



Solvent system selection in counter-current chromatography using conductor-like screening model for real solvents

Elisabeth Hopmann, Wolfgang Arlt, Mirjana Minceva*

Separation Science and Technology, Friedrich-Alexander University Erlangen-Nuremberg, Egerlandstr. 3, 91058 Erlangen, Germany

ARTICLE INFO

Article history:

Received 31 March 2010

Received in revised form 8 September 2010

Accepted 10 November 2010

Available online 21 November 2010

Keywords:

Counter-current chromatography

Solvent system selection

Solvent system screening

COSMO-RS

Two-phase liquid system

ABSTRACT

The aim of this investigation was to minimize the experimental effort for solvent system selection in counter-current chromatography. The separation mechanism in counter-current chromatography is based exclusively on the partitioning of the solute between the two liquid phases. Therefore, a quantum chemical method combined with statistical thermodynamics (conductor-like screening model for real solvents, COSMO-RS) was used for the prediction of the partition coefficient. A comparison of the experimental and predicted data for five model solutes systems demonstrated the potential of the use of COSMO-RS as a screening tool for the solvent system selection.

© 2010 Elsevier B.V. All rights reserved.

1. Introduction

Counter-current chromatography (CCC) is a term introduced by Ito [1] to describe an innovative liquid–liquid chromatographic technique, which employs two almost immiscible liquid phases. One of the phases is kept stationary by the centrifugal force in a specially designed column assembled on a centrifuge [2,3]. The other phase is forced (pushed) through the stationary liquid as the mobile phase. The separation mechanism is based solely on solutes partitioning between the two liquid phases.

The key features of CCC are related to the liquid nature of the stationary phase, which occupies 60–80% of the total column volume. The entire volume of the stationary phase is accessible to the sample solutes, which increases the loading capacity and decreases the mobile phase consumption. The absence of irreversible adsorption provides complete sample recovery and high separation reproducibility. Moreover, both phases of the two-phase liquid system can be used as stationary or mobile phases and their role can be switched during the chromatographic separation. Thus, the same two-phase liquid system can be used to perform a separation in normal or reversed phase mode. Also some unique operating modes, not possible when working with a solid stationary phase, can be used to improve the separation, reduce the separation time and solvent consumption [4,5].

Since the early years of CCC, natural products isolation and purification has been the leading area of CCC applications [6]. CCC has been successfully used for the separation of pharmaceuticals, vitamins, dyes, herbicides, pesticides, and inorganic elements [6,7].

The first and most important step in the development of any CCC separation is the selection of a two-phase liquid system, which can be obtained by mixing of two or more solvents. In fact, the choice of the possible solvent combinations, which form a biphasic system, is almost limitless. Compared to the more popular solid–liquid chromatography, the selection of the solvent system is equivalent to the simultaneous selection of both the stationary phase (column) and the mobile phase. Any change in composition of either phase will affect the composition of the other phase. In CCC, the value of the partition coefficient of the solute of interest should ideally range between 0.4 and 2.5, also called the “sweet spot polarity range” [8]. Partition coefficients lower than 0.4 bring a loss of resolution, while partition coefficients higher than 2.5 cause a long retention time and lead to diluted solutes. Besides the value of the partition coefficient, some physical properties of the solvent system, including the density difference of the two phases, viscosity and interfacial tension, must be considered as additional parameters for the selection of the biphasic solvent system. These properties affect the stationary phase volume, the mass transport and the pressure drop in the CCC column [9]. As stated by the inventor of CCC, Ito, the selection of the solvent system may turn out to be 90% of the entire work needed to set-up a successful separation [10].

The solute distribution (partitioning) between the mobile and stationary phase results from the different solubility in these two phases. According to the “best solvent” approach [11], a system-

* Corresponding author. Tel.: +49 9131 852 7459; fax: +49 9131 852 7441.
E-mail address: mirjana.minceva@cbi.uni-erlangen.de (M. Minceva).

atic method for the selection of a biphasic solvent system, made, in general, by more than 2 compounds, should be started by the selection of a good solvent for the solute (solvent in which the solute of interest is highly soluble), followed by the selection of two other solvents (generally mutually poorly miscible) in which the “best solvent” is soluble. In this case the “best solvent” and solute will partition between the two other solvents in a similar way. After the solvent system is selected the partition coefficient of the solute is measured experimentally for selected solvent system compositions. Based on these results the composition of the solvent system is further adjusted according to the data of the ternary diagram in order to obtain the required partition coefficient of the solute. When this fails, new combinations of the “best solvent” and the other two solvents should be tested. This approach requires a certain degree of skill with solvents and the availability of ternary diagrams, including tie-lines. Ternary diagrams for many solvent systems can be found in [2,12]. Without this know-how and information, this approach turns to a trial and error method.

From the point of view of chromatography users, who are accustomed to selecting a stationary phase from suppliers' catalogues and then fine tuning the mobile phase composition, the lack of a general straightforward approach for the selection of CCC solvent systems is the major disadvantage of this technology.

Currently the selection of the solvent system is mostly done by using already published data or by experimental screening of predefined multisolvent system compositions organized in tables according to the system overall polarity, called solvent system families [10,13]. There is an extensive literature on successful CCC applications for a large variety of compounds. Very useful compilations of previously used solvent systems for broad classes of compounds can be found in [2,10,14].

The solvent family refers to a mixture of 3–5 solvents, forming two almost immiscible phases when mixed in certain proportions. Each solvent system family is ordered in a table with stepwise increasing polarity, obtained by a gradual variation of the solvent proportions in the mixture. The expression “polarity” is not clearly defined and somehow expresses the behavior of a solvent between the cornerstones such as “water” and “hexane”. The solvent system composition leading to a solute partition coefficient in the required CCC range is obtained by experimental screening of the solvent system family table. Solute partition experiments are carried out and the best solvent mixture is selected or further obtained by fine tuning of the solvent mixture composition. When the required partition coefficient of the solute cannot be attained another solvent system family covering a different solvent polarity range is tested.

The most used biphasic CCC solvent system families are: hexane/ethyl acetate/butanol/methanol/water [15], heptane/acetonitrile/butanol/water [2,15], chloroform/methanol/water [16], and heptane/ethyl acetate/methanol/water (“ARIZONA” family) [17].

For a selection of biphasic solvent system for natural products CCC applications, a mixture of predefined compounds called “GUESSmix” (Generally Useful Estimation of Solvent Systems) was recently proposed by Friesen and Pauli [8]. GUESSmix is a mixture of different classes of compounds with varying polarities, functional groups, and structures. This mixture is designed as a representation of a natural product sample and can be used to screen different solvent systems or solvent systems families by performing a single CCC elution experiment [8] or an elution–extrusion experiment [18].

The recent review [19] has shown that despite the many possible solvent combinations forming a two phase system, only a limited number of solvents are used to prepare the mobile and stationary phase in CCC. The most used solvents are *n*-hexane, ethyl acetate, methanol and water. The table of top 10 solvent choices includes also *n*-butanol, chloroform, acetonitrile, ethanol, tert-butyl methyl ether and *n*-heptane, which are used to a much smaller extent.

