LECITHIN LIPOSOMES AND MICROEMULSIONS AS NEW CHROMATOGRAPHIC PHASES

Susana Amézqueta[1,*], Alejandro Fernández-Pumarega[1], Sandra Farré[1], Daniel Luna[1], Elisabet Fuguet[1,2], Martí Rosés[1]

[1] Departament de Química Analítica and Institut de Biomedicina (IBUB), Facultat de Química, Universitat de Barcelona, Martí i Franquès 1-11, 08028, Barcelona, Spain.

[2] Serra Húnter Programme. Generalitat de Catalunya. Spain

* Correspondence:

Susana Amézqueta Pérez

Departament de Química Analítica, Facultat de Química, Universitat de Barcelona

c/ Martí i Franquès 1-11, 08028, Barcelona, Spain

e-mail: samezqueta@ub.edu

Phone: (+34) 93 402 12 77

Fax: (+34) 93 402 12 33

Running title

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ABSTRACT

Lecithins are phospholipidic mixtures that can be part of microemulsions and liposomes. In this work, ready-to-use preparations of lecithin have been tested as pseudostationary and mobile phases in EKC and LC, respectively. The selectivity of two EKC systems, one based on lecithin microemulsions (LMEEKC) and another on liposomes (LLEKC), and of a LC system based on lecithin microemulsions (MELC) has been evaluated through the solvation parameter model. In all cases, solute volume and hydrogen-bond basicity are the main descriptors that drive the partition process. While solute volume favors the retention of solutes, hydrogen-bond basicity has the contrary effect. In lecithin-based EKC systems the hydrogen-bond acidity of the solute leads to a higher retention while in the lecithin-based LC system a minor retention is produced. The three lecithin systems have been compared through the solvation parameter model to other chromatographic systems, most of them containing phospholipids. Principal component analysis reveals that lecithin systems cluster together with the other EKC systems based on phospholipids, with an immobilized artificial membrane (IAM) LC system, with the octanol/water reference partition system, and with a SDS-based microemulsion. Thus, they all show similar selectivity. However, the great advantage of using the ready-to use lecithin systems is that the laborious liposome preparation is avoided, and that their commercial availability makes them more affordable than IAM LC columns. Finally, taking into account that lecithin has a high semblance to the mammalian cell membranes composition, the ability of the three lecithin systems to mimic the pass of the solutes through the membranes has been evaluated. Experimental determinations have demonstrated that the skin partition of neutral solutes can be easily emulated, especially using the lecithin-microemulsion EKC method. The model is robust and shows good prediction ability.

KEYWORDS

Solvation parameter model, physicochemical characterization, lecithin, surrogation, skin partition, chromatography

48 **1 INTRODUCTION**

49 Capillary electrophoresis (CE) is a separation technique used in a wide number of applications that
50 include either the determination of small compounds (pollutants, nutrients, drugs, biomarkers, etc.) and
51 of large ones (enzymes, proteins, DNA, etc.). CE is also used for the physicochemical characterization
52 of chemical substances (pK_a and log $P_{o/w}$ determination); the evaluation of the interaction with other
53 molecules such as the drug-protein binding; or the subrogation of biological properties of environmental
54 or biomedical interest [1–3].

55 Microemulsion electrokinetic chromatography (MEEKC) and liposome electrokinetic chromatography
56 (LEKC) are CE modalities that use a mobile phase containing a microemulsion or liposomes,
57 respectively. Microemulsions are formed by surfactant-coated oil droplets, and usually a cosurfactant
58 that acts as a stabilizer. Liposomes consist of a phospholipid bilayer with an encapsulated inner aqueous
59 cavity. The surfactants or phospholipids used are charged and thus the microemulsion or the liposomes
60 have their own electrophoretic mobility. In both cases, the solutes under analysis will migrate depending
61 not only on their charge-to-size ratio (as in capillary zone electrophoresis (CZE)) but also on the partition
62 with the corresponding pseudostationary phase (microemulsion or liposome) [4]. The partition is
63 analogous to that in reversed-phase liquid chromatography (RPLC) and thus both techniques are
64 considered CE-LC hybrids. Microemulsions can also be part of the mobile phase in reversed-phase
65 chromatography and they give place to microemulsion liquid chromatography (MELC) [5]. As far as we
66 are concerned, liposomes are not usually used as the mobile phase in LC. However, liposomes can be
67 trapped in the pores of gel beads to generate immobilized liposome chromatography (ILC) or
68 immobilized on the stationary phase to form an immobilized artificial membrane (IAM) [6,7].

69 Lecithins are mixtures of phospholipids (phosphatidylcholine, phosphatidylethanolamine,
70 phosphatidylinositol, phosphatidylserine, etc.) that are extracted from different natural sources such as
71 soy or egg. Commercial preparations based on lecithins are available to prepare emulsions and liposomes

72 with cosmetic and pharmaceutical uses (Emulmetik™, Pro-Lipo™, Lipoid™). However, they have not
73 been used yet to prepare chromatographic mobile phases. In the search of new chromatographic systems
74 with different selectivity and taking into account the complexity when preparing a microemulsion or a
75 liposome, it would be of great interest to investigate the viability of the ready-to-use commercial
76 products as chromatographic phases and characterize their selectivity. In the present work we will
77 evaluate the selectivity of a lecithin-based microemulsion in MEEKC (LMEEKC) and MELC
78 (LMELC), and lecithin liposomes in LEKC (LLEKC). In LMEEKC and LLEKC lecithin acts as
79 pseudostationary phase, and buffer as aqueous phase; inversely, in LMELC, C18 acts as stationary phase
80 and lecithin as mobile phase.

81 The solvation parameter model (SPM) proposed by Abraham [8] is a popular quantitative structure-
82 property relationship (QSPR) model to characterize the selectivity of chromatographic systems [9,10].
83 The following equation is used to model the solvation that a neutral solute undergoes in a biphasic system
84 and includes five different molecular descriptors.

