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# Classification of biphasic solvent systems according to Abraham descriptors for countercurrent chromatography

Léa Marlot<sup>1</sup>, Magali Batteau<sup>1</sup>, Karine Faure<sup>1</sup>

<sup>1</sup> Université de Lyon, CNRS, Université Claude Bernard Lyon 1, Institut des Sciences Analytiques, UMR 5280, 5 rue de la Doua, F-69100 VILLEURBANNE, France

## Abstract

The method development of liquid-liquid chromatography, either countercurrent chromatography or centrifugal partition chromatography, is slowed down by the selection of the biphasic solvent system that constitutes its column. This paper introduces a classification of 19 solvent systems, including the most popular systems based on heptane/ethyl acetate/methanol/water, some non-aqueous systems and some greener systems. This classification is based on Abraham descriptors determined through the partition coefficients of 43 probes. Among 21 determined models, nine of them allow an accurate prediction of partition coefficients from solute descriptors and another ten provide a description of the chromatographic interactions at the 5% significance level. A graphical tool (spider diagram) is built for the comparison of the chromatographic columns previously characterized with the solvation parameter model. The position of a solvent system in this spider diagram relates to the interactions at stake, thus the selection of columns offering similar or orthogonal interactions is facilitated, with no previous knowledge of the solute required. This semi-empirical strategy cannot fully predict the retention behavior but can judiciously orientate the user towards a limited number of solvent systems to be experimentally tested.

**Keywords:** Countercurrent chromatography, centrifugal partition chromatography, Spider diagram; Abraham descriptors; Classification methods;

\* Corresponding author : Karine Faure, Institut des Sciences Analytiques, 5 rue de la Doua, F-69100 VILLEURBANNE, France ; phone : +33-437423686 ; e-mail: [karine.faure@isa-lyon.fr](mailto:karine.faure@isa-lyon.fr)

## 30 1. Introduction

31 Support-free liquid-liquid chromatography (LLC), also known as countercurrent  
32 chromatography (CCC) or centrifugal partition chromatography (CPC), has emerged as an  
33 important instrumental approach for the purification and isolation of active compounds from  
34 relatively complex samples, mainly in the natural product industry. The stationary and mobile  
35 phases are the two-phases of a biphasic solvent system. This characteristic offers a wide range  
36 of operating modes as recently reviewed [1]. It also provides a versatility in the nature of the  
37 columns that can be engaged in the process, leading usually to much larger selectivity than  
38 observed in solid-phase chromatography. The number of biphasic systems (called solvent  
39 systems) is tremendous, being generated from the mixture of up to four different solvents in  
40 various proportions and the selection process is hence labor-intensive and highly time-  
41 consuming in the overall method development. The most efficient strategy is to select a  
42 limited number of solvent systems and to conduct partition experiments using the actual  
43 sample to be purified. It is often recommended that the target compound exhibits a partition  
44 coefficient in the range  $0.25 < K < 4$  in elution mode, but alternative modes such as elution-  
45 extrusion, dual-mode or co-current mode have now spread the range of partition coefficient  
46 spectrum that can be separated. Hence, the driving force for solvent selection should now  
47 focus on selectivity.

48 Once the best solvent system is identified, adjustments of its composition have to be made to  
49 obtain the maximal selectivity between compounds of interest and the matrix, using factorial  
50 designs for example [2] or scanning through the biphasic composition diagram. Hence, it is  
51 clear that the primary investigation of the most suitable solvent system candidates has to be  
52 conducted with minimal effort yet guided by chemical considerations.

53 Solvent system screening can be facilitated by the use of databases, solvent system families  
54 and thermodynamic models, as summarized in 2015 by Liu et al. [3]. Skalicka-Wozniak and  
55 Garrard built a comprehensive database containing the solvent systems used from 1984 to  
56 2014 in natural product purification by LLC [4]. Since natural products represent more than 80  
57 % of the CCC applications, this database can be considered as a good overlook of the columns  
58 used. After compiling 2322 isocratic solvent systems, they found out that these were  
59 constituted of 29 different solvents. This number is rather elevated compared to the few  
60 number of solvent used in preparative and reflect the large degree of freedom still available  
61 in LLC development. These databases can be of help to orientate the solvent screening  
62 towards certain solvent families, the HEMWat (heptane/ethyl acetate/methanol/water)  
63 families being the most used in natural products of medium polarity [5].

64 Another approach based on the “best solvent approach” consists in finding a solvent that  
65 easily dissolves the compound of interest and in building around a biphasic system with two  
66 partially miscible solvents [6]. This approach is, unfortunately, highly related to the  
67 scientist’s experience, and limited to binary or ternary mixtures of solvents.