This information clearly shows that one of the main advantages of CCC, the inherent flexibility of the choice of mobile and stationary phases, is not fully exploited. For a long time, CCC was known as a niche chromatographic technique used at a laboratory scale, mainly for isolation and purification of natural products. Recently the CCC hardware was scaled-up to production scale [20]. For the production scale, not only the partition coefficient (thermodynamics) and physical properties of the solvent systems (mass transfer properties) but also the solvent's cost, accessibility, safety considerations, environmental impact as well as the cost of the following unit operation needed for solute recovery from the used solvents must be considered.

In this work, another important advantage of CCC, normally not mentioned in the technology key features, is addressed. This advantage is that it is possible to calculate the thermodynamic equilibrium of the solute, i.e. the partition coefficient. This means that the experimental effort needed for the selection of the solvent system and its composition can be significantly reduced.

Several methods for the calculation of the partition coefficient are available. These methods are widely used for the calculation of the octanol/water partition coefficient, which is an important parameter – used to approximate solute partitioning over a biomembrane. To correlate the partition coefficient and molecular properties of the solute, groups of methods are usually used, as Quantitative Structure–Activity Relationships (QSARs) [21] or Linear Solvation Energy Relations (LSERs) [22]. The parameters of these methods are determined by regression of experimental data and hence the quality of the method highly depends on the accuracy of the used experimental data. Furthermore, the parameters are only valid for the fitted property. Another possibility is the use of thermodynamic based methods as the group-contribution Universal Quasi-Chemical Functional-Group Activity Coefficient (UNIFAC) model [23] and the quantum mechanics based Conductor-like Screening Model for Real Solvents (COSMO-RS) [24]. The disadvantage of the structure-interpolating UNIFAC model is the dependence on group interaction parameters, which are often limited or missing [25].

The COSMO-RS model was chosen because its capability to predict thermodynamic properties based only on the molecular structure. This model, in contrast to UNIFAC, is able to distinguish between steric isomers. The COSMO-RS model was successfully validated for the prediction of the octanol/water partition coefficient of small and complex molecules [26,27]. Recent applications have shown the potential of COSMO-RS as a screening model for the selection of extraction and biocatalytic biphasic reaction systems [28,29]. We were interested in the prediction of the solute partitioning not only between two almost immiscible solvents but also between biphasic solvent systems consisting up to four solvents, which are widely used in CCC. The objective of this work is to test the applicability of the COSMO-RS model as a tool for screening and selection of biphasic solvent systems for CCC applications.

2. Theory

The COSMO-RS was developed by Klamt [30] and introduced to chemical engineering by Arlt et al. and coworkers [31]. The model is based on unimolecular quantum mechanics calculations (COSMO) combined with methods of statistical thermodynamics (COSMO-RS). The main advantage of COSMO-RS is the independence from experimental data. Namely, for the calculation of the partition coefficient only the molecular structure and the composition of each phase (mobile and stationary phase without solute *i*) of the biphasic liquid system are required as input data. In this work the COSMO-RS was used to calculate solute activity coefficients in the upper and lower phase, respectively, which are afterwards used for cal-

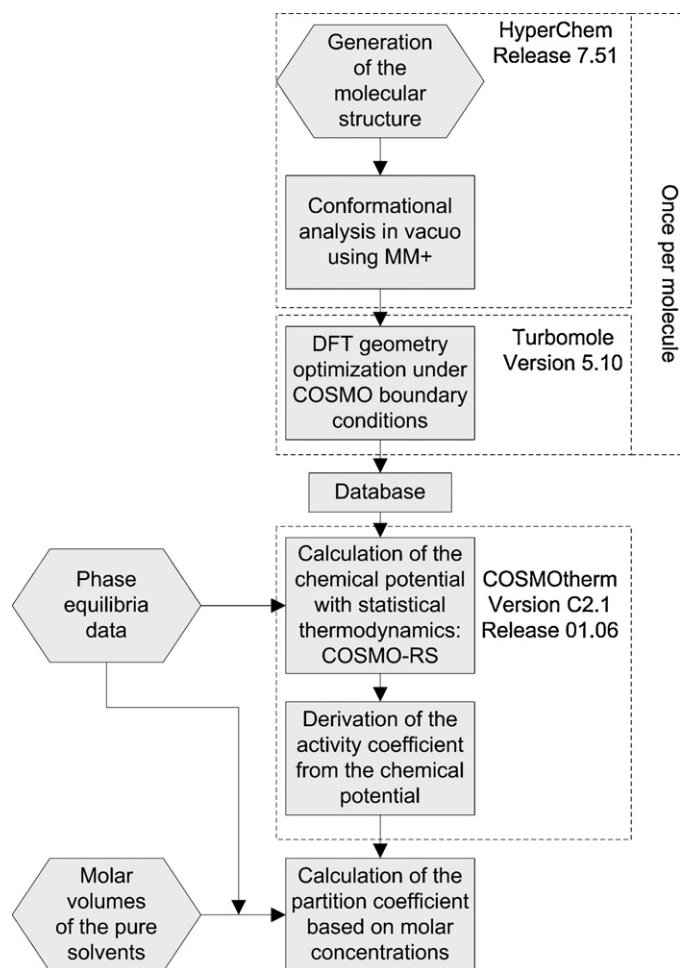


Fig. 1. Scheme of the procedure used for the calculation of the partition coefficient; hexagons represent data input and rectangles represent calculations; DFT, density functional theory; COSMO-RS, conductor-like screening model for real solvents.

calculation of the solute partition coefficient. The calculations of the partition coefficient were carried out according to the procedure presented in Fig. 1. The procedure consists of several steps. The calculations using the programs HyperChem and Turbomole must be performed only once per molecule and the resulting pure compound information can be stored in a database. The calculation using COSMOtherm, which is done in seconds, yields the properties of the mixtures.

Obviously organic molecules have different conformations. The conformations of each molecule have a significant influence on the quality of the COSMO-RS prediction [32,33]. In the present study the drawing of the molecules and the conformational search in vacuum were carried out using MM+ force field implemented in HyperChem (Release 7.51, Hypercube Inc., USA). The numbers of conformers are dependent on the size and complexity of the molecules and can be influenced by the analysis parameter during the conformational search. The analysis parameter and the resulting number of conformers for each molecule are given in the supporting information. Energy within $E=0.05$ kcal/mol to $E=3$ kcal/mol and Root-Mean-Square (RMS) error within $\text{RMS}=0.25$ Å to 2 Å were selected for the conformational analysis. The time needed for the conformational search depends on the size and type of the molecule, for example on a 2.4 GHz CPU for prednisolone acetate 80 min were needed, while for ethylbenzene only 8 min were needed.

The software package Turbomole (Version 5.10, COSMOlogic, Leverkusen, Germany), in which the COSMO model is implemented,

was used to perform the quantum mechanics geometry calculations applying density functional theory (DFT) with an approximate treatment of the electronic Coulomb interactions (resolution of identity, RI-DFT), Becke Perdew (B-P) functional and triple zeta valence polarized (BP-TZVP) basis set [34]. The quantum mechanics calculation is the most time-consuming step, which must be done once for each conformer and the resulting information can be stored in a database. The time needed for the quantum mechanics calculation is dependent on the size and type of the molecules and took no longer than 30 min for small molecules as benzyl alcohol, benzene, toluene, ethyl- and butylbenzene, phenols, vanillin, coumarin, umbelliferone, salicylic acid. Up to 9 h were needed for the bigger molecules (e.g. steroids, hexyl- and dodecylbenzene). If more processors are available, the calculation time can be reduced by parallel calculation. Moreover, due to increasing PC power, the prediction time will also be reduced in the future.