85 $$\log \mathrm{SP} = c + eE + sS + aA + bB + vV \qquad \text{[Eq. 1]}$$

86 Here, SP is the dependent solute property in a given partitioning system, *i.e.* equilibrium constant or
87 some other free energy related property such as the chromatographic retention factor or a membrane
88 partition. The *E*, *S*, *A*, *B* and *V* independent variables are the solute descriptors proposed by Abraham. *E*
89 represents the excess molar refraction, *S* is the solute dipolarity/polarizability, *A* and *B* are the solute's
90 effective hydrogen-bond acidity and hydrogen-bond basicity, respectively, and *V* is McGowan's solute
91 volume. The coefficients of the equation are characteristic of the biphasic system and reflect the
92 difference of the two phases in properties complementary to the ones of solute descriptors. For any
93 system, the coefficients of this equation can be obtained by multiple linear regression analysis between
94 the log SP values acquired for an appropriate group of solutes and their descriptor values. Equations
95 based on the SPM have been reported (some of them are shown in Table SI-1 of the Supplementary

96 Material) to characterize several chromatographic systems. Similarly to the physicochemical systems
97 ruled by partition, the literature proposes equations based on the SPM for different biological processes
98 (Tables SI-2 and SI-3 of the Supplementary Material).

99 Characterizing two systems using the same model (the SPM in this work) makes them comparable, since
100 similar partitioning systems will have similar coefficients [11]. Thus, after characterizing the three new
101 systems with the SPM they will be compared with other physicochemical and biological systems. One
102 parameter to compare their similarity we have proposed is the *d* distance of the SPM coefficients [12].
103 The *d* distance is calculated from the normalized coefficients of the two correlations to be compared. To
104 do so, each system is considered as a five-dimensional vector of system coefficients (*e*, *s*, *a*, *b*, and *v*),
105 with a vector's length (*l*) mathematically defined as:

106 $$l = \sqrt{e^2 + s^2 + a^2 + b^2 + v^2}\ , \qquad \text{[Eq. 2]}$$

107 and each system coefficient is divided by *l* in order to obtain the normalized coefficients (e_u, s_u, a_u, b_u,
108 and v_u).

109 The *d* parameter is the distance between the two normalized vectors, i.e.

110 $$d = \sqrt{(e_{u1}^2 - e_{u2}^2) + (s_{u1}^2 - s_{u2}^2) + (a_{u1}^2 - a_{u2}^2) + (b_{u1}^2 - b_{u2}^2) + (v_{u1}^2 - v_{u2}^2)} \qquad \text{[Eq. 3]}$$

111 where the two subscripts 1 and 2 refer to the two systems to be compared. In previous works [13,14], a
112 *d* distance of 0.25 or less was stablished as adequate for surrogation. When many systems want to be
113 compared, dendrograms of the *d* distance and Principal Components Analysis (PCA) of the normalized
114 coefficients can be used to identify the most similar systems. These approaches are based on simple and
115 fast calculations and allow handling with a high number of data at once. They provide information about
116 the similarity of the systems and are very adequate to compare the selectivity of two different
117 physicochemical systems and to do a first selection of the physicochemical systems that can better

118 emulate a biological property. Detailed information on these comparison tools is described elsewhere
119 [19].

120 Once the most similar coefficients have been identified, the precision of the correlation between two of
121 the systems (i.e. estimation of a biological parameter from a physicochemical one) can also be estimated
122 from the errors of the biological and physicochemical system models and the systems dissimilarity
123 [12,15–17]. Estimation from chromatographic measurements is usually performed through a linear
124 equation of the type of Equation 4.

125 $$\log SP_{bio} = q + p \log SP_{chrom} \qquad \text{[Eq. 4]}$$

126 Here, SP_{bio} is the solute biological property, SP_{chrom} is the solute chromatographic property (in this case,
127 the chromatographic or electrophoretic retention factor), and q and p are the ordinate and slope of the
128 correlation, respectively.

129 In short, the expected precision of the correlation (SD_{corr}^2) can be considered as the sum of three different
130 contributions to the variance of the correlation: the biological data precision (SD_{bio}^2), the
131 chromatographic data precision $(p \times SD_{chrom})^2$ and the error due to the dissimilarity between the
132 correlated systems (SD_d^2). SD_{bio} and SD_{chrom} values are estimated from the respective standard
133 deviations of the SPM characterizations. In order to know p and also SD_d^2 the biological property and
134 the chromatographic property are calculated through their SPM equations and solutes' descriptors. In
135 this way, SD_{bio} and SD_{chrom} are zero. The slope of the correlation of these calculated values provides p,
136 and the SD of the correlation can be entirely attributed to the dissimilarity between both systems.

137 This kind of estimation is more laborious, so it is usually performed only for those pairs of systems that
138 show the highest similarity according to d distances.

139 Those systems closer (with smallest d or closest in the dendrogram and principal components space
140 plots) and with a good estimated correlation precision are identified as good candidates to surrogate each
141 one the other system. In the case of physicochemical-biological pairs, the closest physicochemical

142 system will probably be good surrogates for the biological system. To prove this, the physicochemical
143 property (the retention factor in the chromatographic or the electrophoretic system) is measured and a
144 correlation with the biological property is carried out for a series of representative compounds (Equation
145 4). If a good correlation is established between the properties of these two different systems, the
146 biological property of a new chemical compound can be predicted by measuring the corresponding
147 retention factor in the chromatographic system. The main advantage of this approach over QSPR studies
148 is that it is not necessary to know the molecular descriptor values of the new compound such as in the
149 SPM model. Furthermore, the use of chromatographic and electrophoretic measurements for prediction
150 of biological properties is of main interest due the high level of automatization, speed of analysis, low
151 cost, and high reproducibility of these techniques that lead to the *ex vivo* and *in vivo* tests avoidance.
152 Due to the high structural and compositional similarity between mammalian cell membranes and
153 lecithin-based microemulsions and liposomes it would be very interesting to test the possibility to mimic
154 properties of environmental or biomedical concern using chromatographic measurements. In fact,
155 lecithin-based chromatographic systems have already been used to mimic the intestinal absorption [18].

156

157 ## 2 MATERIAL AND METHODS

158 ### 2.1 Equipment

159 MELC measurements were done using a 10A series chromatograph from Shimadzu (Kyoto, Japan)
160 equipped with a quaternary pump and a diode array detector and fitted with a Gemini C18 column (15
161 cm × 4.6 mm i.d., 5 μm particle size) (Phenomenex, Torrance, CA, US) preceded by the corresponding
162 guard cartridge (1 cm).
163 MEEKC and LEKC measurements were done using the G1600A CE capillary electrophoresis system
164 from Agilent Technologies (Santa Clara, CA, US) equipped with a diode array detector. The fused-silica

capillary (30 cm effective length for MEEKC and 40 cm for LEKC, 38.5 cm total length for MEEKC and 48.5 cm for LEKC, 50 µm i.d.) was obtained from Composite Metal Services Ltd (Shipley, UK).