68 Some theoretical strategies tend to simulate the partition coefficient of a given solute. Its  
69 solubility in each LLC phase is calculated relating to its chemical structure combining solvent  
70 descriptors with solutes characteristics. While considerably decreasing the experimental  
71 work, these theoretical strategies such as COSMO-RS [7, 8] or UNIFAC [9] require the previous  
72 knowledge of the chemical structure of the compound of interest or at least its descriptors,  
73 but also of the impurities that may be present, as well as an expertise on computational tools.

74 The modelling approach based on quantitative structure activity relationships (QSAR) has  
75 been extensively detailed on HEMWat solvent systems [10] with models based on 196

76 descriptors for each test compound. The authors were hence able to predict log K within 0.5  
77 log unit. One of the main advantages of a QSAR model is that it can be run simply using Excel.

78

79 The aim of the present work is to supply a visual mean to compare a large number of solvent  
80 systems and the interactions they involve. For this purpose, a classification of the most  
81 common solvents systems is performed using Abraham descriptors. While being less precise  
82 than a complete QSAR model, with only 5 descriptors per solvent system, the model could be  
83 used to estimate the partition coefficient of a neutral solute if its Abraham descriptors are  
84 known. More importantly this classification aims at illustrating similar (correlated) and  
85 complementary (orthogonal) columns by their respective position. It provides a faster decision  
86 support in CCC/CPC development method for unknown compounds. The classification  
87 presents here 21 biphasic solvent systems (hence providing 42 columns depending on the role  
88 of each phase), amongst which 3 totally organic solvent systems.

89

## 90 2. Experimental

### 91 2.1 Chemicals

92 The solvents used were analytical grade provided by Acros organic (1-butanol, methyl tert-  
93 butyl ether) or Sigma-Aldrich (methanol, acetonitrile, ethanol, limonene, heptane, ethyl  
94 acetate). The initial compositions (v/v) of each studied solvent system are listed in Table 1.

95 The composition of the upper and lower phases of each solvent system has been described in  
96 several papers [8; 11-14]

97 [Table 1]

98 A set of 27 compounds was extracted from a previous publication by West et al. [15] who use  
99 them to classify SFC columns. They represent homologous series that easily describe some  
100 LSER descriptors. Fourteen compounds, from the GUESS list [16], were added to the set since  
101 they are very often encountered in LLC column testing, covering a wide range of polarity, and  
102 supplying a better description of the terms A and B. Their LSER descriptors *E*, *S*, *A*, *B* and *V*  
103 were extracted from ACD web database. All solutes were purchased from Sigma-Aldrich.  
104 These 43 solutes were pooled into 7 groups, mainly by chemical families, to ensure their  
105 chromatographic separation (Table 2). A second set comprising 5 solutes was gathered to  
106 validate the models (Table 2: validation set) and their partition coefficients were measured  
107 using partition experiments (shake-flasks) and CCC and CPC separations.

108 [Table 2]

## 109 2.2 Determination of partition coefficient: shake-flask procedure

110 Each solvent system (Table 1) was prepared using thermostated solvents and allowed to settle  
111 for three hours in a thermostated bath at 24 °C ( $\pm 1$  °C). A shake-flask constituted of 2 mL of  
112 each phase was arranged for each group of solutes. This means that each solvent system to  
113 be characterized requires 7 shake-flasks. Then 2 mg of each solute of the group of concern  
114 was added to its shake-flask. It is noteworthy that these partition tests have to be performed  
115 at high dilution to avoid any saturation of a phase. After dissolution through ultrasonic bath,  
116 the shake flasks were allowed to sit for 30 min in a thermostated bath at 24 °C ( $\pm 1$  °C) to ensure  
117 that thermodynamic equilibrium was reached.

118

## 119 2.3 Analytical conditions

120 Aliquots of the upper and lower phases of the shake-flasks were diluted by a factor 10 in  
121 methanol. The UHPLC analysis was performed using a Waters Acquity instrument, equipped  
122 with a 20  $\mu\text{L}$  injection loop and for which the dwell volume is estimated at 0.11 mL (loop  
123 excluded). The column Acquity CSH Phenylhexyl (100 x 2.1 mm i.d., 1.7  $\mu\text{m}$ ) was employed at  
124 30 °C. The mobile phase was composed of (A) water + 0.1 % formic acid and (B) acetonitrile +  
125 0.1 % formic acid. The flow rate was 0.4 mL/min and UV detection was set at 254 nm. The  
126 injection volume was 1  $\mu\text{L}$ . For each group a UHPLC gradient method was optimized using  
127 OSIRIS software (Datalys, Grenoble). Resulting methods are providing in Table S1. Peak areas  
128 were monitored thanks to Empower software and partition coefficients were established as  
129 the ratio of the upper phase peak area on the lower phase peak area, considering that the  
130 sample solvent does not influence the detector response.

