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Computational solvent system screening for the separation of tocopherols with centrifugal partition chromatography using deep eutectic solvent-based biphasic systems☆

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ABSTRACT

Tocopherols are a class of molecules with vitamin E activity. Among those, α-tocopherol is the most important vitamin E source in the human diet. The purification of tocopherols involving biphasic liquid systems can be challenging since these vitamins are poorly soluble in water. Deep eutectic solvents (DES) can be used to form water-free biphasic systems and have already proven applicable for centrifugal partition chromatography separations. In this work, a computational solvent system screening was performed using the predictive thermodynamic model COSMO-RS. Liquid-liquid equilibria of solvent systems composed of alkanes, alcohols and DES, as well as partition coefficients of α-tocopherol, β-tocopherol, γ-tocopherol, and σ-tocopherol in these biphasic solvent systems were calculated. From the results the best suited biphasic solvent system, namely heptane/ethanol/choline chloride-1,4-butanediol, was chosen and a batch injection of a tocopherol mixture, mainly consisting of α- and γ-tocopherol, was performed using a centrifugal partition chromatography set up (SCPE 250-BIO). A separation factor of 1.74 was achieved for α- and γ-tocopherol.

1. Introduction

Tocopherols are a class of molecules which are known for their vitamin E activity. Their antioxidative capacity and the fact that they act as free radical scavenger make tocopherols an interesting class of molecules for human health and food supplement industry [1,2]. Among the tocopherols, α-tocopherol is the most important vitamin E source in the human diet [3]. Hence, there is a great interest in separation methods that can provide purified α-tocopherol [4–12]. Besides chromatographic methods, liquid-liquid separation technologies, such as countercurrent distribution or countercurrent chromatography, have been applied for tocopherol purification [13,14]. The purification of tocopherols involving biphasic liquid systems can be challenging since these vitamins are poorly soluble in water. Therefore, a water-free separation method is favorable. Deep eutectic solvents (DES) can be used to form water-free biphasic systems and have already proven applicable for centrifugal partition chromatography (CPC) separations [15]. DESs are formed when hydrogen bond donor (HBD) and hydrogen bond acceptor (HBA) molecules are combined in a certain molar ratio. The resulting mixture has a substantially lower melting point than the pure components. The term refers to such combinations with a melting point below 100 °C. They gained increasing attention after Abbott et al. described properties of DESs in 2003 [16,17]. The high solvation capacity, low volatility, and low cost of DESs led to many new applications in extraction and separation processes [18–20]. The number of possible combinations of HBA and HBD is nearly limitless. Typical HBA are quaternary ammonium or phosphonium salts, which are combined with HBD, such as urea, carboxylic acids and sugar-based polyols [8]. The resulting mixtures are considered biocompatible and less environmentally harmful green solvents [3].

In previous work, we have shown that the predictive thermodynamic model COSMO-RS (Conductor-like Screening Model for Realistic Solvation) can be used to drastically reduce the experimental effort in the solvent system selection process for centrifugal partition chromatography [21–23]. With this model, thermodynamic properties, such as liquid-liquid equilibria and partition coefficients can be calculated based on the molecular structure of the compounds in the system. This method has also proven applicable for DES-based biphasic solvent systems [24]. In this work, the computational screening method is applied for the selection of

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a DES-based biphasic solvent system for the separation of a mixture consisting primarily of α-tocopherol and γ-tocopherol, with β-tocopherol and σ-tocopherol in small amounts. In the screening, 21 solvent systems composed of alkanes, alcohols and DESs were considered. The most promising system from the computational solvent system screening was then used in a centrifugal partition chromatography experiment in a 250 ml CPC (SCPE 250-BIO, also called centrifugal partition extractor). The separation performance was compared to a reference DES-based biphasic solvent system, which has been used for the separation of hydrophobic natural compounds in our previous work [15].

2. Material and methods

2.1. Chemicals

Ethanol and *n*-heptane (liquid-chromatography grade) were obtained from Merck KGaA and VWR International GmbH. Choline chloride, betaine, 1,4-butanediol, and levulinic acid were purchased from Alfa Aesar with purities ≥98%. Commercial α-tocopherol with a purity of ≥97% was obtained from Alfa Aesar and a synthetic tocopherol mixture was kindly provided by Sigma-Aldrich Chemie GmbH. The tocopherol content was 37 wt% of α-, and 37 wt% of γ-tocopherol, 1 wt% of β-tocopherol and 7 wt% of δ-tocopherol. Structures of the tocopherol homologues contained in the mixture are shown in Fig. 1.