2.2 Reagents

Methanol (HPLC-grade), hydrochloric acid (25 % in water), sodium hydroxide (>99%), sodium dihydrogenphosphate monohydrate (>99%), disodium hydrogenphosphate (>99%), and phenanthrene (>97%) were from Merck (Darmstadt, Germany). Potassium bromide (≥99.5%), 1-butanol (≥99.7%), heptane (99%), and dodecanophenone (98%) were from Sigma-Aldrich (St. Louis, MO, US). Water was purified by a Milli-Q plus system from Millipore (Bedford, MA, US), with a resistivity of 18.2 MΩ cm. The lecithin products to prepare the microemulsions and the liposomes were Emulmetik™ 300 and Pro-Lipo™ Neo, respectively, from Lucas Meyer Cosmetics (Champlan, France). They were kindly supplied by Comercial Química Jover (Terrassa, Spain).

Tested substances were reagent grade or better and obtained from several manufacturers (Merck, Sigma-Aldrich, Carlo Erba (Milano, Italy), Baker (Center Valley, PA, US), Panreac (Castellar del Vallès, Spain), Thermo Fisher Scientific (Waltham, MA, US), and Scharlab).

2.3 Microemulsion and liposomes preparation

Lecithin microemulsions contained 0.5% (w/v) of lecithin and were prepared using Emulmetik™ 300 as starting material, product that contains 97% of phospholipids. In the case of MEEKC, lecithin (0.25 g) was mixed with 35 mL of phosphate buffer (20 mM, pH 7.0) in a magnetic stirrer at low speed (200 rpm). Next, 1-butanol (8.15% (v/v)) was added drop by drop and the solution was stirred for 30 min. Then, heptane (1.15 % (v/v)) was added and the suspension stirred for 30 min more. This suspension was transferred to a 50 mL volumetric flask and diluted with buffer. Finally, it was stirred for 1-2 h at low speed (200 rpm) until the mixture turned semitransparent. In the case of MELC, a higher volume

189 (0.5 L) was prepared using the proportional amounts of the reagents and stirring at higher speed (650
190 rpm).
191 Lecithin liposomes contained 0.5% (w/v) of lecithin and were prepared using the preliposome Pro-
192 Lipo^{TM} Neo as starting material, product that contains around 20% of phospholipids. Preliposome (0.5
193 g) was mixed with 20 mL of buffer in a magnetic stirrer at high speed (1000 rpm) for 45 min at 25-30
194 ºC. The use of other brands of ready-to-use lecithin products to form microemulsions or liposomes
195 should not provide very different results from those obtained in the present work. Notwithstanding, note
196 that a new calibration curve has to be done for every batch-to-batch or brand-to-brand analysis.
197
198 **2.4 Preparation of the test compounds solutions**
199 For MELC, stock solutions (2000 mg/L) of the solutes and the hold-up time marker (potassium bromide)
200 were prepared in methanol. Working solutions were prepared from stock solutions at a concentration of
201 50 mg/L, solving the corresponding amount of stock solution in the microemulsion.
202 In the case of MEEKC, stock solutions of the solutes and the microemulsion marker (phenanthrene)
203 (2000 mg/L) were prepared in methanol. Working solutions were prepared from the stock solutions and
204 contained 400 mg/L of the solutes and 200 mg/L of the microemulstion marker. They were diluted to
205 obtain a final methanol:buffer relation of 1:1. Methanol was used as the electroosmotic flow marker.
206 For LEKC, a stock solution containing the solutes (1000 mg/L) and the liposome marker
207 (dodecanophenone) was prepared in methanol. Work solutions were prepared by direct dilution of the
208 stock solution with buffer, and contained 200 mg/L of the solutes or the liposome marker. The final
209 methanol:buffer relation was 1:4. Again methanol was used as the electroosmotic flow marker.
210 Finally, all solutions were passed through a 0.45 μm nylon syringe filter obtained from Filter-Lab (Sant
211 Pere de Riudebitlles, Spain).
212
213 **2.5 Analysis by LC**

214 Target compounds were analyzed using the microemulsion as mobile phase at 1 mL/min. The injection

215 volume was 50 µL and the column temperature 25ºC. After a preliminary scan, detection wavelengths

216 were set at 200 and 254 nm depending on the compound absorption profile. All measurements were

217 taken in triplicate. After analyses, the system was cleaned by passing through water for 60 min,

218 acetonitrile:water for 60 min and acetonitrile for 30 min.

219 The LC retention factor (*k*), was calculated according to Eq. 5.

220 $$k = \frac{t_R - t_0}{t_0 - t_e} \qquad \text{[Eq. 5]}$$

221 where t_R corresponds to the solute retention time, t_0 is the column hold-up time, and t_e is the extra column

222 time determined by an analysis that excludes the chromatographic column. To measure t_e a

223 chromatographic correction with negligible hold-up volume has been used. t_0 and t_e have been

224 determined using an aqueous potassium bromide solution.

225

226 **2.6 Analysis by EKC**

227 Target compounds were analyzed using the microemulsion or the liposomes as pseudostationary phase.

228 Before the first use, the capillary was activated by the following washing sequence: water (5 min), 1 M

229 NaOH (20 min), water (2 min), 0.1 M NaOH (10 min), water (2 min) and microemulsion or liposome

230 suspension (20 min). As daily conditioning, the capillary was flushed with water for 6 min, followed by

231 methanol (3 min), water (3 min), 1 M NaOH (5 min), water (3 min), 0.1 M NaOH (3 min), water (1 min)

232 and microemulsion or liposome suspension (5 min). Before each separation, the capillary was flushed

233 with water (2 min), methanol (2 min), water (1 min), 0.1 M NaOH (2 min), water (1 min), and

234 microemulsion or liposome suspension (4 min).

235 The injection was done during 3 s at 50 mbar, the capillary temperature was 25ºC, and the voltage was

236 +15 kV. After a preliminary scan, wavelengths were set at 200, 214, and 254 nm depending on the

237 compound absorption profile. All measurements were done in triplicate. In terms of practicality,

238 LMEEKC and LLEKC analytical procedures were similar, and both systems were robust in batch-to-
239 batch analyses. Also, we did not detect run-to-run differences after applying the cleaning protocols
240 described.
241 The retention factor (k), was calculated according to Eq. 6.

