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A priori selection of the mobile and stationary phase in centrifugal partition chromatography and counter-current chromatography☆

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ABSTRACT

The selection of the mobile and the stationary phase in support-free liquid–liquid chromatography (centrifugal partition chromatography and counter-current chromatography) is equivalent to a selection of a biphasic liquid system and its global composition. There is an immense number of choices of biphasic liquid systems. On one hand what makes this technique extremely versatile, on the other hand turns the selection of the appropriate system for a particular separation problem into a challenging and demanding task. In this work a systematic procedure for the selection of biphasic liquid systems for preparative scale separations is presented. The procedure is adaptable to the production scale requirements including production cost and safety. The experimental effort of different stages of the selection procedure is minimized by using a fully predictive method, the conductor-like screening model for real solvents (COSMO-RS). The COSMO-RS is used to assess properties relevant for the selection of a biphasic liquid system, such as the solute solubility and the partition coefficient.

1. Introduction

Support-free liquid–liquid chromatography, centrifugal partition chromatography (CPC) and counter-current chromatography (CCC), combines the principles of extraction and chromatography. The separation is achieved due to the different partitioning of the solutes between two liquid phases, while one of the phases is kept stationary by means of centrifugal forces in a specially designed "column" [1–3].

In contrast to any other chromatographic technique, in support-free liquid–liquid chromatography the user prepares not only the mobile phase but also the stationary phase. This is done, simply, by mixing different portions of two or more solvents that form a biphasic liquid system. The selection of a biphasic liquid system is therefore equivalent to a simultaneous selection of the mobile and stationary phase. The choice of biphasic liquid systems is almost limitless, what makes this technique extremely versatile and allows for a tailor-made system. Surprisingly, the recent review [4] has shown that in CPC/CCC only a restricted number of solvents is used. The most used solvents are *n*-hexane, ethyl acetate, methanol and water. The top 10 solvent choices include *n*-butanol, chloroform, acetonitrile, ethanol, methyl-tert-butylether and *n*-heptane, which are used in a much smaller extent.

The selection of the biphasic solvent systems in CPC/CCC is mostly done from previously published literature data for similar separation problems or by experimental screening of predefined biphasic multi-solvent system compositions organized in tables according to the overall polarity of the system, so called solvent system families [5–7]. The most frequently used biphasic CCC solvent system families are: hexane/ethyl acetate/butanol/methanol/water [8], heptane/acetonitrile/butanol/water [6,8], chloroform/methanol/water [9] and heptane/ethyl acetate/methanol/water ("ARIZONA" family) [5]. For a selection of a biphasic solvent system for separation of natural products, Friesen and Pauli [10] proposed to use a mixture of predefined compounds, with different polarities, functional groups, and structures, called "GUESSmix" (Generally Useful Estimation of Solvent Systems).

The partition coefficient (i.e. distribution constant) is used as a screening and prerequisite parameter for the selection of a biphasic liquid system. The target of the screening is to find a system composition for which the partition coefficients of the solutes of interest are in a range between 0.4 and 2.5 [10]. The experimental procedure normally includes the determination of the partition coefficient of the target solute and related impurities in pre-selected biphasic solvent systems with the shake flask method. The equilibrium concentration of the solute in each phase (upper and lower) phase is commonly determined with GC (gas chromatography) or HPLC (high performance liquid chromatography) analysis.

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By combining a robotic liquid handling system and an analytical system (HPLC or GC) the above described experimental procedure can be fully automated and used to perform high-throughput screening [11]. Another more advanced, but rarely explored option is to calculate the solute partition coefficient using well established models for the description of the thermodynamic equilibrium of multicomponent liquid–liquid systems. Using this approach the experimental effort and cost needed to select a biphasic liquid system and to tune its composition can be significantly reduced, if not eliminated.

In our previous work [12], we have shown that the conductor-like screening model for real solvents (COSMO-RS) can be used as a tool for screening and selection of biphasic liquid systems in support-free liquid–liquid chromatography. The only information needed for the prediction of the partition coefficient is the molecular structure of the solute and the solvents, and the composition of the two liquid phases.

In summary, currently in CPC/CCC only a limited number of solvents is used. The selection of the biphasic liquid systems is mostly made by experimental screening procedures, in which the partition coefficient of the target solutes is used as the only selection criterion. The preparative scale relevant parameters, related to productivity, cost and safety, are rarely addressed. Separation method transfer to production scale is occasionally addressed [13–18], even though preparative scale equipment is available since the beginning of 2000 [19,20].

