Journal of Chromatography A, xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Enhancing silymarin fractionation using the conductor-like screening model for real solvents☆

Emma C. Brace[a,b], Abigail S. Engelberth[a,b,c,*]

[a] Department of Agricultural & Biological Engineering, Purdue University, 125 S. State St., West Lafayette, IN 47907, United States
[b] Laboratory of Renewable Resources Engineering, Purdue University, 500 Central Dr., West Lafayette, IN 47907, United States
[c] Environmental & Ecological Engineering, Purdue University, 500 Central Dr., West Lafayette, IN 47907, United States

ARTICLE INFO

Article history:
Received 5 October 2016
Received in revised form 17 January 2017
Accepted 22 January 2017
Available online xxx

Keywords:
Silymarins
Flavonolignans
Solvent system selection
Countercurrent chromatography
Conductor-like screening model for real solvents

ABSTRACT

A significant hurdle for discovery of plant-derived products is the numerous trial-and-error experiments required to develop an effective purification strategy. To overcome the experimental burden, a quantum mechanics-based molecular modeling approach – known as the COnductor-like Screening Model for Real Solvents (COSMO-RS) – was used to predict a suitable two-phase solvent system to purify six silymarins from an aqueous mixture. Silymarins, a class of flavonolignans found in milk thistle (*Silybum marianum* L.), are well suited for assessing the use of a molecular modeling approach to predict partitioning in a countercurrent chromatography (CCC) separation because they are well characterized and previous studies report low purity fractionation in liquid–liquid solvent systems. They also present an opportunity to evaluate the use of COSMO-RS in predicting the partitioning of structurally similar isomeric compounds that are present together in an aqueous solution upon extraction from their native source. The COSMO-RS model results predicted the partition coefficients in: three traditional ARIZONA solvent systems (composed of heptane, ethyl acetate, methanol, and water), nine additional variations of this quaternary solvent system, and two chloroform, methanol, and water solvent systems. Predicted results were concise but not accurate when compared to experimental results determined by the shake flask method. The 1:4:3:5 *n*-heptane:ethyl acetate:methanol:water (v/v/v/v) system was identified to be an improvement on the 1:4:3:4 system previously reported. The present study verified the ability of COSMO-RS to hone in on one or two solvent systems that will yield the best fractionation using CCC.

1. Introduction

The U.S. Departments of Energy and Agriculture have each recognized a need to foster the emerging biorefinery industry, which could produce fuels, chemicals, nutraceuticals, and other bioproducts [1,2]. Nutraceuticals are naturally occurring plant compounds that exhibit properties that make them attractive for additives in food or medicine. Biomass-derived nutraceuticals can be sourced from a variety of plants, but the complexity of both the biomolecules and the source from which they must be removed may impede the pace of research. The primary objectives of this study were to evaluate the use of a Conductor-like Screening Model for Real Solvents (COSMO-RS) in predicting partition coefficients of the six silymarin compounds to quickly eliminate unacceptable and hone in on suitable solvent systems. The motivation for studying silymarins and COSMO-RS is to further develop theoretical methodology that will allow for faster identification of the best solvent system for fractionation of plant-derived biomolecules using countercurrent chromatography (CCC). Innovative bioseparations techniques such as this are needed to improve the economics of the biorefinery and improve the feasibility of developing new bioproducts through purification of biomolecules that are used as precursors for commodity chemicals and other products. Liquid-liquid extraction techniques are often used for biomolecule extraction and purification, in order to maximize product yield and reduce the risk of losing product to the stationary phase. CCC is a form of liquid chromatography in which two liquid phases under a centrifugal field are used to fractionate target compounds from each other. Various forms of CCC have been used for fractionation of molecules from plant extracts, including various flavonoid compounds [3–9].

☆ Selected paper from the 9th International Counter-current Chromatography Conference (CCC 2016), 1–3 August 2016, Chicago, IL, USA.

* Corresponding author at: 500 Central Dr., West Lafayette, IN 47907, United States.

E-mail addresses: ebrace@purdue.edu (E.C. Brace), aengelbe@purdue.edu (A.S. Engelberth).

http://dx.doi.org/10.1016/j.chroma.2017.01.058

Please cite this article in press as: E.C. Brace, A.S. Engelberth, Enhancing silymarin fractionation using the conductor-like screening model for real solvents, J. Chromatogr. A (2017), http://dx.doi.org/10.1016/j.chroma.2017.01.058

The first, and arguably most critical step, to successful fractionation of biomolecules in countercurrent chromatography is selecting solvents that will yield relatively pure fractions to minimize the need for further downstream processing. Traditionally, biphasic solvent systems can be selected through solubility experiments to identify the solvent the compounds are most soluble in, and then choosing a solvent that is more polar and less polar to create a biphasic ternary solvent system [10]. In an effort to ease the experimental burden, families of solvent systems have been developed including the ARIZONA, HEMWat, and ChMWat [11,12]. However, these families target separation of compounds based on polarity, which is not beneficial for compounds of similar structure and polarity. There remains a need to be able to quickly hone in on a suitable solvent system based solely on molecular differences in the compounds. This critical solvent screening and selection step is often very costly due to the use of high volumes of expensive solvents and standards, and requires a significant amount of time. COSMO-RS is a molecular modeling tool for predicting partition coefficients and can serve as a method to more efficiently and effectively select solvents. The present work evaluates how COSMO-RS can predict the partition coefficient and in turn, hone in on an appropriate solvent system for complex molecules that are present in a mixture extracted from a plant.