242
$$k = \frac{t_m - t_{eof}}{\left(1 - \frac{t_m}{t_{ps}}\right) t_{eof}} \qquad \text{[Eq. 6]}$$

243

244 where t_m is the solute migration time, t_{eof} corresponds to the migration time of methanol, and t_{ps} is the
245 migration time of the pseudostationary phase marker.

246

247 **2.7 Data analysis**

248 PCA and dendrogram plots were performed with Matlab package from MathWorks (Natick, MA, USA).
249 Excel from Microsoft (Redmond, WA, US) was used for data calculations and multiple linear regression
250 analyses. The Abraham descriptors of the substances were the same used in a previous study [19]. The
251 biological data have been extracted from literature [20,21].

252

253 **3 RESULTS AND DISCUSSION**

254 **3.1 Characterization of the lecithin-based systems through the SPM model**

255 The retention factor of 59 solutes has been determined. These solutes were selected from a total of 71
256 set in a previous work [19] due to their variety of descriptors magnitude, their representability of the
257 physicochemical space, and their compatibility with the lecithin systems. These solutes are neutral in
258 the pH of work. Table 1 shows the set of compounds together with the retention factors obtained in the
259 three chromatographic systems (an example of the chromatograms and electropherograms obtained is
260 included in Figures 1-3 of the Supplementary Information). The results are an average of a minimum of
261 3 determinations and the RSD is under 5%. Next, the logarithm of the retention factor determined in the

corresponding chromatographic system has been correlated to the descriptors *A*, *B*, *S*, *E* and *V* of the solutes also presented in Table 1. Multiple linear regression between log*k* and the descriptors provide the coefficients and statistics for the physicochemical systems considered in the present work, which are shown in Table 2.

The systems are properly characterized by the SPM model: the determination coefficient (R^2) is higher than 0.90 in all cases, the standard error is low and in the order of that of other physicochemical systems characterized through this same model [19] , F_{cal} value is much higher than F_{tab} at a 95% confidence level, and all the SPM coefficients present statistical significance. Outliers, those compounds that present a residual value over |2.5|, non-detectable substances, and compounds that coeluted with the markers were not considered to set the model.

The direct comparison between the normalized coefficients of the SPM of each system (a graphical representation is shown in Figure 1) and the evaluation of the distance between these coefficients (Table 3) shows that the three lecithin based systems are very similar. The two electrophoretic systems (LMEEKC and LLEKC) are the most similar ($d_{LMEEKC/LLEKC}$=0.15), whereas the chromatographic LMELC is slightly less similar ($d_{LMELC/LMEEKC}$=0.18, $d_{LMELC/LLEKC}$=0.20). The big similarity of LMELC to the two electrophoretic systems is somewhat surprising because in LMEEKC and LLEKC lecithin acts as pseudostationary phase and the aqueous buffer as mobile phase, but in LMELC C18 acts as stationary phase and lecithin as mobile phase. We speculate that due to its structure, lecithin probably has intermediate properties between the aqueous buffer and C18 and thus the partition C18/lecithin (LMELC) is similar in properties to the partition lecithin/water, being in any case C18 and water saturated with lecithin.

Solute volume (*V*) and hydrogen-bond basicity (*B*) present high coefficients for the three systems. The cavity contribution is more favorable to partition to the stationary or pseudostationary phase than to the mobile phase or to the buffer ($v > 0$), and the hydrogen-bond acidity of the stationary (C18) or pseudostationary phase (lecithin microemulsion and liposomes) is much lower, especially for the lecithin

287 microemulsion, than that of the aqueous phase. All systems show a moderate negative polarizability
288 value (*s*), so the lecithin mobile phase is more polar than C18 stationary phase in LMELC, whereas
289 lecithin pseudostationary phases are less polar than water in LMEEKC and LLECK especially in the
290 latter system.
291 The liposomes system shows the highest *e* value, which means that the liposomes are highly polarizable.
292 The two electrophoretic systems show positive hydrogen-bond basicity value (*a*), so they are more
293 hydrogen-bond basic than the aqueous buffer, while the LMELC system presents a negative value, which
294 means that lecithin microemulsion is more basic than the C18 stationary phase. Among other factors,
295 the constant (*c*) of the correlations is related to the phase ratio for the separation system, and this one
296 depends on the microemulsion/liposome concentration and the molar volume of these suspensions [19].
297

298 **3.2 Comparison of the lecithin-based systems with other chromatographic systems**

299 The three lecithin-based systems (LMELC, LMEEKC, LLEKC) have been compared to other
300 chromatographic systems included in Table 4. The selected systems use any of the three techniques of
301 separation – MELC, MEEKC, LEKC - (SDS0.8, SDS1.6, Brij, and BrijSDS for MELC; SDSME for
302 MEEKC; DGDCChol, DGDC, PAAU, and PSUA for LEKC), or contain phospholipids in the stationary
303 phase (IAM, PLM) or in the pseudostationary phase (DHP, DHPChol and POPCPS), In addition, the
304 octanol/water partition (OW) has been included as reference system in the evaluation of partition
305 processes [22].
306 The similarity of these systems with the lecithin-based ones has been evaluated from the distance
307 between the corresponding normalized SPM coefficients (Table 3). The systems that are more similar to
308 the lecithin-based ones show a distance value under 0.25 units and are OW, SDSME, DHP, DHPChol,
309 and all the systems formed by phospholipids (IAM, PLM, DGDC, DGDCChol, and POPCPS)
310 independently of the technique used. The MELC systems that contain SDS in the mobile phase (SDS0.8,
311 SDS1.6 and BrijSDS) show the biggest differences (in general, d>0.45).