2.2. Preparation of the DES

To prepare the DES, HBA and HBD were weighed in at a specific molar ratio listed in Table 1 for each DES, and the sealed flask was heated to 80–85 °C. The mixtures were stirred and heated until a clear homogenous liquid was obtained. A more detailed description of the procedure can be found in [15]. Choline chloride was used as HBA. The HBD levulinic acid was used in the first and 1,4-butanediol in the second experiment.

2.3. Biphasic solvent systems

The biphasic solvent systems considered in the computational screening are listed in Table 1. The proportions of the components of the biphasic solvent systems were chosen from the experimental ternary diagram of heptane/ethanol/CLA [25]. The compositions 30/40/30 wt/wt/wt and 37.5/25/37.5 wt/wt/wt of different alkane/alcohol/DES-systems for the screening were chosen because they have been used in our previous work for shake flask experiments, and with heptane/ethanol/CLA a good stationary volume retention ratio has been achieved for the composition of 30/40/30 wt/wt/wt [15]. For the experimental validation of the selected biphasic solvent systems, DES and solvents were mixed in the respective amounts. The system was stirred in a sealed flask for at least one hour at room temperature and allowed to settle for a minimum time of 30 min afterwards. The phases were then separated into two reservoirs for their use as mobile and stationary phases of the chromatographic separation.

2.4. Partition coefficient

For the calculation of solute partition coefficients in a biphasic solvent system, the composition of the phases (solute-free) has to be known. The equilibrium phase composition can be taken from literature or determined by solving the liquid-liquid equilibrium (LLE) conditions defined by Eq. (1)

$$x_i^{SP}\gamma_i^{SP} = x_i^{MP}\gamma_i^{MP} \tag{1}$$

where x_i is the mole fraction, γ_i the activity coefficient of compound *i*, and *SP* and *MP* are the two phases of a biphasic solvent system used as stationary phase (*SP*) and mobile phase (*MP*) for the chromatographic separation.

The partition coefficient *K* of a solute *i* is defined as the ratio of the mole fraction of the solute in the phases at thermodynamic equilibrium:

$$K_i = \frac{x_i^{SP}}{x_i^{MP}} = \frac{\gamma_i^{\infty,MP}}{\gamma_i^{\infty,SP}} \tag{2}$$

where γ_i^{∞} is the limiting activity coefficient of the solute *i*.

In this work, the partition coefficients were all calculated for descending mode, i.e. *SP* is the upper phase and *MP* the lower phase of the considered biphasic system. At low solute concentrations, the distribution shows linear behavior and the partition coefficient is assumed to be constant.

For centrifugal partition chromatography the partition coefficient is expressed using concentrations *c* instead of mole fractions (see Eq. (3)). K_i can be converted to P_i using the molar densities ρ of the two phases.

$$P_i = \frac{c_i^{SP}}{c_i^{MP}} = \frac{x_i^{SP}\rho^{SP}}{x_i^{MP}\rho^{MP}} \tag{3}$$

The partition coefficient P_i can be experimentally determined by pulse injections in centrifugal partition chromatography. It is calculated from the chromatogram using the following equation:

$$P_i = \frac{V_R - V_{MP}}{V_{SP}} \tag{4}$$

where V_R is the retention volume, V_{MP} is the volume of the mobile phase and V_{SP} is the volume of stationary phase in the column. In centrifugal partition chromatography moderate partition coefficients in the range of $-0.4 < \log P_i < 0.4$ or close to that range are preferred. Smaller partition coefficients result in early eluting peaks with low resolution, whereas for high partition coefficients the target components elute late and in broad peaks.

The separation factor is calculated as the ratio of the partition coefficient of two consecutive peaks of compounds *i* and *j* where $P_j > P_i$.

$$\alpha_{ij} = \frac{P_j}{P_i} \tag{5}$$

2.5. Computational details

2.5.1. COSMO-RS

In this work, the Conductor-Like Screening Model for Realistic Solvation (COSMO-RS) was used to calculate LLE, i.e. the composition of the phases of the investigated biphasic solvent systems, and the partition coefficients of tocopherol homologues in these systems. The predictive thermodynamic model COSMO-RS combines quantum mechanical calculations and statistical thermodynamics. For each molecular conformer of the solutes and solvents of the biphasic systems, the screening charge density is computed using the density functional theory (DFT). These calculations only have to be performed once per molecule. From the screening charge density, the charge distribution (σ-profile) is obtained. From the σ-profile thermodynamic properties, such as activity coefficients and chemical potential can be obtained based solely on the molecular structure of the molecules in the mixture. From these properties LLE and partition coefficients can be computed. Detailed information about COSMO-RS can be found in [26].

2.5.2. Modelling of the DES in COSMO-RS

For the computational screening of partition coefficients of the tocopherol homologues in DES-based biphasic systems, the DES

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