#### 131 2.4 Data analysis

132 For the determination of the coefficients  $e$ ,  $s$ ,  $a$ ,  $b$  and  $v$  of each solvent system, multilinear  
133 regression was performed using Excel, as well as statistical analysis and graphic  
134 representations. The number of solutes used for the multilinear regression of each solvent  
135 system is indicated in Table 3.

#### 136 2.5 Centrifugal partition chromatography and countercurrent chromatography

137 The LLC separation was conducted on the validation set solutes with three different columns:  
138 #SS2 (heptane/ethyl acetate/methanol/water 1/1/1/1), #SS7 (octanol/water) and #SS15  
139 (heptane/methanol/water 50/33.5/16.5), the first two in descending mode; i.e. with the  
140 upper phase used as stationary phase, the latter in ascending mode, i.e. with the lower phase  
141 used as stationary phase.

142 The CPC experiments were set with a Spotprep II pumping system from Gilson (Saint-Avé,  
143 France) connected to a FCPC-A frame equipped with a 33.1 mL rotor from Kromaton  
144 Rousselet-Robatel (Annonay, France) thermostated at 25 °C ( $\pm 3$  °C) and detection was set up  
145 at 254 or 280 nm. Operating conditions were in elution mode, at 2000 rpm and 10 mL/min,  
146 with an injection volume of 0.5 mL. Because of the large expected K values for #SS7, an  
147 artificial stationary phase ratio of 10 % of the volume column was induced.

148 The CCC experiments were set with a Waters 600E pumping system (Waters, Milford,  
149 Massachusetts, USA ) on a CCC apparatus (Spectrum, Dynamic Extractions, Slough, UK) with a  
150 65.5 mL column (polytetrafluoroethylene bore tubing of 3.2 mm,  $\beta$  range 0.52-0.86)  
151 thermostated at 25 °C ( $\pm 2$  °C) and a SPD20A detector (Shimadzu, Noisel, France) set up at 210  
152 nm. The rotational speed was 1000 rpm. A classical elution at 5 mL/min of mobile phase for  
153 the first 6 minutes was followed by a co-current mode, with the addition of a flow of  
154 “stationary” phase of 1 mL/min, in order to elute compounds with very large K values.

155

### 156 3. Results and discussion

157 The linear solvation energy relationships (LSERs) framework relates the properties of a solute  
158 and a solvent to the strength of the interactions that generate retention mechanism in such  
159 an environment [17,18]. In a partition process, molecular interactions take place in each phase  
160 and the overall interaction strength provides the partition coefficient K. Hence, the LSER  
161 approach for neutral solutes can be transposed as equation 1.

$$162 \log K = c + eE + sS + aA + bB + vV \quad \text{Eq. (1)}$$



163  $K$  is the partitioning coefficient of a solute in the biphasic system. The capital letters E, S, A, B  
164 and V are the solute descriptors highlighting its ability to participate in a given interaction. The  
165 lowercase letters c, e, s, a, b and v are the coefficients that describe, in a specific biphasic  
166 system, the difference in the strength of interactions with the solutes in the two immiscible  
167 phases. While c is a system constant, the descriptor E represents the excess molar refractivity,  
168 i.e. the electron lone pair interaction, the descriptor S measures the dipole-type interaction  
169 solute, A and B are the hydrogen bond acidity and basicity respectively, and V is the solute's  
170 McGowan characteristic volume.

171 It is very important to consider that the system parameters (noted as lowercase letters)  
172 reflects the differences in the properties of the two phases and not the properties of a specific  
173 phase. Hence, a coefficient with a zero value for a biphasic solvent system that does not mean  
174 that this type of interaction is absent, but that the same strength of interaction is actually  
175 provided in each phase, meaning that this specific interaction does not contribute to the  
176 overall free energy of transfer and hence does not influence the partition coefficient.

### 177 3.1 Descriptors

178 To properly describe the constants of a given system, a minimum number of 20 solutes is  
179 recommended, providing a wide range of interactions, preferentially with at least 4 solutes  
180 per studied interaction [17]. Figure S1a in supplementary material presents the repartition of  
181 the 43 solutes according to their polarity ( $\log P$ ) and molecular weight. The solutes are mostly  
182 below 300 g/mol, and polarity  $\log P$  exhibits a wide range of 0–6 with few polar compounds  
183 (chlorogenic acid  $\log P = -0.36$ ). This set of solutes is representative of most applications that  
184 are dealt with in centrifugal partition chromatography. The selected compounds provide a

185 good general cover of LSER descriptor space (Figure S1b) between 0-3.9 for E, 0-5 for S and V,  
186 0-2.0 for A and 0-3.0 for B (41 values for each descriptor, except A that has 19 values). The  
187 adjunction of GUESS solutes [16] provides A and B values above 1 which was not the case if  
188 only conventional LC/SFC test solutes were used. Absence of cross –correlation between the  
189 descriptors was proven through a covariance matrix with values far below 1 (Table S2).