A noteworthy difference between this study and previously published work [13–15] using COSMO-RS for solvent system prediction is that we are studying six nearly-identical compounds that occur naturally together. Previously published reports compared the partition coefficients predicted by COSMO-RS to those determined by the shake flask method or countercurrent chromatography using molecules of low molecular weight, such as phenols and *n*-alkyl benzenes [13,14]. This demonstrated the ability of COSMO-RS to distinguish between stereoisomers such as hydroquinone and pyrocatechol, which is useful, but does not necessarily translate to larger stereoisomers such as the silymarins, which have a molecular weight greater than 400 Da. Additionally, analysis of a GUESS-mix containing caffeine, estradiol, coumarin, vanillin, and other molecules showed successful use of COSMO-RS, but this ideal mixture contains molecules of various polarities, molecular weights, and other properties [13]. Successful modeling of the silymarins is challenging not only because they are large molecules (>400 Da) in an aqueous solution but also because they are naturally found together and exhibit similar properties.

2. Experimental methods

2.1. Reagents and equipment

Purified standards (purity >93%) of the six silymarin compounds were purchased from Cerrilliant (Round Rock, TX). Silymarin extract was purchased from Sigma-Aldrich (St. Louis, MO). All solvents were of HPLC grade and water was deionized in house. *n*-Heptane was purchased from Alfa Aesar (Ward Hill, MA). Methanol was purchased from OmniSolv (Gibbstown, NJ). Ethyl acetate, dichloromethane, and chloroform were purchased from J.T. Baker (Center Valley, PA).

HPLC analyses were performed using a Waters e2695 model equipped with a Waters 2489 UV detector and a Waters Symmetry C18 column (Waters Corporation, Milford, MA).

Gas chromatography analyses were performed using an Agilent Technologies 7693A auto-sampler and 7820A system equipped with a flame ionization detector (Agilent Technologies, Santa Clara, CA).

2.2. Experimental determination of partition coefficients

The conventional shake flask method was used to experimentally determine the partition coefficients of the six silymarin compounds in *n*-heptane:ethyl acetate:methanol:water solvent systems and in chloroform:methanol:water solvent systems. Each solvent system was prepared to a volume of 10 mL and mixed using a vortex genie for 2 min before adding 30 mg of powdered silymarin extract. Each tube was then mixed using a vortex genie for 3 min and allowed to settle for 2 h. A 1 mL aliquot of each phase was extracted from each solvent system tested. Each sample was dried under nitrogen at 70 °C and then reconstituted in 1 mL of methanol for HPLC analysis to determine the concentration of each of the six silymarins in both the upper and lower phases. The partition coefficient, K_D, is the ratio of the concentration in the upper phase divided by the concentration in the lower phase.

2.3. HPLC analysis

HPLC analyses were carried out based on previously published methods [6,16,17], using a Waters e2695 model equipped with a Waters 2489 UV Detector and a Waters Symmetry C18 column (150 mm × 4.6 mm, 5 μm) set at 40 °C. Solvent A was 80% water, 20% methanol; Solvent B was 20% water, 80% methanol. The UV detector was set at 290 nm. The mobile phase flow rate was 0.75 mL/min and the injection volume was 10 μL.

2.4. Gas chromatography analysis of phase composition

As a precursor to molecular modeling, the composition of each phase of each of the bi-phasic solvent systems was determined using gas chromatography or available references [18–20]. This data was input for calculation of the activity coefficient of molecules in each phase and derivation of the partition coefficient, and was the only experimental data required for the theoretical calculations of the partition coefficient. The phase composition of the ternary chloroform-methanol-water used phase diagrams [18]. The phase composition of ARIZONA G, P, and U were taken from gas chromatography determination by Garrard et al. [20]. The remaining *n*-heptane:ethyl acetate:methanol:water solvent system phase compositions were determined experimentally via gas chromatography. Solvent systems were made up to a volume of 10 mL and mixed for 3 min using a vortex genie. After equilibration for a minimum of 2 h, 1.5 mL of each phase was removed and added to a GC vial and analyzed using an Agilent HP-5 column (30 m × 0.32 mm × 0.25 μm, 7-in. cage). The oven initial temperature was 45 °C and increased at a rate of 20 °C/min to a final temperature of 70 °C. The total run time was 7 min and the split mode was used with a split ratio of 20:1 to avoid overloading the flame ionization detector (FID), which was held at 250 °C. Helium was used as the carrier gas. The GC was calibrated for *n*-heptane, ethyl acetate, and methanol by mixing each solvent with dichloromethane at the following volume percentages: 1, 2, 5, 10, 15, 25, 45, 65, 85, and 100%. The volume percent of each solvent in each phase was determined and it was assumed that the remaining volume fraction in either phase was water.

3. Theoretical method: COSMO-RS

The COSMO-RS method for calculating the partition coefficient is unique because it requires only the molecular structure of the solute molecules, and the composition of the phases in the bi-phasic solvent system [21]. Other theoretical models for relating molecular structure to the partition coefficient include Quantitative Structure-Activity Relationships (QSARs) [22], however,

Please cite this article in press as: E.C. Brace, A.S. Engelberth, Enhancing silymarin fractionation using the conductor-like screening model for real solvents, J. Chromatogr. A (2017), http://dx.doi.org/10.1016/j.chroma.2017.01.058