Using the results of the quantum mechanics calculations and the composition of each phase (mobile and stationary phase without solute i) the chemical potential of solute i infinitely diluted in a mixture was calculated with statistical thermodynamics (COSMO-RS). Subsequently the activity coefficient, γ_i , of solute i was derived from the chemical potential. The statistical thermodynamics calculations were completed using the COSMOtherm program (Version C2.1 Release 01.06, COSMOlogic, Leverkusen, Germany). A weighted Boltzmann mixture of the conformers, implemented in the COSMOtherm program, is used. The required phase equilibrium data can be taken from literature or determined experimen-

tally. An extensive collection of ternary diagrams is available in [12].

The partition coefficient of solute i in stationary (S) and mobile (M) phase (K_i^{SM}) was calculated from the thermodynamic equilibrium as follows:

$$K_i^{SM} = \frac{x_i^S}{x_i^M} = \frac{\gamma_i^M}{\gamma_i^S}; \quad x_i \rightarrow 0, \quad (1)$$

where x_i^S is the molar fraction of solute i in the stationary phase and x_i^M is the molar fraction of solute i in the mobile phase.

To compare the calculations with the experimental data the partition coefficient based on molar fraction (Eq. (1)) was converted into the partition coefficient based on molar concentration (P_i^{SM}) using Eq. (2). The excess volume of mixing was neglected and hence the molar volume of the mobile, v^M , and stationary, v^S , phase, respectively, was approximated as the weighted sum of the molar volumes of the pure compounds v_{0j} . The data for the molar volume of the pure compounds were taken from the DIPPR (“Design Institute for Physical Properties”) database [35].

$$P_i^{SM} = \frac{c_i^S}{c_i^M} = K_i^{SM} \cdot \frac{v^M}{v^S} = K_i^{SM} \cdot \frac{\sum x_j^M \cdot v_{0j}}{\sum x_j^S \cdot v_{0j}} \quad (2)$$

where j = compounds of the upper and lower phase, respectively. c_i^M and c_i^S are the concentration of the solute i in mobile phase and stationary phase, respectively, in mol/l.

2.1. Solvent systems

For the prediction of the partition coefficient some of the most commonly used multisolvent systems in CCC were selected. Namely, the solvent system family hexane/ethyl acetate/methanol/water (“HEMWat”) and solvent system family – with the less toxic heptane instead of hexane – heptane/ethyl acetate/methanol/water (“ARIZONA”) were chosen.

HEMWat solvent system family, given in Tables 1a and 1b, was proposed by Friesen and Pauli [8], who had actually modified and adapted the original solvent system family defined by Oka [15]. Beside the numeration used by Friesen the notation used by two other authors is also included in Table 1a. In the HE MWat solvent system family the volume of hexane and ethyl acetate is constant and equal to the volume of methanol and water. The polarity of the systems increases with increasing number of the systems (–7 to +8). The composition of the upper and lower phase for the HE MWat systems were measured by gas chromatography (GC) in our laboratory.

Table 1a
Hexane/ethyl acetate/methanol/water (HEMWat) global composition (v/v/v/v).

[8]	[37]	[15]	Hexane	Ethyl acetate	Methanol	Water
–7	X	–	9	1	9	1
–6	U	–	8	2	8	2
–5	–	–	7	3	7	3
–4	–	–	7	3	6	4
–3	Q	–	6	4	6	4
–2	–	4	7	3	5	5
–1	–	5	6	4	5	5
0	N	6	5	5	5	5
1	–	–	4	6	5	5
2	–	–	3	7	5	5
3	L	–	4	6	4	6
4	–	–	3	7	4	6
5	–	–	3	7	3	7
6	G	–	2	8	2	8
7	C	–	1	9	1	9
8	A	11	0	10	0	10

Table 1b
Global composition of the heptane/ethyl acetate/methanol/water (ARIZONA) systems (v/v/v/v).

[36]	[2]	Heptane	Ethyl acetate	Methanol	Water
6	A	0	1	0	1
7	B	1	19	1	19
8	C	1	9	1	9
9	D	1	6	1	6
10	F	1	5	1	5
11	G	1	4	1	4
12	H	1	3	1	3
13	J	2	5	2	5
14	K	1	2	1	2
15	L	2	3	2	3
16	M	5	6	5	6
17	N	1	1	1	1
18	P	6	5	6	5
19	Q	3	2	3	2
20	R	2	1	2	1
21	S	5	2	5	2
22	T	3	1	3	1
23	U	4	1	4	1
24	V	5	1	5	1
25	W	6	1	6	1
26	X	9	1	9	1
27	Y	19	1	19	1
28	Z	1	0	1	0

The heptane/ethyl acetate/methanol/water system, given in Table 1b, was introduced by Margraff and named ARIZONA system [2]. Additionally the notation from Garrard et al. [36] is included. The volume of heptane and ethyl acetate is constant and equal to the volume of methanol and water, as in the HE MWat solvent system family. In contrast to the HE MWat solvent system numbering, the polarity of the ARIZONA systems decreases as the system number increases. Liquid–liquid equilibrium (LLE) data for this system were taken from Berthod et al. [37] for case study 2 and 5, and from Garrard et al. [36] for case study 3.

The liquid system heptane/methanol/water was used for case study 1. This system was used by Berthod because of its easy preparation [38].

3. Material and methods

3.1. Chemicals

Benzyl alcohol (purity $\geq 98\%$, GC) was purchased from Sigma–Aldrich, Germany. Phenol, hydroquinone and pyrocatechol with a purity $\geq 99\%$ were purchased from Sigma–Aldrich, USA. The above components were used as solutes in the partition equilibria experiments.

Solvents employed in the experiments included: hexane, heptane, methanol (all of analytical grade) purchased from Merck, Germany. Ethyl acetate (analytical grade) was purchased from VWR, France. Water was deionised. Tetrahydrofuran (THF, analytical grade) – used for dilution – was purchased from VWR, France.

Methanol used in the high-performance liquid chromatography (HPLC) analysis (gradient grade for liquid chromatography, $\geq 99.9\%$) was purchased from Merck. Deionised water used in the HPLC analysis was filtered through 0.45 μm Whatman membrane filters manufactured from regenerated cellulose.

3.2. Equipment

HPLC analyses were performed on a Waters gradient system consisting of Waters 717 auto sampler, Waters 510A pump, Knauer degasser and Shimadzu SPD-10A VP UV Detector.

An Agilent Technology 7890A system equipped with an Agilent technology 7683D auto sampler was used for all GC analyses.