312 Figure 2 presents the dendrogram of *d* distances, and the PCA of the normalized coefficients for all the
313 selected chromatographic systems. Plots show three different clusters at *d*=0.25 level; the first one
314 includes the lecithin-based systems (OW, SDSME, DHP, DHPChol, IAM, PLM, DGDC, DGDCChol,
315 and POPCPS). The others include PSUA and PAAU (second cluster, polymeric-based LEKC systems);
316 and SDS0.8, SDS1.6, Brij, BrijSDS (third cluster, surfactant-based MELC systems). Systems of the first
317 cluster show negative PC1 and negative PC2. They differ from the third cluster mainly in the first
318 principal component (PC1) and from the second cluster in the second principal component (PC2). The
319 descriptors that have the main impact on PC1 are the solute's effective hydrogen-bond basicity (*B*) and
320 the McGowan's solute volume (*V*). In PC2, all of the descriptors have some influence except for the
321 McGowan's solute volume.
322 A relevant outcome is that all the systems containing phospholipids cluster together and are very similar
323 to the reference OW, with independence of the chromatographic approach used. The lecithin-based
324 LEKC system (LLEKC) behaves like the other LEKC systems based on phospholipids (DGDC, DHP,
325 DHPChol and DGDCChol). It shows also high semblance to IAM, an immobilized artificial membrane
326 of phospholipids used as stationary phase in RPLC. On the contrary, it shows different selectivity than
327 the polymeric-based LEKC systems (PAAU and PSUA), probably due to the chemical difference
328 between natural phospholipids and synthetic polymers. Generally, liposome preparation is laborious,
329 time-consuming and it requires the characterization of the liposomes formed. The main advantage of
330 using LLEKC over the other liposome systems is that the former is prepared by direct dilution of a ready-
331 to-use commercial product and does not imply a mandatory liposome characterization due to the
332 simplicity of the preparation process. It is also more affordable than IAM columns.
333 In the case of MEEKC, the two systems evaluated (LMEEKC and SDSME) cluster together ($d<0.25$)
334 and are different from the pure surfactant MELC systems (SDS0.8, SDS1.6, Brij, BrijSDS) that use C18
335 as stationary phase and form the second cluster. Probably, the contribution of the type of surfactant in
336 the ME properties is negligible in favor of the presence of other components such as 1-butanol or

337 heptane. Therefore, the partition is mainly influenced by the presence or absence of the C18 column.
338 Despite the clustering of the two microemulsion EKC systems, LMEEKC and SDSME, they show some
339 slight differences in selectivity. LMEEKC is more similar to LECK systems based on phospholipids,
340 and to the phospholipid membrane LPS used as stationary phase in RPLC. Whereas, SDSME is more
341 similar to LMELC. Surprisingly, LMELC system does not cluster with the surfactant-based MELC
342 systems in the second cluster. A reason could be that lecithin in MELC is probably mostly adsorbed to
343 the surface of the stationary phase and thus it is more similar to IAM (immobilized artificial membrane
344 formed of phospholipids), PLM (phospholipid membrane) and SDSME (SDS microemulsion used as
345 pseudostationary phase in EKC), also in the same cluster, than to surfactant-based MELC systems.
346

347 **3.3 Similarity of the lecithin-based systems and the biological systems**

348 Forty-two biological systems characterized through the SPM model have been considered in the present
349 study (their SPM characterization is described in the Tables SI-2 and SI-3 of the Supplementary
350 Material). They evaluate different properties of pharmaceutical interest related with the blood-tissue
351 partition, permeation or absorption and others of environmental interest related to aquatic toxicity, cell
352 permeation and soil absorption.
353 As before, the similarity of these systems with the lecithin-based ones has been evaluated through the *d*
354 distance parameter (Table 5). According to this criterion, the nineteen systems with the lowest *d*
355 distances have been selected. They include five and fourteen systems of pharmaceutical and
356 environmental interest, respectively. A previous study used a lecithin-based system to predict intestinal
357 absorption [18], however our predictions do not consider any of the three lecithin systems as candidates
358 to model this parameter (*d*~0.85).
359 Next, the dendrogram of *d* distances, and the PCA of the normalized coefficients of these nineteen
360 systems have been plotted (Figure 3). These plots show that the biological systems that are closer, and
361 hence show similar characteristics to the lecithin ones, are toxicity to rana tadpoles and blood brain

362 barrier permeability estimated by the parallel artificial membrane permeability assay (PAMPA) in the
363 case of LLEKC and LMELC, and skin partition, blood-lung partition, and toxicity to several aquatic
364 species in the case of LMEEKC. In concrete and according to both the distance and the clustering
365 criteria, the biological systems that probably will be best emulated by the lecithin systems are toxicity
366 to tadpoles and skin partition. Our group of research has recently reported a method using MEKC to
367 surrogate the toxicity to tadpoles [13] and other species [23] and a method using RPLC to surrogate skin
368 partition [24]. The RPLC method uses a C18 stationary phase and 10 mM phosphate buffer (pH 7) :
369 acetonitrile 60:40 as mobile phase and requires two descriptors to model the property, the retention factor
370 in the chromatographic system and the McGowan volume of the solute. Thus now we will test the
371 possibility to emulate the skin partition (log K_{SC}) using a direct method that only requires the retention
372 factor determination, and more ecofriendly analyses that do not use high volume of organic solvents.
373 To this end, the variance of the final correlation ($SD_{corr\ cal}^2$) between skin partition and the
374 physicochemical property data (the retention factor) of the selected systems has been estimated. Results
375 are shown in Table 6. The $SD_{corr\ cal}^2$ value is very low and little variance is added due to the dissimilarity
376 between compared systems (SD_d^2). Thus, skin partition is a promising system to be surrogated by the
377 three lecithin-based systems, probably best by LMEEKC and LLEKC, which show low dissimilarity
378 with the biological system and the lowest SD_d^2 and $SD_{corr\ cal}^2$ values. This fact, together with the higher
379 complexity in the preparation of high volumes of microemulsion, and the intensive cleaning protocols
380 needed when working with microemulsions in LC systems have led to the suppression of the LMELC
381 system as candidate for the surrogation. As indicated in Section 2.6, LMEEKC and LLEKC did not show
382 big differences in terms of practicality. Therefore, the ability of the LMEEKC and LLEKC systems to
383 surrogate the skin partition of neutral solutes has been evaluated.
384

385 **3.4 Evaluation of the performance of lecithin systems to estimate skin partition**

386 To evaluate the skin partition the retention factor of the solutes included in Table 1 with known skin
387 partition values (K_{SC}) [21] has been determined (Eq. 3) by LLEKC and LMEEKC. Also other
388 compounds with known K_{SC} values have been incorporated into the analysis to get more statistical
389 significance in the further skin partition estimation study (Table 7). They present different K_{SC} values
390 [21], physicochemical representability and detectability in the UV-Vis, and are neutral at the working
391 pH. Then, a regression analysis between the biological property logarithm values and the retention factor
392 logarithm values (k_{LLEKC} and k_{LMEEKC} for LLEKC and LMEEKC systems, respectively) has been done
393 according to Eq. 4. Eq. 7 and Eq. 8 include the regression parameters and the statistics for LEKC and
394 MEEKC, respectively. SD^2_{correl} stands for the variance of the correlation log K_{SC} *vs* log k.