### 190 3.2 Partition coefficients

191 The partition coefficient K of each solute was determined in every studied biphasic systems  
192 using the shake-flask procedure (see experimental section for details) and calculated  
193 conventionally according to equation 2.

$$194 \quad K = [A]_{up}/[A]_{low} \quad \text{Eq. (2)}$$

195 Where  $[A]_{up}$  is the solute concentration in the upper phase and  $[A]_{low}$  is the concentration in  
196 the lower phase. This K value corresponds to the retention factor in CPC when used in  
197 descending mode, i.e. the stationary phase is the upper phase. When using the ascending  
198 mode, i.e. the lower phase as stationary phase, the retention factor is the inverse of K. Hence  
199 it would be possible to measure K values of solutes in a solvent system directly during a CPC  
200 procedure [19].

201 Repeatability experiments (3 shake-flasks and 3 HPLC determinations per shake-flask) were  
202 carried out on group 7 (five solutes) in solvent system #2 (heptane/methanol/ethyl  
203 acetate/water 1/1/1/1) and it was found that relative standard deviation was below 2 %.  
204 While errors can occur when the concentration in a phase is very low, the partition coefficient  
205 measured in shake-flask is considered as reliable for log K between -3 and +3 [20]. In the set  
206 we selected, only 1 % of the log K values are over +3 and 2 % below -3. When solutes did not

207 properly partition i.e. when HPLC peaks were too small to be quantified or a solubility issue  
208 occurred, the solute descriptors were discarded from the model.

### 209 3.3 Multilinear regression

210 For each solvent system a multilinear regression was performed and the 21 solvent system  
211 descriptors  $e$ ,  $s$ ,  $a$ ,  $b$  and  $v$  are presented in Table 3, along with the number of solutes  $n$  that  
212 were considered in the regression. Indeed, some solutes were discarded due to solubility  
213 issues.

214 [Table 3]

215  $n$  number of solutes considered in the multilinear regression;  $u$  vector length;  $p$ -value  
216 probability value

217 Over the 21 studied solvent systems, 9 models exhibit  $R^2$ adjusted over 0.8, meaning that these  
218 models can be used to accurately predict the partition coefficient of any solute for which the  
219 LSER coefficients are known. For 10 models, the endogeneous variance is not fully explained  
220 by the model ( $R^2$ adjusted below 80%), but the models are globally significant at the 5% level  
221 ( $p$ -value below 0.05). For these models, there is a significant linear relationship between the  
222 response and the prediction, with a 5% risk of false positive. These models cannot be used to  
223 accurately predict the partition coefficient of a known compound, but they can illustrate the  
224 global chromatographic interactions. In practice, these models can be used to compare two  
225 solvent systems in terms of selectivity and increase the chance to select orthogonal solvent  
226 systems without any knowledge of the solutes to be separated.

227 Two models were found not significant at the 5% level. Systems #19 and #21 exhibit  $p$ -values  
228 around 8.6 % and 6.5 % which means that there is over 5 % risk that the correlation is not

229 significant. The residuals for these models were found to vary with the retention value, a  
230 statistic phenomenon called heteroscedasticity, which indicates that the LSER model is not  
231 adequate, probably due to the lack of a variable. These two solvent systems are hence  
232 discarded from the rest of the study.

233

234 To illustrate the correlation between the log K values calculated through the model and the  
235 experimental log K values and the residuals, Figure 1 presents the results obtained with the  
236 most common solvent system, heptane/ethyl acetate/methanol/water 1/1/1/1, here named  
237 system #2. The analysis of residuals [21] shows that they have a normal distribution (the mean  
238 value  $-1.10^{-16}$  being close to the median value  $-1.10^{-2}$ ). Two outliers (quercetine and  
239 propriophenone) were identified above the residuals distribution range ( $-0.426$ ;  $+0.394$ ) and  
240 two others (umbelliferone and ferulic acid) were found below this range. These solutes were  
241 not properly modeled with the LSER model due to either experimental error or inadequate  
242 model.