The huge choice of solvents combinations to make the biphasic liquid system can be an advantage, but turns into disadvantage if it is done by trial and error. It can be extremely time consuming to make the right choice. Therefore, having a general approach for selecting biphasic solvent systems and a clear method for comparing the relative merits of different systems is very important and needed.

The “best solvent” approach, proposed by Foucault [3], is at the moment the most systematic method. Unfortunately, it is rarely used. The first step in this approach is to find a solvent in which the sample is highly soluble. After that, two other preferably mutually poorly miscible solvents are selected. The “best solvent” should be soluble in these two solvents and a two phase system should be formed. It is expected that the “best solvent” and the sample would partition between the two liquid phases in a similar way. Therefore, the tie lines of the ternary diagram of the selected ternary solvent system can be used to anticipate the solute partitioning. This approach, even though systematic, uses only the sample solubility as a relevant parameter for the selection of the biphasic liquid system and requires experimental determination of the partition coefficient.

In this work, a general approach for a systematic selection of the mobile and stationary phase (two-phase liquid system) in support-free liquid–liquid chromatography (CPC/CCC) is presented. The approach takes into account preparative scale separation relevant parameters related to the production cost and safety. The main focus is given to the use of an a priori method, COSMO-RS, for the prediction of thermodynamic properties, which are important for the selection of an appropriate biphasic liquid system for a particular separation task.

2. Theory

The procedure for the selection of a biphasic liquid system proposed in this work includes several steps. Some of these steps involve considerable experimental effort, time and cost. These could be reduced significantly by using predictive models for the assessment and calculation of biphasic liquid system related thermodynamic properties, such as the solute solubility and partition coefficient. In this section the predictive method used in this work, COSMO-RS, and its application to the proposed procedure is described.

2.1. COSMO-RS

The conductor-like screening model for real solvents (COSMO-RS) has been developed by Klamt [21] to predict thermophysical data of pure and mixed fluids. The model combines a quantum mechanical treatment of solutes and solvents and methods of statistical thermodynamics for the description of the molecular surface interactions [22]. All thermodynamic properties, which can be derived from the chemical potential, can be predicted using the molecular structure of the components as starting information. More details about COSMO-RS and its application can be found in [12,22,23].

In this work COSMO-RS is used to calculate the solvent capacity and the solute partition coefficient in multicomponent biphasic liquid systems.

2.1.1. Capacity as a qualitative measure of a solute solubility in a solvent

One of the factors influencing the productivity of a CPC/CCC separation is the sample solubility in the biphasic liquid system. It is possible to calculate the solubility of a solute in a single solvent and mixture of solvents using COSMO-RS. This has been done successfully for neutral solid compounds and salts in water and non-aqueous media [24].

For the selection of a biphasic liquid system for a CPC/CCC process the knowledge of the precise value of the solute solubility in a single solvent is very useful, but not necessarily needed. However, it is important to know if a given solute is poorly or substantially soluble in a given solvent.

To get an idea of the solute solubility in a given solvent, the solvent capacity is used. The capacity (C_i) is defined as a reciprocal value of the activity coefficient of solute i infinitely diluted in a solvent (γ_i^∞) [25]:

$$C_i(\text{solvent}) = \frac{1}{\gamma_i^\infty(\text{solvent})} \tag{1}$$

The capacity should be regarded only as qualitative measure of the solubility. Namely, the solute solubility will be higher in a solvent with higher capacity than in a solvent with lower capacity.

The activity coefficient of the solute at infinite dilution (in Eq. (1)) is calculated with COSMO-RS, using the following procedure. In the first step, the molecular structure of the solute and the solvent is generated, and a conformational analysis for each molecule using the MM+ force field is performed in HyperChem (Release 7.51, Hypercube Inc., USA). After that the software package Turbomole (Version 5.10, COSMOlogic, Leverkusen, Germany) is used to perform the quantum mechanics geometry calculations. The geometry of molecules and the screening charge density on their surface is calculated by applying the density functional theory (DFT) under COSMO boundary conditions. Using COSMOtherm, (Version C2.1 Release 01.06, COSMOlogic, Leverkusen, Germany), the chemical potential and consequently, the activity coefficient is calculated [23].

2.1.2. A priori prediction of partition coefficient

The partition coefficient, P_i^{UL}, is defined as ratio of the concentration of solute i in the upper phase (c_i^U) and its concentration in the lower phase (c_i^L). The partition coefficient can also be expressed