Journal of Chromatography A, 1377 (2015) 55–63

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Qualitative and quantitative evaluation of solvent systems for countercurrent separation☆

J. Brent Friesen[a,b,c,*], Sana Ahmed[a], Guido F. Pauli[b,c]

[a] Physical Sciences Department, Rosary College of Arts and Sciences, Dominican University, River Forest, IL 60305, USA
[b] Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, 833 S. Wood Street, Chicago, IL 60612, USA
[c] Institute for Tuberculosis Research, College of Pharmacy, University of Illinois at Chicago, 833 S. Wood Street, Chicago, IL 60612, USA

ARTICLE INFO

Article history:
Received 12 September 2014
Received in revised form
22 November 2014
Accepted 27 November 2014
Available online 4 December 2014

Keywords:
Countercurrent chromatography
Counter-current chromatography
CCC
centrifugal partition chromatography
CPC
GUESSmix

ABSTRACT

Rational solvent system selection for countercurrent chromatography and centrifugal partition chromatography technology (collectively known as countercurrent separation) studies continues to be a scientific challenge as the fundamental questions of comparing polarity range and selectivity within a solvent system family and between putative orthogonal solvent systems remain unanswered. The current emphasis on metabolomic investigations and analysis of complex mixtures necessitates the use of successive orthogonal countercurrent separation (CS) steps as part of complex fractionation protocols. Addressing the broad range of metabolite polarities demands development of new CS solvent systems with appropriate composition, polarity (π), selectivity (σ), and suitability. In this study, a mixture of twenty commercially available natural products, called the GUESSmix, was utilized to evaluate both solvent system polarity and selectively characteristics. Comparisons of GUESSmix analyte partition coefficient (K) values give rise to a measure of solvent system polarity range called the GUESSmix polarity index (GUPI). Solvatochromic dye and electrical permittivity measurements were also evaluated in quantitatively assessing solvent system polarity. The relative selectivity of solvent systems were evaluated with the GUESSmix by calculating the pairwise resolution (α_{ip}), the number of analytes found in the sweet spot (N_{sw}), and the pairwise resolution of those sweet spot analytes (α_{sw}). The combination of these parameters allowed for both intra- and inter-family comparison of solvent system selectivity. Finally, 2-dimensional reciprocal shifted symmetry plots ($ReSS^2$) were created to visually compare both the polarities and selectivities of solvent system pairs. This study helps to pave the way to the development of new solvent systems that are amenable to successive orthogonal CS protocols employed in metabolomic studies.

1. Introduction

1.1. Basic research in solvent system selection

Solvent system selection plays a major role in the application of countercurrent (also spelled counter-current) chromatography and centrifugal partition chromatography technologies collectively known as countercurrent separation (CS). The choice of an appropriate biphasic solvent system for a particular application is comparable to the simultaneous choice of both the liquid mobile phase and the chromatographic media in conventional liquid chromatography. Therefore, solvent system selection strategies abound in the countercurrent separation literature [1–11].

For every solvent system selection method, whether it is thermodynamically or empirically based, it is necessary to identify a solvent system or set of solvent systems which will be tested. The set of solvent systems to be tested is often based on the practitioner's familiarity with them, or based on a literature analysis of solvent systems employed for a certain type of source organism or class of compounds. As a result, solvent system families such as hexane/ethyl acetate/methanol/water (HEMWat), chloroform/methanol/water (ChMWat), and ethyl acetate/butanol/water (EBuWat) tend to be favored. In fact, a review of CS methods used in the separation of natural products reported that HEMWat alone was used in 29% of the protocols [12]. The abbreviated nomenclature for CS has been systematized according to previously established definitions, which are summarized in Section 2.1.

☆ Presented at the 8th International Conference on Countercurrent Chromatography - CCC 2014, 23–25 July 2014, Uxbridge, United Kingdom.

* Corresponding author. Tel.: +1 708 524 6972.
E-mail address: jbfriesen@dom.edu (J.B. Friesen).

http://dx.doi.org/10.1016/j.chroma.2014.11.085
0021-9673/

If the commonly used solvent systems are sufficient for the task at hand, then the practitioner need not look further. However, this approach tends to limit analytical rigor as well as creativity and may systematically overlook truly superior solvent system formulations in favor of tried and true recipes. To this end, basic research on the characteristics of solvent systems and their potential usefulness for CS promotes a greater understanding of the attributes of the most suitable solvent system.

1.2. Solvent system polarity, selectivity and performance

The essential attributes of a solvent system in terms of usefulness for CS are polarity, selectivity, and performance. It is very important that the polarity of the solvent system matches the polarity of the target compound(s). Moreover, CS is most effective when the target compounds elute in the "sweet spot" polarity range of optimal separation, i.e. the range where the partition coefficients (K values) are between 0.25 and 16 [13]. Selectivity refers to the resolution of compounds with similar structural and/or polarity characteristics. Compared with other liquid chromatography methods, CS has shown to be a very selective technique capable of separating constitution isomers, diastereomers, congeners and homologs [14–19]. Performance mainly refers to the ability of the chosen stationary phase of the solvent system to be retained in the CS column, leading to high stationary phase volume retention ratio values and resulting in optimized separation.