243 [Figure 1]

244 The root mean square error (RMSE) is calculated over the 39 experimental measurements  
245 using equation 3. Its value that should be as low as possible was found to be  $9.10^{-16}$ , showing  
246 a good accuracy of the model. The open circles in Figure 1 represents the results obtained for  
247 the validation set that was not included to build the model. For these solutes, it was found a  
248 RMSE of 0.06, which is lower than the prediction accuracy reported for 580 compounds of the  
249 heptane-water partition coefficients (RMSE = 1.45) [22]

250 
$$RMSE = \sqrt{\frac{\sum(\log K_c - \log K_{exp})^2}{n}}$$
 Eq. (3)

251 where  $K_c$  is the calculated value and  $K_{exp}$  the experimental value and  $n$  is the number of  
252 observations.

### 253 3.4 Representation of solvent systems with spider diagram

254 The system constants provide information on the nature and extent of the interactions  
255 between the solute and the solvent. However, comparing two systems requires the  
256 comparison of the five coefficients of each system. While this is still achievable for two  
257 systems, it becomes almost impossible for the comparison of several systems because of the  
258 number of data to compare. Many representations exist [23] but the representation on a 5-  
259 axis diagram (called spider diagram) [24] is the easiest way to visualize similarity and  
260 differences simply according to the distance between dots. These diagrams are simple to  
261 represent and very illustrative to interpret the results. The mathematical treatment to build  
262 spider diagrams was described elsewhere [15]. Each column (the combination of stationary  
263 and mobile phases) is represented as a vector, where the coordinates of the vector are the  
264 values provided by the system constants. The overall strength of interactions is estimated  
265 through the length  $u$  of the solvation vector (equation 4) and represented as the size of the  
266 dot [25]. This vector length is a useful tool to compare the strength of the interaction  
267 capabilities for each solvent system.

$$268 \quad u = \sqrt{e^2 + s^2 + a^2 + b^2 + v^2} \quad \text{Eq. (4)}$$

269 It is important to note that in the case of CPC, a single biphasic system can generate two  
270 opposite chromatographic columns, depending on the phase selected as stationary phase.  
271 Hence, in descending mode, the stationary phase is the upper phase of the biphasic system.  
272 Its system constants are indicated in Table 3. In ascending mode, the lower phase is selected

273 as the stationary phase, and hence the ascending system constants exhibit opposite values to  
274 the corresponding descending column constants. In Figure 2 are represented the descending  
275 solvent systems numbered as in Table 1, and their ascending counterparts (same colour but  
276 no numbering).

277 [Figure 2]

278

279 Two close dots represent solvent systems that exhibit similar interactions for a given solute, a  
280 distant point provides different interactions. Hence we can expect that in CPC, the selection  
281 of two close solvent systems may provide similar elution order, i.e. that the retention  
282 mechanisms are correlated, while two distant solvent systems should offer different types of  
283 interactions and hence orthogonal separations with different elution order. This was  
284 illustrated by comparing solvent systems #2, #7 and #15asc (solvent system #15 used in  
285 ascending mode, meaning the aqueous phase is used as stationary phase). A closer look on  
286 these solvent systems coefficients on a radar plot (Figure 3a) clearly indicates the similarities  
287 and differences in the strength of the interactions that lead the separations. When running  
288 these columns for the CCC separation of validation set solutes (Figure 3b), the shift in retention  
289 order and hence the non-correlation between solvents system #15asc and the two other is  
290 obvious, while some correlation exist between solvent systems #2 and #7.

291 [Figure 3]

292

293 3.5 Validation set

294 The LSER models using Abraham descriptors have limited predictive ability. However, it was  
295 interesting to verify, with a set of solutes that are not homologous to the model solutes, how  
296 predictable their partition coefficients or their CPC/CCC retention are. To do this, five solutes  
297 were tested (group 8; Abraham descriptors in Figure S2), with their partition coefficient  
298 measured via shake-flask in the 19 solvent systems, and their retention factors measured  
299 using centrifugal partition chromatography (CPC) and countercurrent chromatography (CCC)  
300 on system solvents #2, #7 and #15asc. Figure 4a reports the results from the 5 x 19  
301 experimental partition coefficients plotted versus their predicted values. The large majority of  
302 them fall close to the first bisector, with only 9 % of the values outside the  $\pm 0.5$  log unit. The  
303 RMSE is calculated at 0.448, a value of less than 0.5 being considered as acceptable [10, 22].

304 The validation set was injected in CPC and CCC instruments with 3 solvent systems. Catechol  
305 was not injected in #15asc as its retention factor was predicted at 353, which was considered  
306 as not feasible. Because some predicted values of retention factors reach nearly 20, the LLC  
307 methods were adapted to reduce the run duration, which is a great benefit of the liquid nature  
308 of the stationary phase. The CPC instrument was operated with a reduced amount of  
309 stationary phase. A 10 % column volume of stationary phase was introduced before  
310 equilibration with the mobile phase and a conventional elution mode was performed. The  
311 partition coefficient  $K$  was deduced from the observed retention volume  $V_R$  (Equation 5).

$$312 \quad V_R = V_C - (K-1) \cdot V_S \quad \text{Eq. (5)}$$

313 with  $V_C$  the column volume and  $V_S$  the stationary phase volume.