3.3. Methods

3.3.1. Determination of partition coefficients (shake flask method)

The shake flask method was used for measurement of the partition coefficient at room temperature ($22 \pm 2^\circ\text{C}$). The sample was introduced in a test tube containing the two liquid phases. The resulting total volume was 8 ml. The maximum concentration of the sample in either phase did not exceed 0.02 mol/l. The test tube was shaken manually and additionally mixed with a vortex until equilibrium was reached. After centrifugation for 3 min at 1500 rpm using a Sigma 2-16KC centrifuge with a swing out rotor number 11192 an aliquot of each layer was transferred to a vial and analyzed by HPLC or GC as described below. P_i^{SM} values were calculated by dividing the measured absorbance peak area of the solute in the upper phase by the measured absorbance peak area of the solute in the lower phase. This means that in a possible CCC separation the stationary phase will be the upper phase.

3.3.2. HPLC analysis

The amount of phenol, hydroquinone and pyrocatechol, in each phase was determined by HPLC. Samples of upper and lower phases were diluted with methanol prior to HPLC measurement.

The analysis was performed isocratically using a Nucleosil 100-5 C18 column (125 mm \times 3 mm i.d.) at 40°C and 280 nm. A mixture of 15% methanol and 85% water was used as a mobile phase. The mobile phase flow rate was 0.6 ml/min. The sample injected volume was 5 μl .

3.3.3. Gas chromatography analysis

The amount of benzyl alcohol, pyrocatechol, phenol and hydroquinone, respectively in each phase was determined by gas chromatography (GC) with an Agilent HP-5 column (30 m \times 0.32 mm \times 0.25 μm) and helium as a carrier gas. The injector temperature was 250°C . The oven temperature program was: 60°C for 3.5 min, $20^\circ\text{C}/\text{min}$ temperature ramp to 250°C and then 1 min at 250°C . An exception was made for the temperature program used for analysis of hydroquinone, as the injector temperature was 300°C and the initial oven temperature was 60°C for 3.5 min, then a ramp rate of $20^\circ\text{C}/\text{min}$ to 300°C with a final hold time of 1 min. A flame ionization detector was used for sample analysis. A sample volume of 1 μl was injected using a split injector system with a split ratio of 25:1. Samples were diluted with THF 1:10 (v/v) prior to analysis.

3.3.4. Phase composition determination

8 ml of each of the solvent systems in the HEMWat family (Table 1a) was prepared in a test tube at room temperature ($22 \pm 2^\circ\text{C}$). The test tube was shaken manually and additionally mixed with a vortex until equilibrium was reached. After centrifugation for 3 min at 1500 rpm an aliquot of each layer was transferred to a vial and analyzed by GC using a Restek packed column Silico HP-D80/100 (2 mm ID, 1.8 m) and helium as carrier gas. The

injector temperature was 250°C and the oven temperature program was: 60°C for 2 min and followed by ramp of $20^\circ\text{C}/\text{min}$ to 250°C with a final hold time of 15 min. A sample volume of 1 μl was injected using a splitless injector. A thermal conductivity detector was used in these analyses. Solutions of known compositions were used to construct the calibration curves for each solvent.

4. Results and discussion

The COSMO-RS model is validated regarding the prediction of the partition coefficients of homologues and different test mixtures. Five different mixtures of model solutes were selected and subdivided into five case studies. The solute/s and solvent systems used in each case study are summarized in Table 2.

The quality of the COSMO-RS prediction of common homologues, which are simple molecules, was first evaluated (case study 1). Then the partition coefficient of one solute (benzyl alcohol) in different solvent system compositions was calculated (case study 3). In the second stage of our study, more complex solutes like steroids (case study 2) and molecules of the GUESS mix (case study 5) were considered. In addition, a model mixture consisting of steric isomers, hydroquinone and pyrocatechol, and phenol (case study 4) was chosen.

For the case studies 1, 2 and 5 partition coefficients experimental data were taken from the literature. Whereas for the case studies 3 and 4 the partition coefficients were measured in our laboratory.

4.1. Case study 1: *n*-alkylbenzenes in heptane/methanol/water

Due to their stability and easy detection, *n*-alkylbenzenes were used in CCC as a test mixture to compare different solvent systems in terms of selectivity and separation efficiency [39]. In this work, the partition coefficient of five alkylbenzene homologues was calculated in one heptane/methanol/water system composition only.

A comparison of partition coefficients of *n*-alkylbenzenes homologous series in heptane/methanol/water 50:45:5 (v/v/v) obtained experimentally [39] at $22 \pm 0.5^\circ\text{C}$ and predicted by COSMO-RS at 20°C is presented in a parity plot given in Fig. 2. LLE data at 20°C for heptane/methanol/water system used for calculation of the partition coefficients were taken from Berthod and Billardello [38]. The experimental values of the partition coefficient were measured by a coil planet centrifuge Model CPHV 2000 from Société Française de Chromato Colonne [39]. In the CCC experiment the upper phase was used as a mobile phase.

As can be seen in Fig. 2, the partition coefficient decreases with increasing alkyl chain length. This trend was reflected by the prediction using COSMO-RS.

The difference between predicted and measured $\log(P_i^{SM})$ values is the smallest for dodecylbenzene. With decreasing alkyl chain length, the difference between predicted and measured values increases ($\Delta \log(P_{\text{benzene}}^{SM}) = 0.28$, $\Delta \log(P_{\text{toluene}}^{SM}) =$

Table 2
Summary of the used solute/s and solvent systems.

Case study	Solute/s	Solvent systems	Phase designation
1	<i>n</i> -Alkyl benzenes: Benzene, toluene, ethyl-, butyl-, hexyl-, dodecylbenzene	Heptane/methanol/water	Stationary lower phase
2	Steroids: Estrone, prednisone, prednisolone acetate, testosterone	Heptane/methanol/ethyl acetate/water	Stationary upper phase
3	Benzyl alcohol	Heptane/methanol/ethyl acetate/water	Stationary upper phase
4	Phenols: Hydroquinone, phenol, pyrocatechol	Heptane/methanol/ethyl acetate/water	Stationary upper phase
5	GUESS mix: Beta-ionone, caffeine, carvone, coumarin, estradiol, naringenin, quercetin, salicylic acid, umbelliferone, vanillin	Alkane/methanol/ethyl acetate/water	Stationary upper phase

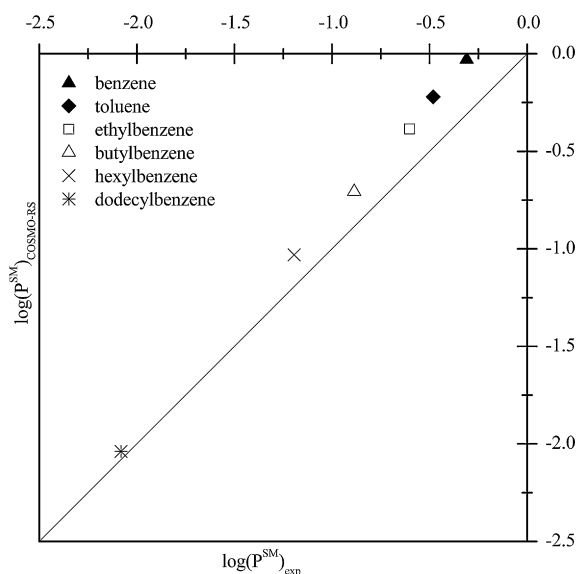


Fig. 2. Comparison of experimental ($22 \pm 0.5^\circ\text{C}$) [39] and predicted partition coefficients (20°C) of *n*-alkylbenzenes in the system heptane/methanol/water 50:45:5 (v/v/v); the line indicates the slope unity.