395 $$\log K_{SC} = 1.24(0.08) + 0.59(0.05)\ \log k_{LLEKC} \quad (SD^2_{correl} = 0.089;\ n = 21\ (2\ outliers);\ R^2 =$$
396 $$0.671;\ F = 39) \qquad \text{[Eq. 7]}$$

397 $$\log K_{SC} = 1.29(0.06) + 0.71(0.08)\ \log k_{LMEEKC} \quad (SD^2_{correl} = 0.050;\ n = 24;\ R^2 = 0.789;\ F =$$
398 $$82) \qquad \text{[Eq. 8]}$$

399

400 As expected considering the initial predictions, it has been possible to surrogate skin partition directly
401 from the retention of the solute in the chromatographic system. Significant coefficients and good
402 statistical parameters (determination coefficient over 0.60, standard deviation in the order of the
403 biological data, significant F value) have also been obtained. In accordance with the clustering in Figure
404 3, all statistics are slightly better for the LMEEKC system. Furthermore, the number of solutes
405 considered is higher and it does not contain outliers. Therefore, we have selected the LMEEKC system
406 as best candidate to model the skin partition of the solutes. Figure 4 shows the graphical representation
407 of the regression of log K_{SC} vs log k_{LMEEKC}.
408 The LMEEKC system has been validated to prove its robustness and ability to predict skin partition
409 following the method proposed previously [25]. To perform the model's validation, the set of solutes
410 (24 compounds) has been divided into a training set (15 compounds, around 2/3 of the compounds) and

411 a test set (9 compounds, around 1/3 of the compounds). This selection has been done considering the
412 solutes distribution in a PCA plotted using the SPM descriptors that represents the chemical space. In
413 this way, compounds are distributed in the scores plot according to their physicochemical properties,
414 and a representative selection of compounds of different nature has been done for both, the training set
415 and the test set. For the internal validation, the model is established again, but only with the solutes of
416 the training set.

417 $$\log K_{SC} = 1.33(0.08) + 0.77(0.10)\log\ k_{LMEEKC} \quad (SD^2_{correl} = 0.052;\ n = 15;\ R^2 = 0.828;\ F =$$
418 $$63;\ Q^2_{LMO} = 0.98) \qquad \text{[Eq. 9]}$$

419 Eq. 9 shows the correlation parameters obtained. Equations' coefficients are similar to those of the model
420 with all solutes (Eq. 8), which is indicative of the robustness of the model. Adequate determination
421 coefficient, standard deviation, F value, and leave-multiple-out cross-validation coefficient (over 0.90)
422 have also been obtained.

423 Finally, the external validation has been carried out. A regression between the experimental skin partition
424 parameter and the one predicted through the training set equation has been done for the compounds of
425 the test set (Eq. 10). According to statistics, the model considered shows good prediction ability: the
426 slope of the trend line is not significantly different from unity and the intercept from zero at 95%
427 confidence level by the Students t-test; the variance (SD^2) is of the same order of that of the biological
428 data ($SD^2 = 0.047$), the determination coefficient (R^2) is above 0.70; the correlation cross-validation
429 coefficient ($QLMO^2$) is above 0.6; and the Fisher's F parameter is significant.

430 $$\log K_{SC,\ predicted} = 0.12(0.25) + 0.93(0.23)\log\ K_{SC,\ experimental} \quad (SD^2_{correl} = 0.063;\ n =$$
431 $$9;\ R^2 = 0.711;\ F = 17;\ Q^2_{LMO} = 0.66) \qquad \text{[Eq. 10]}$$

432

433 ## 4 CONCLUSION

434 Three different chromatographic systems based on lecithin have been characterized through the SPM
435 model. They are quite similar regarding the interaction with neutral compounds, and the main driving

436 forces implied are hydrophobicity and the hydrogen-bond acidity of the systems. The main differences
437 between them lie in the hydrogen-bond basicity and polarizability. They also show very similar partition
438 physicochemical characteristics to other phospholipid-based chromatographic systems, while they offer
439 more easy preparation and availability because they are based on ready-to-use commercial products.
440 Chemometric evaluation has shown that all three are good candidates to model skin partition of
441 compounds, especially that of LMEEKC. Moreover, EKC systems are more practical in terms of
442 technical issues compared to the LC one. Experimental evaluation has confirmed that the electrokinetic
443 system based on lecithin microemulsions (LMEEKC) is able to model skin partition through a direct
444 correlation between the logarithms of the chromatographic retention factor and the skin partition
445 parameter. Therefore, it offers an alternative to skin *in vivo* or tissue *in vitro* testing.
446