314 The CCC separation was run in a co-current mode, which consists in slowly pushing the  
315 stationary phase out while the elution goes on. In order to analyze low-retained and highly

316 retained compounds in the same run, the co-current mode was started after a few minutes  
317 delay. Equation 6 is derived from the co-current equation [26] to consider this delay.

$$318 \quad K = \frac{t_R x F_M - V_M}{V_S - (t_R - t_{delay}) x F_S} \quad \text{Eq. (6)}$$

319 With  $t_R$  the retention time,  $F_M$  the mobile phase flowrate,  $V_M$  the mobile phase volume,  $t_{delay}$   
320 the starting time for co-current mode and  $F_S$  the stationary phase flowrate during this co-  
321 current mode.

322 The RMSE was found to be 0.36 for CPC measurements and 0.47 for CCC measurement (n=12).  
323 Once again, the experimental values are close to the calculated values as seen in Figure 4b  
324 with a deviation of  $\pm 0.5$  log units, with the exception of catechol, for which the experimental  
325 value was always far below the calculated value. Despite Marsden-Jones's statement [10], a  
326 0.5 log unit deviation does not seem acceptable to predict the most suitable solvent system  
327 in CCC or CPC, but it may be sufficient to select few of them on which further studies can be  
328 conducted to confirm solvent system selectivity. The concordance between the partition  
329 coefficients observed in LLC techniques and the one measured using shake-flask procedure is  
330 notoriously limited [27,28]. While CCC provides usually closer values than CPC to the expected  
331 shake-flasks values, we suspect the temperature control in LLC instruments being a general  
332 source of deviation from the prediction.

333

#### 334 4. Conclusion

335 Based upon the Abraham descriptors of a set of probes, we have presented here the LSER  
336 classification of biphasic solvent systems that can act as CCC or CPC columns for neutral  
337 species. The Abraham model was found accurate for 9 out of 21 biphasic solvent systems



338 and can provide a significant correlation for 10 other solvent systems. A validation set of five  
339 solutes was used to confirm the strong correlation between the model and the experimental  
340 values. While the partition experiments in a static environment follow the model, the  
341 observed partition coefficients in CPC or CCC experiments show some deviation.  
342 Nonetheless, the visualization as a spider diagram contributes to the selection of similar or  
343 orthogonal columns. While the exploration of various compositions within a chosen family  
344 must still be performed, this semi-empirical strategy reduces the workload by directing the  
345 selection towards orthogonal systems, hence aiming at a faster method development or an  
346 easier investigation of 2D configurations, including LLC techniques alone or in combination  
347 with LC techniques.