$$0.26, \Delta \log(P_{\text{ethylbenzene}}^{\text{SM}}) = 0.22, \Delta \log(P_{\text{butylbenzene}}^{\text{SM}}) = 0.18, \Delta \log(P_{\text{hexylbenzene}}^{\text{SM}}) = 0.16, \Delta \log(P_{\text{dodecylbenzene}}^{\text{SM}}) = 0.04.$$

The root mean square error (RMSE) in $\log(P_i^{\text{SM}})$, which is calculated according Eq. (3), is 0.21. This value is smaller than the prediction accuracy (RMSE = 1.45) of the heptane–water partition coefficient reported for 580 compounds [40]. It should be pointed out that the difference between the experimental and predicted values of the partition coefficient (P_i^{SM}) is bigger than the one calculated in terms of $\log(P_i^{\text{SM}})$. Therefore, the predicted partition coefficient should be used with care when precise determination of the solute elution time is required.

$$\text{RMSE} = \left[\frac{1}{n} \sum_i^n (\log_{10}(P_{i,\text{exp}}^{\text{SM}}) - \log_{10}(P_{i,\text{pred}}^{\text{SM}}))^2 \right]^{1/2} \quad (3)$$

4.2. Case study 2: steroids in heptane/ethyl acetate/methanol/water

Steroids were used in CCC literature to evaluate the alkane effect in the Arizona system [37] and to validate different CCC operation modes, such as elution–extrusion and the co-current method [41,42].

The prediction of the partition coefficients of more complex molecules such as a mixture of steroids consisting of four rings and different functional groups was validated ($T = 22^\circ\text{C}$). Fig. 3 shows the partition coefficient of estrone, prednisone, prednisolone acetate and testosterone in three different compositions of the heptane/ethyl acetate/methanol/water solvent system. These compositions relate to the Arizona systems 14, 16 and 17. The LLE data at room temperature ($22 \pm 2^\circ\text{C}$) used for calculations were taken from Berthod et al. [37]. The experimental measurements ($T = 22 \pm 2^\circ\text{C}$) were performed again using CCC on a SFCC 2000 chromatograph from SEAB (Villejuif, France) [37,41], the lower phase was used as a mobile phase.

Also the trend of the partition coefficient obtained experimentally for these more complex molecules could be correctly predicted. Higher deviations between predicted and measured values can be seen for predicted $\log(P_i^{\text{SM}})$ greater than 0.5. The RMSE in $\log(P_i^{\text{SM}})$ is 0.19. This value is also smaller than the prediction

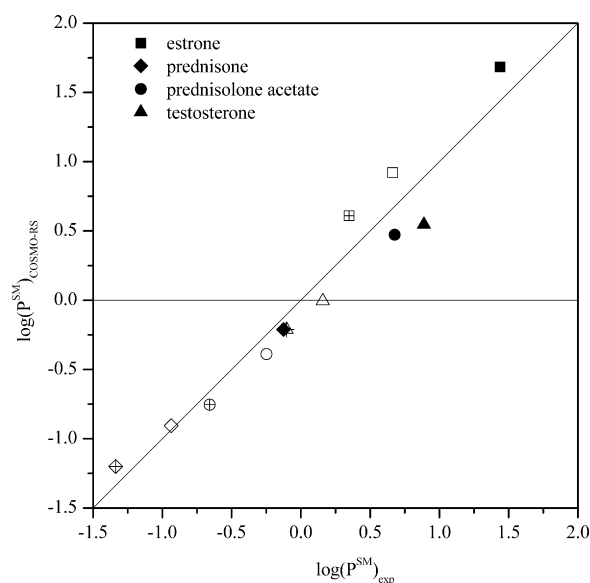


Fig. 3. Comparison of experimental ($22 \pm 2^\circ\text{C}$) [37,41] and predicted partition coefficients (22°C) of four steroids in different heptane/ethyl acetate/methanol/water systems; black symbols: 1:2:1:2 (ARIZONA 14), white symbols: 5:6:5:6 (ARIZONA 16); crossed symbols: 1:1:1:1 (ARIZONA 17).

accuracy (RMSE = 1.45) of the heptane–water partition coefficient reported for 580 compounds [40].

4.3. Case study 3: benzyl alcohol in heptane/ethyl acetate/methanol/water

Benzyl alcohol was used as a model solute during the CCC instruments development and scaling up evaluations [43,44].

In the third case study the partition coefficient of benzyl alcohol in different solvent system compositions was predicted at a temperature of 22°C and measured using the shake flask method at $22 \pm 2^\circ\text{C}$. The solvent system used was heptane/ethyl acetate/methanol/water. The mean standard deviation of the partition coefficient, which was measured by shake flasks experiments, was 0.017. LLE data ($T = 22^\circ\text{C}$) used for calculations

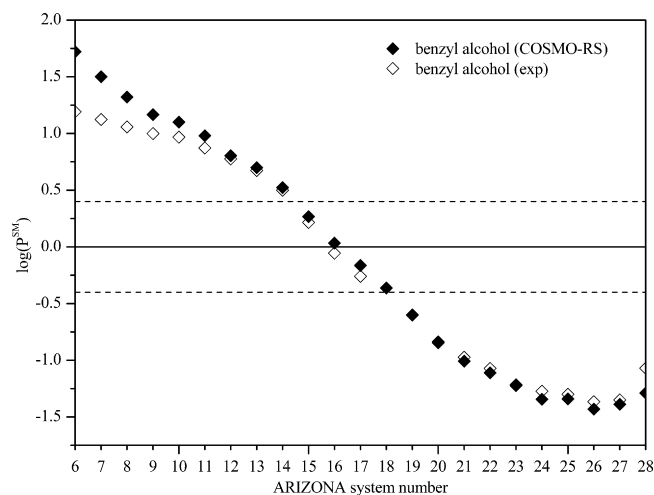


Fig. 4. Comparison of experimental ($22 \pm 2^\circ\text{C}$) and predicted (22°C) partition coefficients of benzyl alcohol in different heptane/ethyl acetate/methanol/water systems (Table 1b); the dashed horizontal lines indicate the optimal CCC range of the partition coefficient.

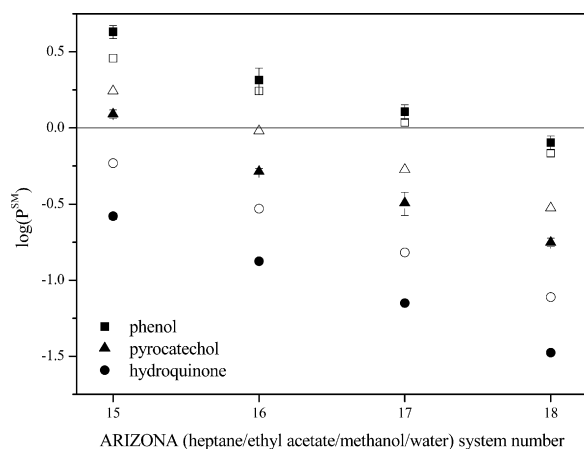


Fig. 5. Comparison of experimental ($22 \pm 2^\circ\text{C}$) and predicted (22°C) partition coefficients of phenols in different heptane/ethyl acetate/methanol/water systems; closed symbols: experimental data, open symbols: predicted data. Error bars represent the standard deviation of the experimental data.

of the partition coefficient were taken from Garrard et al. [36].