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448

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455

456

457 **CONFLICT OF INTEREST**

458 The authors declare no competing financial interest.
459

460

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530

531

532 **TABLES**

533 **Table 1**

534 SPM descriptors and logarithm of the retention factor of the solutes used to characterize the LMELC,

535 LMEEKC and LLEKC systems based on lecithin

	E	*S*	*A*	*B*	*V*	log k_{LMELC}	log k_{LMEEKC}	log k_{LLEKC}
1,2,3-Trihydroxybenzene	1.165	1.350	1.350	0.620	0.8925	-0.37	-1.16	-0.68
2,3-Benzofuran	0.888	0.830	0.000	0.150	0.9053	1.89	0.14	0.44
2,3-Dimethylphenol	0.850	0.850	0.520	0.360	1.0569	-	-0.20	0.29
2,4-Dimethylphenol	0.843	0.800	0.530	0.390	1.0569	-	-0.23	0.31
2-Naphthol	1.520	1.080	0.610	0.400	1.1441	1.67	0.30	0.73
2-Nitroaniline	1.180	1.370	0.300	0.360	0.9904	1.20	-0.58	-0.08
2-Nitroanisole	0.968	1.340	0.000	0.450	1.0902	1.20	-0.72	-0.25
3-Chloroaniline	1.053	1.100	0.300	0.300	0.9386	1.35	-0.37	0.00
3-Methylphenol	0.822	0.880	0.570	0.340	0.9160	1.28	-0.56	-0.20
3-Nitroaniline	1.200	1.710	0.400	0.350	0.9904	1.00	-0.73	-0.27
4-Aminobenzamide	1.340	1.940	0.800	0.940	1.0726	0.02	-1.30	-1.18
4-Chloroacetanilide	0.980	1.470	0.640	0.510	1.2361	1.33	-0.45	-0.06
4-Chloroaniline	1.060	1.130	0.300	0.310	0.9386	1.32	-0.38	-0.06
4-Chlorophenol	0.915	1.080	0.670	0.200	0.8975	1.56	-0.04	0.10
4-Nitroaniline	1.220	1.930	0.460	0.350	0.9904	0.95	-0.75	-0.23
Acetanilide	0.900	1.390	0.480	0.670	1.1137	0.65	-1.25	-0.93
Acetophenone	0.818	1.010	0.000	0.480	1.0139	1.16	-0.94	-0.73
Aniline	0.955	0.960	0.260	0.410	0.8162	0.66	-1.22	-0.92
Anisole	0.708	0.750	0.000	0.290	0.9160	1.61	-0.41	-0.15
Antipyrine	1.320	1.500	0.000	1.480	1.4846	0.17	-	-1.61
α-Pinene	0.446	0.140	0.000	0.120	1.2574	1.23	-	0.44
Benzaldehyde	0.820	1.000	0.000	0.390	0.8730	1.08	0.76	-0.76
Benzamide	0.990	1.500	0.490	0.670	0.9728	0.42	-	-1.10
Benzene	0.610	0.520	0.000	0.140	0.7164	1.19	-0.41	-0.10
Benzyl benzoate	1.264	1.420	0.000	0.510	1.6804	-	1.17	-
Methyl benzoate	0.733	0.850	0.000	0.460	1.0726	1.56	-0.46	-0.20
Benzophenone	1.447	1.500	0.000	0.500	1.4808	2.00	0.40	0.83
Benzonitrile	0.742	1.110	0.000	0.330	0.8711	1.13	-0.94	-0.66
Bromobenzene	0.882	0.730	0.000	0.090	0.8914	1.86	0.43	0.58
Butyrophenone	0.797	0.950	0.000	0.510	1.2957	-	-0.08	0.23
Caffeine	1.500	1.720	0.050	1.280	1.3632	0.04	-	-

Catechol	0.970	1.100	0.880	0.470	0.8338	0.65	-1.04	-0.56
Chlorobenzene	0.718	0.650	0.000	0.070	0.8388	-	0.26	0.46
Corticosterone	1.860	3.430	0.400	1.630	2.7389	1.21	-0.61	0.10
Cortisone	1.960	3.500	0.360	1.870	2.7546	0.84	-1.02	-0.38
Estradiol	1.800	1.770	0.860	1.100	2.1988	1.17	0.37	-
Estratriol	1.970	1.740	1.060	1.630	2.2575	-	-0.74	-
Ethylbenzene	0.613	0.510	0.000	0.150	0.9982	-	0.53	0.83
Phenol	0.805	0.890	0.600	0.300	0.7751	0.95	-0.92	-0.51
Furan	0.369	0.510	0.000	0.130	0.5363	-0.47	-	-
Geraniol	0.513	0.630	0.390	0.660	1.4903	-	0.19	0.36
Heptanophenone	0.720	0.950	0.000	0.500	1.7184	-	1.56	1.64
Hydrocortisone	2.030	3.490	0.710	1.900	2.7976	0.91	-0.90	-0.31
Hydroquinone	1.063	1.270	1.060	0.570	0.8338	0.31	-	-
Monuron	1.140	1.500	0.470	0.780	1.4768	1.20	-0.78	-0.46
Naphthalene	1.340	0.920	0.000	0.200	1.0854	2.05	0.82	1.09
Nitrobenzene	0.871	1.110	0.000	0.280	0.8906	1.37	-0.61	-0.27
o-Toluidine	0.966	0.920	0.230	0.450	0.9571	0.94	-0.98	-0.75
Pyrimidine	0.606	0.930	0.000	0.670	0.6342	0.03	-	-
Pyrrole	0.613	0.910	0.220	0.250	0.5774	-0.03	-	-1.09
Propylbenzene	0.604	0.500	0.000	0.150	1.1391	1.24	1.07	-0.14
Propiophenone	0.804	0.950	0.000	0.510	1.1548	1.56	-0.51	-0.22
p-Xylene	0.613	0.520	0.000	0.160	0.9982	-	0.57	0.74
Quinoline	1.268	0.970	0.000	0.540	1.0443	1.37	-0.81	-0.55
Resorcinol	0.980	1.110	1.090	0.520	0.8338	0.52	-1.11	-0.56
Thymol	0.822	0.790	0.520	0.440	1.3387	1.92	0.44	0.91
Thiourea	0.840	0.820	0.770	0.870	0.5696	-0.03	-	-
Toluene	0.601	0.520	0.000	0.140	0.8573	-	0.08	0.36
Valerophenone	0.795	0.950	0.000	0.500	1.4366	2.07	0.42	0.55

536

537 -: Compounds not detected in the chromatographic system

538

539

540

541 **Table 2**

542 Coefficients and statistics of the SPM model for the systems based on lecithin

543

	LSER coefficients						Normalized LSER coefficients					Statistics				
	c (SD_c)	*e* (SD_e)	*s* (SD_s)	*a* (SD_a)	*b* (SD_b)	*v* (SD_v)	e_u	s_u	a_u	b_u	v_u	n	R^2	SD	F	$n_{outliers}$
LMELC	0.525 (0.046)	0.250 (0.067)	-0.591 (0.039)	-0.212 (0.039)	-1.984 (0.059)	2.084 (0.059)	0.085	-0.200	-0.072	-0.671	0.705	39	0.984	0.078	410	9[a]
LMEEKC	-2.217 (0.098)	0.431 (0.104)	-0.624 (0.067)	0.330 (0.074)	-3.709 (0.161)	3.317 (0.115)	0.085	-0.124	0.065	-0.735	0.658	47	0.937	0.131	246	3[b]
LLEKC	-1.869 (0.083)	0.885 (0.118)	-0.809 (0.078)	0.339 (0.065)	-2.865 (0.112)	2.809 (0.092)	0.211	-0.193	0.081	-0.682	0.669	46	0.968	0.125	241	5[c]