348

349

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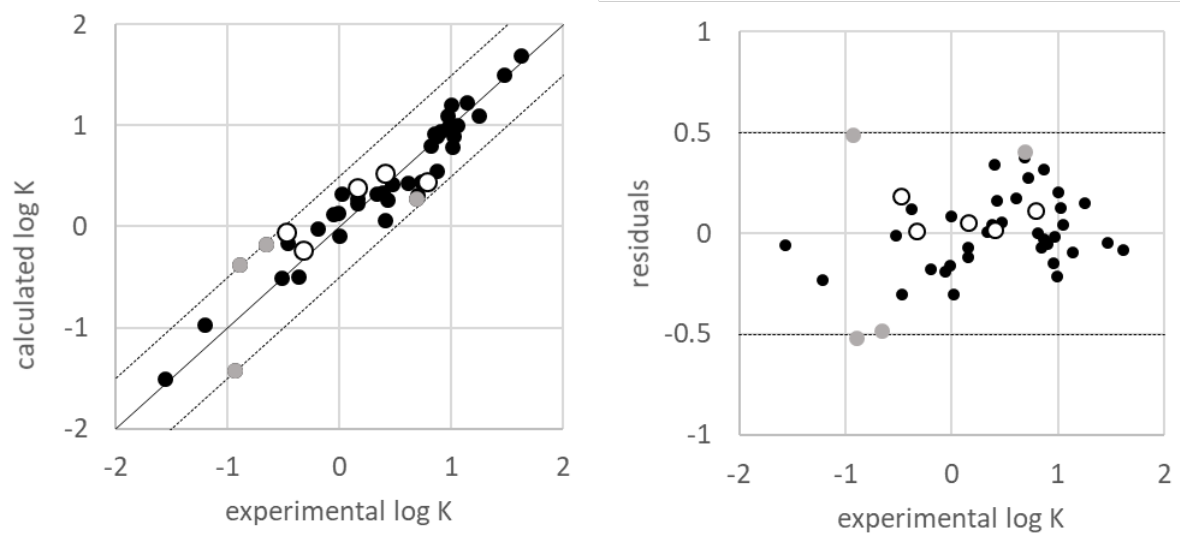
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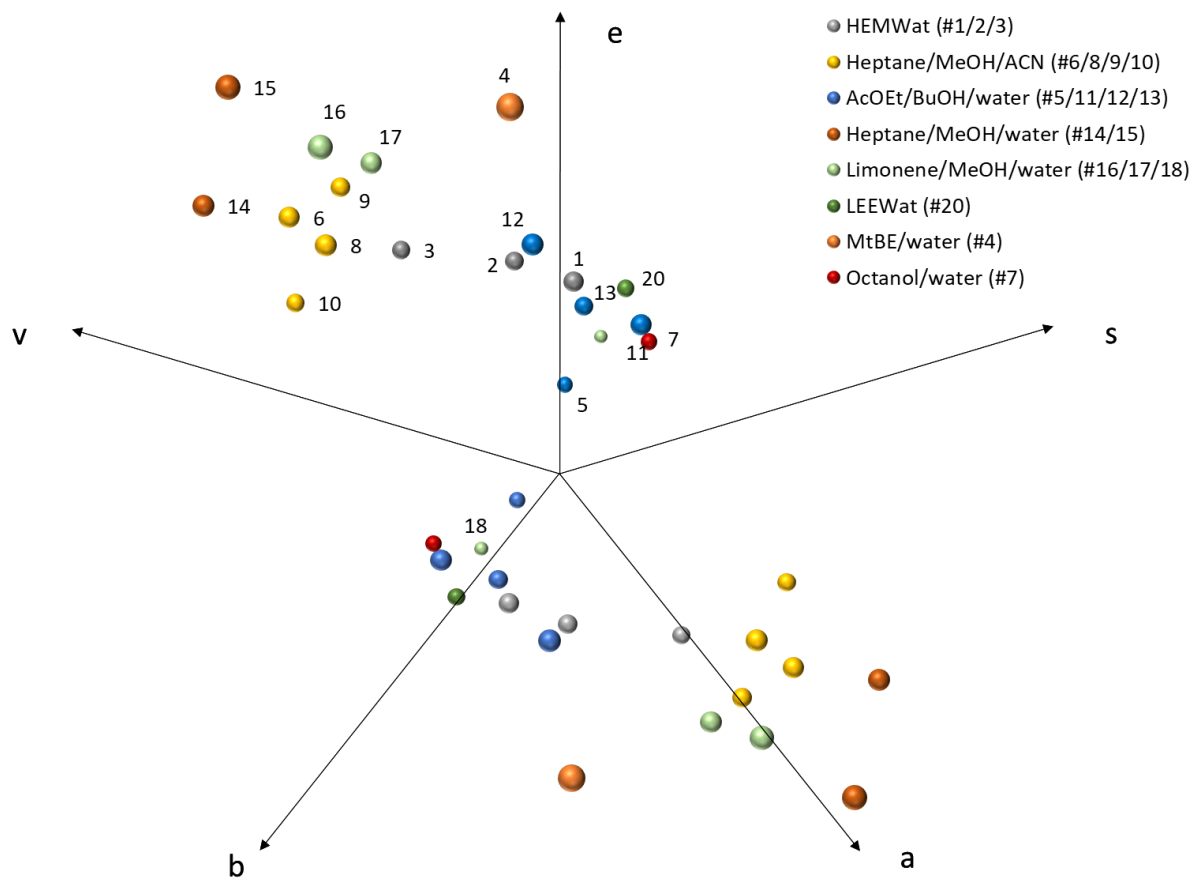