In Fig. 4 the experimental and calculated partition coefficients are plotted versus the solvent system number, given in Table 1b. In the optimal CCC range of the partition coefficient values, i.e. between $+0.4 < P_i^{SM} < +2.5$ and $-0.4 < \log(P_i^{SM}) < +0.4$, respectively [8], the agreement between predicted and measured values is very good. The deviation of the predictions increases for high partition coefficients.

Because a partition coefficient of the target compound close to one is desirable, the prediction quality is sufficient. According to the experimental and predicted results system 16 would be the optimal system for a CCC separation, since the $\log(P_i^{SM})$ value is close to 0.

4.4. Case study 4: phenols in heptane/ethyl acetate/methanol/water

In the literature a model mixture of phenol, hydroquinone and pyrocatechol was applied to test the CPC instrument performance [Armen instrument CPC application note No. 070707] and the effect of the sample volume on the separation [45].

In case study 4 the partition coefficients of phenol, pyrocatechol and hydroquinone in four different compositions of a heptane/ethyl acetate/methanol/water solvent mixture were measured experimentally and compared to the COSMO-RS predictions ($T=22^\circ\text{C}$). The shake flask method was used for the determination of the partition coefficient and hence the partition coefficient is defined as the concentration of the solute in the upper phase divided by the concentration of the solute in the lower phase. The solute concentration in each phase was analyzed by GC and HPLC. LLE data for heptane/ethyl acetate/methanol/water systems measured in our own laboratory were used in the COSMO-RS calculations (Tables A1 and A2 in Appendix A). Due to experimental error, differences between the data measured in our laboratory and published values [36,37] can be observed. The mean deviation in the $\log(P_i^{SM})$ resulting from the use of different LLE data is 0.08. This deviation does not affect the prediction of the optimal solvent system.

In Fig. 5 the partition coefficients of phenol, pyrocatechol and hydroquinone are plotted versus the system number of the heptane/ethyl acetate/methanol/water solvent mixture, given in Table 1b. The experimental partition coefficient values (mean values obtained from GC and HPLC measurements) are represented by closed symbol and the predicted values by open symbols. Addi-

tionally the standard deviation of the experimental values is given in the figure as error bars.

One of the advantages of the COSMO-RS model is that steric isomers can be distinguished. Fig. 5 illustrates the potential of the COSMO-RS model to predict different partition coefficients for hydroquinone and pyrocatechol.

For all compositions of the studied solvent system, the value of the partition coefficient of phenol is the highest, followed by the partition coefficient of pyrocatechol and hydroquinone.

A decrease in the polarity of the solvent system results in a decrease in the partition coefficient, because as the upper phase becomes less polar, the solutes prefer the lower aqueous phase. This observation is in agreement with the COSMO-RS predictions. To demonstrate the applicability of COSMO-RS for selecting the solvent system for a particular separation, a screening target was defined. The screening target was a $\log(P_i^{SM})$ value of pyrocatechol close to zero (P_i^{SM} close to 1) and between the $\log(P_i^{SM})$ values of phenol and hydroquinone. According to the experimental data, system 15 meets the required conditions. The COSMO-RS prediction deviates by one system and predicts system 16.

4.5. Case study 5: GUESS mix in alkane/ethyl acetate/methanol/water

GUESS mix is a mixture of commercially available compounds proposed by Friesen and Pauli [8]. GUESS mix simulates the most common chemical classes encountered in a natural product extract. The solutes differ in terms of polarity, functional groups and molecular mass. GUESS mix was developed in order to compare the effectiveness of different biphasic solvent systems applied in the isolation and purification of natural products.

The predicted partition coefficients of several GUESS mix compounds are plotted in Fig. 6 versus the hexane/ethyl acetate/methanol/water system number ($T=23^\circ\text{C}$), given in Table 1a. LLE data was measured in our laboratory and is presented in the appendix (Tables A3 and A4).

Even for these complex molecules the partition coefficient change in different solvent system compositions can be predicted. For some components, the $\log(P_i^{SM})$ value calculated by COSMO-RS does not increase continuously from system -7 to system 8. Similar behavior of the $\log(P_i^{SM})$ was obtained experimentally by Friesen and Pauli [8] and is most probably the result of the lower phase polarity remaining approximately constant since the

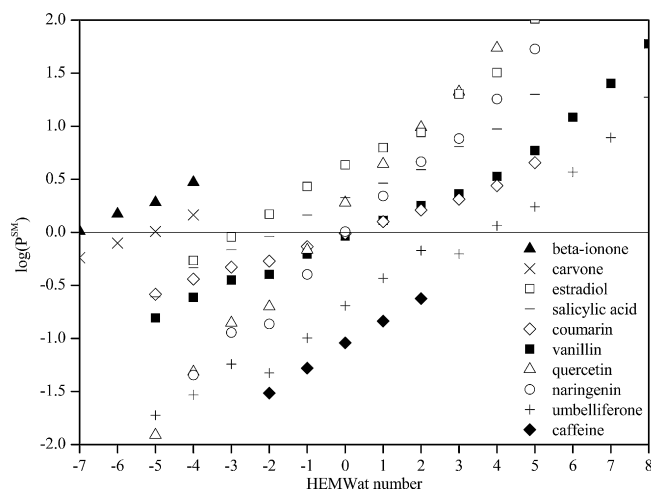


Fig. 6. Prediction of partition coefficients of several GUESS mix compounds in different HEMWat compositions (Table 1a) at 23°C .

Table 3

Comparison of the perfectly suited solvent system number determined by shake flask method at room temperature [8] with the COSMO-RS prediction at 23 °C.

Compound	Experiment	COSMO-RS
β -Ionone	-7	-7
Carvone	-6	-5
Salicylic acid	-2/-3	-2
Coumarin	-1	0
Estradiol	-1	-3
Quercetin	0	0/-1
Naringenin	0/+1	0
Vanillin	+2	0
Umbelliferone	+2/+3	+2/+3/+4