Solvent system polarity is typically measured by comparing the K values of a compound in different solvent systems. This is similar to the measurement of eluotropic polarity in LC. In reversed phase mode, a more polar mobile phase will give a higher K value. Solvatochromic dyes, such as Reichardt's dye, have been used to measure the polarity of CS solvent system phases [20]. In some cases, solvent system polarity has been predicted by the linear combination of polarity parameters established for pure solvents such as Abraham's descriptors, octanol/water partition coefficients (Log P), molar refraction (MR), dipole moment (μ), energy of hydration (E_H), Rohrschneider–Snyder polarities (p'), and Hildebrand polarity values [10,21–23].

Selectivity comes into play when a solvent system of appropriate polarity does not adequately resolve the target analyte(s) from each other or from impurities. In this case, an orthogonal solvent system is sought that has a similar polarity as the original but different selectivity characteristics. To this date, no method of measuring solvent system selectivity in CS has been proposed. In other LC fields such as HPLC, it is common to demonstrate the selectivity of a stationary phase by publishing examples where the media have been employed to separate a test mixture of compounds of interest. For this purpose, frequently employed analytes are mixture of pharmaceutical APIs and natural products such as opioids from *Papaver somniferum*, α- and β-acids in hop extract, ginkgolides from *Ginkgo biloba*, or gingerols and shogaols from *Zingiber officinale* [24,25].

Solvent system performance in CS is determined by running the solvent system in an instrument. In fact, the retention of solvent systems by a given instrument is an indicator for both solvent system suitability and instrument design. Solvent system characteristics that influence the stationary phase volume retention ratio (Sf) are density, viscosity, interfacial tension, and settling time [3,26–34].

2. Experimental

2.1. Solvent system family abbreviations

Solvent abbreviations have been combined to create solvent system family names: Ac = acetonitrile, Bu = n-butanol, Ch = chloroform, Di = dichloromethane, E = ethyl acetate, H = hexane, M = methanol, and *ter* = *tert*-butylmethylether, and Wat = water [1,2,13,35]. Therefore, solvent system combinations may be written and pronounced in a manageable fashion, such as HEMWat (pronounced "hemwat") and ChMWat (pronounced "kemwat"). Solvents are arranged in order of polarity: from least polar to most polar. Volume ratios are given in whole numbers.

2.2. Sources for K values

The experimental procedures for obtaining the GUESSmix K values have been published previously for the 17 solvent systems of the original HEMWat family [13,35,36]. In addition, the GUESSmix K values for the eight *ter*AcWat, nine H*ter*AcWat, six EBuWat solvent systems, as well as the portal systems HBuMWat 5:5:5:5, *ter*BuMWat 5:5:5:5, and DiEMWat 5:5:5:5 were obtained according to previously published procedures [2]. The formulation of the eight solvent system ChMWat family is described in [1]. The experimental procedures for the eight ChMWat solvent systems and eight DiMWat solvent systems are described below.

2.3. Instrumentation

The high-speed countercurrent chromatograph (HSCCC) instrument employed in the present study was a TBE-300A (Shanghai Tauto Biotech Co. Ltd, Shanghai, China) with three multilayer coil separation columns connected in series (1.6 mm tubing i.d.), giving 280 mL total column volume, with a 20 mL sample loop. The revolution radius or the distance between the holder axis and the central axis of the centrifuge (R) was 5 cm, and the β values of the multilayer coil varied from 0.5 at the internal terminal to 0.8 at the external terminal ($\beta = r/R$. where r is the distance from the edge of the coil to the holder shaft). The rotational speed of the apparatus could be regulated with a speed controller with the range 0–1000 rpm. A Neslab RTE7 constant temperature-circulating bath (Thermo Electron Corporation) was used to control the separation temperature within the range of 5–35 °C. The HSCCC system was equipped with a ChromTech Series I digital single-piston solvent pump, a JMST Systems VUV-14D fixed wavelength UV–vis detector with preparative flow cell, and a Advantec CHF122SC fraction collector. Data was recorded on a PEAK-ABC Chromatography Data Handling System and then transferred to an Excel worksheet for further treatment.

Analytical TLC was performed at room temperature on SIL G-25 precoated 0.25 mm thick silica gel UV_{254} glass plates (20 × 10 cm; Macherey-Nagel, Düren, Germany). TLC experiments were carried out in duplicate. Plates were dipped in the general-purpose reagent *p*-anisaldehyde/sulfuric acid/acetic acid 1/1/48, drained, and heated on a Camag TLC Plate Heater III at 95 °C for about 5 min. All TLC chromatograms were scanned for digital preservation at 150 dpi with a Canon CanoScan N670U scanner.

2.4. Solvents and reagents

All solvents were HPLC grade from Pharmco-AAPER, Shelbyville, KY. Chemicals were purchased from Sigma–Aldrich-Fluka.

2.5. High-speed countercurrent chromatography (HSCCC)

GUESSmix samples were prepared as previously described in the form of a stock solution with a final concentration of approximately 0.1 g/mL of combined compounds [1]. The stock solution was stored at −30 °C and warmed to room temperature before use. Unless stated otherwise, the GUESSmix was prepared for a chromatographic run by obtaining 2.2 mL of the stock solution and evaporating it to dryness under forced air. The resulting residue was