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438 Figure 2 Spider diagram of the solvent systems in descending (numbered dots) and ascending  
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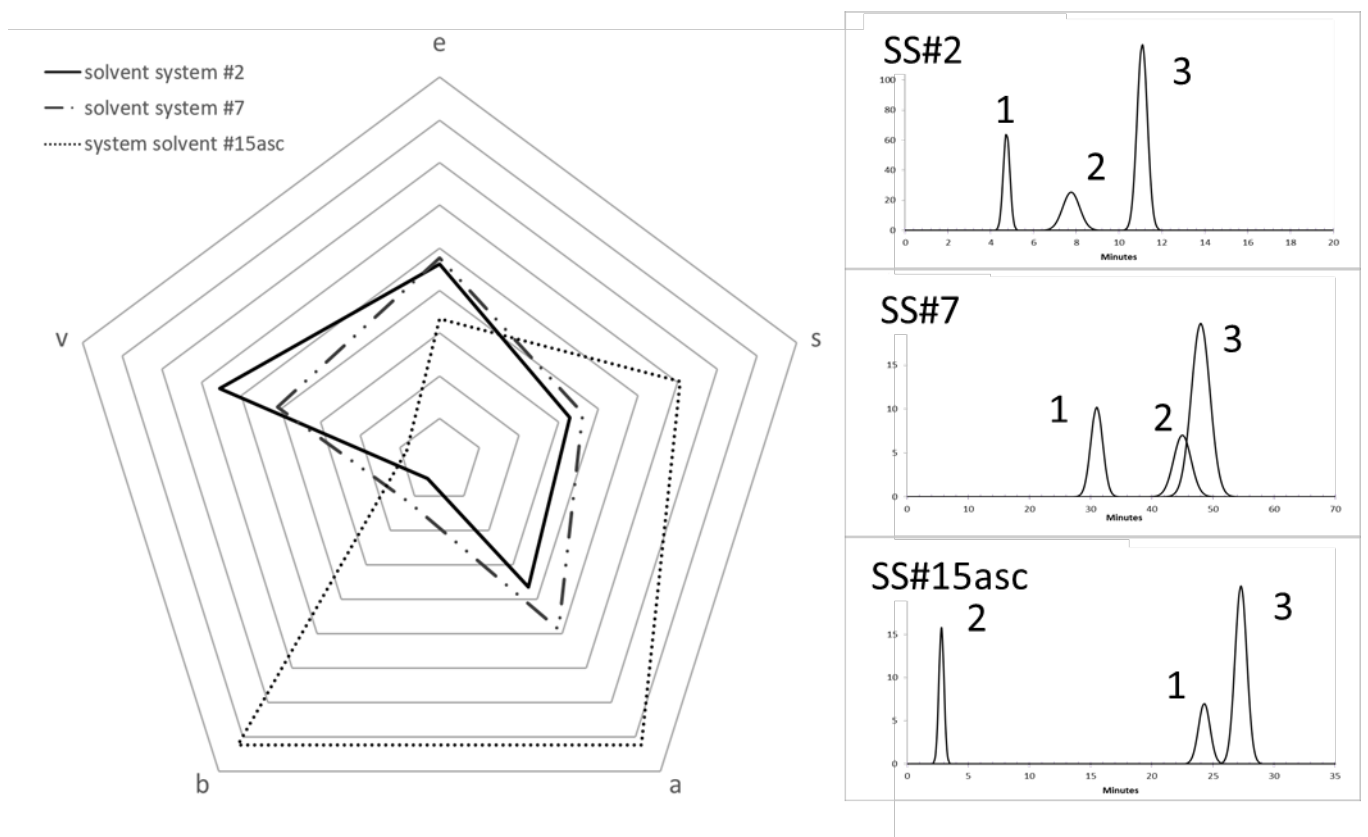
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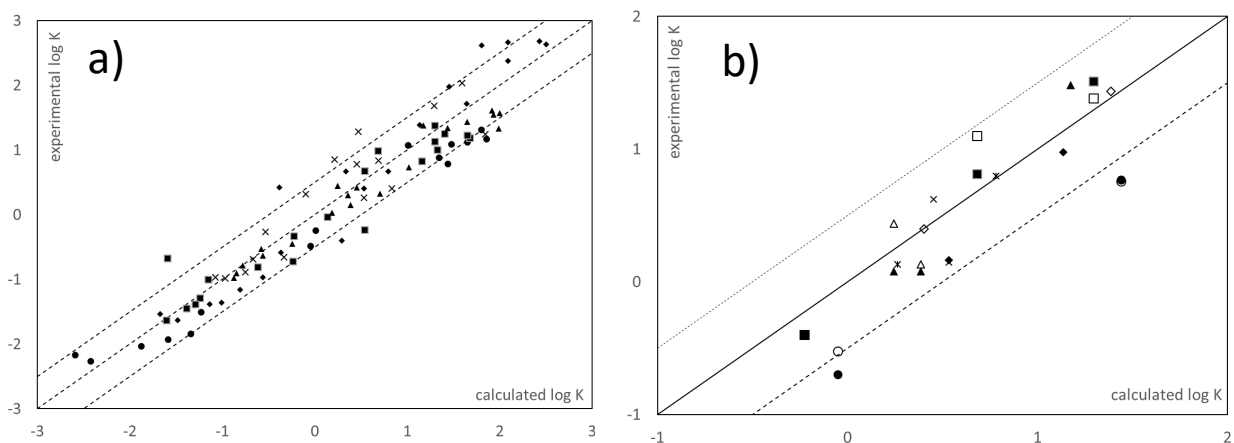
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