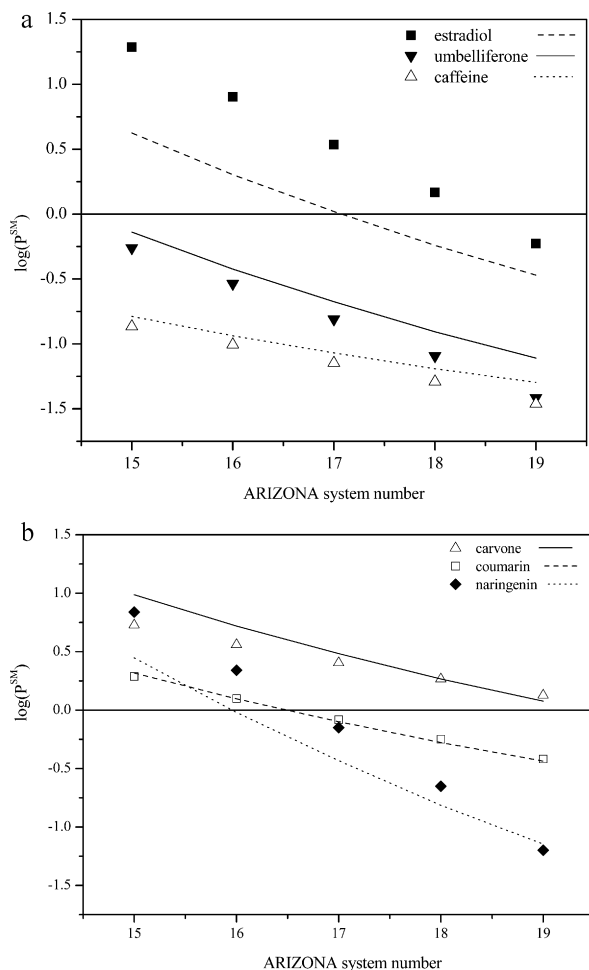


Fig. 7. Comparison of experimental [46] and predicted partition coefficients of some GUESS mix compounds in heptane/ethyl acetate/methanol/water systems between system 15 and system 19 (Table 1b): (a) umbelliferone, estradiol, caffeine and (b) carvone, coumarin, naringenin. Lines: experimental data, points: predicted data.

relative proportion of methanol and water, the most polar solvents, remain roughly constant between systems -2 and 2, see Table A4.

However, the most important goal of using the COSMO-RS method as a screening tool is the correct prediction of a solvent system composition where the partition coefficient is close to one. The solvent system is named here perfectly suited whenever the partition coefficient of the solute is close to 1. A comparison of the predicted perfectly suited HEMWat systems by COSMO-RS with

the perfectly suited systems determined experimentally is shown in Table 3. The experimental data was taken from Fig. 5 of Friesen and Pauli [8]. In cases where the perfectly suited system is between two solvent system compositions, both systems are presented in the table.

For most solutes, the deviation between the predicted and experimentally determined perfectly suited solvent system is only one single solvent system composition. The two exceptions are estradiol and vanillin where the prediction differs from the experiment by two solvent system compositions.

The partition coefficients of some GUESS mix compounds were also calculated for heptane/ethyl acetate/methanol/water (ARIZONA) systems between system 15 and system 19. The experimental data were extracted from the data presented in Table 3 in [46]. Namely, the partition coefficient of GUESS solutes in the systems 15–19 were calculated from the correlation of the experimental $\log(P_i^{SM})$ with the water content in the lower phase of these ARIZONA system obtained by Lu et al. [46]. In Fig. 7 the experimental $\log(P_i^{SM})$ data are presented as a line and $\log(P_i^{SM})$ data predicted by COSMO-RS are presented as points. Going from system 15 to system 19 a linear decrease of the values was observed for all components. This indirectly confirms the linear dependence of the $\log(P_i^{SM})$ on the water content in the lower phase of these ARIZONA systems, pointed out by Lu et al. [46]. For coumarin, caffeine, umbelliferone and carvone (see Fig. 7) a close match between the predicted and experimental data was obtained, while for estradiol and naringenin the values of the $\log(P_i^{SM})$ are higher or lower than the experimental data. It is interesting to observe that the slopes of the experimental and predicted data are quite similar even for these solutes.

5. Conclusion

The first and most important step for performing a successful CCC separation is the selection of a suitable biphasic solvent system, i.e. the mobile and the stationary phase. This work demonstrates the potential of COSMO-RS as a tool for predicting the partition coefficients of compounds with known structure, which is used as a prerequisite parameter for screening and selection of biphasic solvent systems for CCC applications. It was shown that COSMO-RS can successfully predict the change of the solute partition coefficient with the change of the solvent system compositions for simple molecules as well as for complex molecules. The only required information for the calculation of the partition coefficient is the molecular structure of the solute and the compounds of the two-phase liquid system as well as the composition of the two liquid phases, which can be obtained from available literature, measured experimentally or calculated using well-established thermodynamic models. By using COSMO-RS, the laborious experimental work normally needed for the screening of the solvent systems and the selection of an optimal solvent system composition can be reduced significantly.

The validation of the COSMO-RS method is a step towards the development of a systematic method for the selection of solvent systems based on thermodynamics.

Acknowledgement

The authors thank Petra Kiefer for carrying out the experimental work.

Appendix A.

Tables A1–A4.

Table A1

Measured composition of the upper phase of ARIZONA systems by GC analysis at $22 \pm 2^\circ\text{C}$ [vol%]; mean total percentage 100.11 vol%, relative standard deviation: 1 vol%.

	Heptane	Ethyl acetate	Methanol	Water
15	49.02	48.51	3.52	0.89
16	56.73	40.09	3.16	0.54
17	62.80	34.17	2.73	0.27
18	68.72	27.35	2.28	0.19

Table A2

Measured composition of the lower phase of ARIZONA systems by GC analysis at $22 \pm 2^\circ\text{C}$ [vol%]; mean total percentage 100.7 vol%, relative standard deviation: 0.87 vol%.

	Heptane	Ethyl acetate	Methanol	Water
15	0.11	17.13	34.69	48.09
16	0.19	18.47	37.45	44.24
17	0.18	19.83	40.31	40.73
18	0.40	19.70	42.71	37.12

Table A3

Measured composition of the upper phase of HEMWat systems by GC analysis at $22 \pm 2^\circ\text{C}$ [vol%]; mean total percentage 100.46 vol%, relative standard deviation: 0.88 vol%.

	Hexane	Ethyl acetate	Methanol	Water
-7	94.40	3.12	1.95	0.01
-6	90.06	7.45	1.90	0.05
-5	83.85	14.01	1.74	0.07
-4	81.33	17.12	1.57	0.08
-3	74.33	23.42	2.29	0.17
-2	79.39	20.23	1.42	0.09
-1	70.40	28.08	2.17	0.19
0	60.51	36.54	3.27	0.49
1	50.44	45.32	4.69	0.94
2	37.72	54.74	6.90	1.87
3	45.43	50.17	3.93	0.97
4	33.33	60.47	5.39	1.80
5	31.65	64.29	3.59	1.46
6	20.89	74.45	2.59	1.79
7	10.61	87.01	1.35	2.14
8	0.00	96.83	0.00	2.58

Table A4

Measured composition of the lower phase of HEMWat systems by GC analysis at $22 \pm 2^\circ\text{C}$ [vol%]; mean total percentage 101.82 vol%, relative standard deviation: 1.3 vol%.

