE-250-BIO from Armen Instrument (France), with a total column volume of 250 ml. The column was composed of 11 stainless steel disks coated with PTFE and was thus suitable for applications involving biomolecules. Each disk had 20 twin-cells with a cell volume of 0.961 ml. The maximum achievable rotational speed was 3000 rpm. The column could withstand a pressure drop of 100 bar.

The centrifugal partition extractor was connected to two isocratic HPLC pumps (Model 306 50 SC, Gilson, USA), which could deliver flow rates of up to 50 ml min^{-1} . The effluent was monitored with a diode-array detector (Model 171 from Gilson, USA). The sample was introduced through a six port manual injection valve.

2.3. Methods

2.3.1. Preparation of DES

The DESs were prepared by mixing the HBA (choline chloride or betaine) with the corresponding HBD according to the molar ratios provided in Table 1. After weighing in the particular amounts of HBA and HBD, the 20 ml vial was closed with a crimp cap. The mixture was stirred with a magnetic stirrer and heated at 80 – 85°C until a single clear liquid phase was formed. In case of the preparation for the separation of the multicomponent mixture with the CPE,

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