	Hexane	Ethyl acetate	Methanol	Water
-7	8.79	6.98	74.58	8.80
-6	3.07	12.80	66.66	19.20
-5	1.70	17.28	56.04	25.46
-4	0.79	15.30	50.78	35.51
-3	1.01	19.60	48.27	33.93
-2	0.16	12.22	44.52	45.77
-1	0.24	15.47	42.43	44.23
0	0.40	18.74	41.17	42.33
1	0.51	21.62	39.14	40.92
2	0.65	24.70	37.20	40.00
3	0.12	15.42	34.44	52.98
4	0.10	16.24	32.55	53.61
5	0.00	11.32	25.63	65.41
6	0.00	9.64	17.50	74.74
7	0.00	9.03	8.90	82.49
8	0.00	8.70	0.00	91.18

Appendix B. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2010.11.018

References

- [1] Y. Ito, M. Weinstein, I. Aoki, R. Harada, E. Kimura, K. Nunogaki, *Nature* 212 (1966) 985.
- [2] A. Foucault (Ed.), *Centrifugal Partition Chromatography*, Chromatographic Science Series, vol. 68, M. Dekker, New York, 1995.
- [3] N.B. Mandava, Y. Ito, *Counter-Current Chromatography, Theory and Practice* Chromatographic Science Series, vol. 44, M. Dekker, New York, 1988.
- [4] M. Agnely, D. Thiebaut, *J. Chromatogr. A* 790 (1997) 17.
- [5] A. Berthod, M.J. Ruiz-Angel, S. Carda-Broch, *Anal. Chem.* 75 (2003) 5886.
- [6] A. Berthod, M.J. Ruiz-Angel, S. Carda-Broch, *J. Chromatogr. A* 1216 (2009) 4206.
- [7] Y. Ito, W.D. Conway (Eds.), *High-Speed Countercurrent Chromatography*, Chemical Analysis, vol. 132, Wiley, New York, 1996.
- [8] J.B. Friesen, G.F. Pauli, *J. Liq. Chromatogr. Relat. Technol.* 28 (2005) 2777.
- [9] P.L. Wood, D. Hawes, L. Janaway, I.A. Sutherland, *J. Liq. Chromatogr. Relat. Technol.* 26 (2003) 1373.
- [10] Y. Ito, *J. Chromatogr. A* 1065 (2005) 145.
- [11] A.P. Foucault, L. Chevolot, *J. Chromatogr. A* 808 (1998) 3.
- [12] J.M. Sørensen, W. Arlt, E.A. Macedo, P. Rasmussen, *Liquid-Liquid Equilibrium Data Collection*, DECHEMA, Frankfurt/Main, 1979.
- [13] A. Berthod (Ed.), *CCC, The Support-Free Liquid Stationary Phase*, Comprehensive, Analytical Chemistry, vol. 38, Elsevier, Amsterdam/New York, 2002.
- [14] G. Gunawardana, J. McAlpine, in: J.M. Menet, D. Thiebaut (Eds.), *Chromatographic Science Series*, vol. 82, M. Dekker, New York, 1999, p. 249.
- [15] F. Oka, H. Oka, Y. Ito, *J. Chromatogr.* 538 (1991) 99.
- [16] A. Berthod, *J. Chromatogr.* 550 (1991) 677.
- [17] E. Camacho-Frias, A. Foucault, *Analisis* 24 (1996) 159.
- [18] J.B. Friesen, G.F. Pauli, *J. Chromatogr. A* 1151 (2007) 51.
- [19] G.F. Pauli, S.M. Pro, J.B. Friesen, *J. Nat. Prod.* 71 (2008) 1489.
- [20] I.A. Sutherland, *J. Chromatogr. A* 1151 (2007) 6.
- [21] C. Hansch, A. Leo, *Substituent Constants for Correlation Analysis in Chemistry and Biology*, Wiley, New York, 1979.
- [22] R.W. Taft, J.L. Abboud, M.J. Kamlet, M.H. Abraham, *J. Solution Chem.* 14 (1985) 153.
- [23] A. Fredenslund, J. Gmehling, P. Rasmussen, *Vapor-Liquid Equilibria using UNIFAC. A Group-Contribution Method*, Elsevier, Amsterdam, 1977.
- [24] A. Klamt, F. Eckert, M. Hornig, *J. Comput. Aid. Mol. Des.* 15 (2001) 355.
- [25] F. Chen, Y. Zhou, Z. Li, *J. Liq. Chromatogr. Relat. Technol.* 26 (2003) 1431.
- [26] M. Buggert, *Modelling of Partition Coefficients of Pharmaceuticals and Active Agents in Colloidal Systems*, Ph.D. Thesis, TU Berlin, 2008.
- [27] S. Wille, M. Buggert, L. Mokrushina, W. Arlt, I. Smirnova, *Chem. Eng. Technol.* 33 (2010) 1075.
- [28] A.C. Spiess, W. Eberhard, M. Peters, M.F. Eckstein, L. Greiner, J. Buechs, *Chem. Eng. Process.* 47 (2008) 1034.
- [29] B. Burghoff, J. Schiferli, J. Sousa Marques, A.B. de Haan, *Chem. Eng. Sci.* 64 (2009) 2887.
- [30] A. Klamt, *J. Phys. Chem.* 99 (1995) 2224.
- [31] S. Maassen, W. Arlt, A. Klamt, *Chem. Eng. Technol.* 67 (1995) 476.
- [32] C. Jork, C. Kristen, D. Pieraccini, A. Stark, C. Chiappe, Y.A. Beste, W. Arlt, *J. Chem. Thermodyn.* 37 (2005) 537.
- [33] M. Buggert, C. Cadena, L. Mokrushina, I. Smirnova, E.J. Maginn, W. Arlt, *Chem. Eng. Technol.* 32 (2009) 977.
- [34] A. Schaefer, A. Klamt, D. Sattel, J.C.W. Lohrenz, F. Eckert, *Phys. Chem. Chem. Phys.* 2 (2000) 2187.
- [35] T.E. Daubert, R.P. Danner, *Physical and Thermodynamic Properties of Pure Chemicals: Data Compilation*, Hemisphere, New York, 1989.
- [36] I.J. Garrard, L. Janaway, D. Fisher, *J. Liq. Chromatogr. Relat. Technol.* 30 (2007) 151.
- [37] A. Berthod, M. Hassoun, M.J. Ruiz-Angel, *Anal. Bioanal. Chem.* 383 (2005) 327.
- [38] A. Berthod, B. Billardello, *J. Chromatogr. A* 902 (2000) 323.
- [39] A. Berthod, M. Bully, *Anal. Chem.* 63 (1991) 2508.
- [40] C. Wittekindt, A. Klamt, *QSAR Comb. Sci.* 28 (2009) 874.
- [41] A. Berthod, M. Hassoun, G. Harris, *J. Liq. Chromatogr. Relat. Technol.* 28 (2005) 1851.
- [42] A. Berthod, M. Hassoun, M.J. Ruiz-Angel, *Abstracts of Papers, 231st ACS National Meeting*, Atlanta, GA, United States, March 26–30, ANYL-285, 2006.
- [43] T.A. Maryutina, S.N. Ignatova, B.Y. Spivakov, I.A. Sutherland, *J. Anal. Chem. (Translation of Zhurnal Analiticheskoi Khimii)* 58 (2003) 762.
- [44] P. Wood, S. Ignatova, L. Janaway, D. Keay, D. Hawes, I. Garrard, I.A. Sutherland, *J. Chromatogr. A* 1151 (2007) 25.
- [45] C. Zhao, C. He, *J. Chromatogr. A* 1146 (2007) 186.
- [46] Y. Lu, A. Berthod, R. Hu, W. Ma, Y. Pan, *Anal. Chem.* 81 (2009) 4048.