544

545 [a] Furan, α-pinene, pyrogallol (1,2,3-trihydroxybenzene), pyrrole, propylbenzene, thiourea, aniline, benzene, *o*-toluidine; [b] Benzaldehyde, 4-

546 aminobenzamide, β-estradiol; [c] propylbenzene, α-pinene, monuron, quinolone, 4-aminobenzamide

547

548 **Table 3**

549 *d* distance values between lecithin-based and other physicochemical systems, according to the SPM

550 coefficients comparison. Values under 0.25 are in bold format

551

System	LMELC	LMEEKC	LLEKC
LMELC	**--**	**0.18**	**0.20**
LMEEKC	**0.18**	**--**	**0.15**
LLEKC	**0.20**	**0.15**	**--**
OW	**0.08**	**0.14**	**0.14**
IAM	**0.18**	**0.12**	**0.04**
PLM	**0.16**	**0.07**	**0.10**
SDS0.8	0.36	0.50	0.50
SDS1.6	0.54	0.69	0.69
Brij	0.27	0.42	0.45
BrijSDS	0.34	0.48	0.50
SDSME	**0.07**	**0.13**	**0.18**
DGDCChol	**0.17**	**0.05**	**0.12**
DGDC	**0.25**	**0.10**	**0.17**
PAAU	**0.18**	**0.11**	**0.15**
PSUA	**0.17**	**0.08**	**0.11**
DHP	0.32	0.42	0.41
DHPChol	0.44	0.42	0.48
POPCPS	**0.15**	**0.11**	**0.11**

552 **Table 4**

553 Composition of the physicochemical systems evaluated in the present work

Acronym	Technique of separation	Mobile phase	Stationary or Pseudostationary phase	Reference
OW	Liquid/liquid partition	Octanol/Water		[27]
IAM	LC	CH_3CN:phosphate buffer, gradient	IAM	[12]
PLM	LC	Phosphate buffer:CH_3OH 8:2	PLM	[28]
LMELC	MELC	Lecithin-based microemulsion	C18	Present work
SDS0.8	MELC	SDS, butanol, 0.80% heptane	C18	[29]
SDS1.6	MELC	SDS C18, butanol, 1.60% heptane	C18	[29]
Brij	MELC	Brij 35, butanol, heptane	C18	[29]
BrijSDS	MELC	Brij 35 SDS, butanol, heptane	C18	[29]
LMEEKC	MEEKC	Phosphate buffer	Lecithin-based microemulsion	Present work
SDSME	MEEKC	Phosphate/Borate buffer	SDS, butanol, heptane	[30]
LLEKC	LEKC	Phosphate buffer	Lecithin-based liposomes	Present work
DGDCChol	LEKC	2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid buffer	DPPG:DPPC:Chol	[31]
DGDC	LEKC	2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid buffer	DPPG:DPPC	[31]
PAAU	LEKC	Phosphate/Borate buffer	PAAU	[32]
PSUA	LEKC	Phosphate/Borate buffer	PSUA	[32]
DHP	VEKC	Tris(hydroxy- methyl)aminomethane buffer	DHP	[33]
DHPChol	VEKC	Tris(hydroxy- methyl)aminomethane buffer	DHP:Chol	[33]
POPC/PS	VEKC	2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid buffer	POPC/PS	[34]

554 Brij 35: polyoxyethylene(23) dodecyl ether; Chol: cholesterol; DGDC: dipalmitoylphosphatidyl glycerol + dipalmitoylphosphatidyl choline; DGDCChol:

555 dipalmitoylphosphatidyl glycerol + dipalmitoylphosphatidyl choline + cholesterol; DHP: dihexadecylphosphate; DHPC: 1-palmitoyl-2-oleyl-sn-glycero-3-

556 phosphocholine + cholesterol; DPPC: dipalmitoylphosphatidyl choline; DPPG: dipalmitoylphosphatidyl glycerol; IAM: immobilized artificial membrane;

557 LEKC: liposome electrokinetic chromatography; LLEKC: lecithin liposome electrokinetic chromatography; LMEEKC: lecithin microemulsion electrokinetic

558 chromatography; LMELC: lecithin microemulsion liquid chromatography; MEEKC: microemulsion electrokinetic chromatography; MEKC: micellar

559 electrokinetic chromatography; OW: octanol/water; PAAU: poly(sodium 11-acrylamidoundecanoate); PLM: phospholipid modified; POPC: 1-palmitoyl-2-

560 oleyl-sn-glycero-3-phosphocholine; PS: phosphatidyl serine; PSUA: poly(sodium 10-undecylenate); SDS: sodium dodecyl sulfate; VEKC: vesicle

561 electrokinetic chromatography

562

563

Table 5

d distance values between biological and chromatographic systems, according to the SPM coefficients comparison. Values under 0.25 are in bold format

System[a]		LMELC	LMEEKC	LLEKC
BBD	Blood-brain distribution	0.63	0.77	0.70
BBP	Blood-brain permeation	0.58	0.73	0.73
IA	Intestinal absorption	0.78	0.82	0.89
SPA	Skin partition	**0.18**	**0.10**	**0.19**
SPE	Skin permeation	**0.21**	**0.23**	0.34
BBI	Blood–brain partition /in vitro	0.55	0.70	0.67
BMI	Blood–muscle partition/in vitro	0.71	0.81	0.80
BLII	Blood–liver partition /in vitro	0.62	0.78	0.75
BLUI	Blood–lung partition /in vitro	0.26	**0.17**	0.31
BKI	Blood–kidney partition /in vitro	1.08	1.22	1.18
BHI	Blood–heart partition /in vitro	0.56	0.60	0.59
BFI	Blood–fat partition /in vitro	0.52	0.61	0.67
PBB	PAMPA-BBB[b] permeability	**0.20**	0.26	**0.23**
PPO	PAMPA-Po[c] permeability	0.34	0.50	0.49
PDS	PAMPA-DS[d] permeability	**0.23**	0.39	0.39
PH	PAMPA-HDM[e] permeability	0.42	0.56	0.59
PDO	PAMPA-DOPC[f] permeability	0.35	0.48	0.51
PC	PAMPA-COS[g] permeability	0.62	0.76	0.78
PP16	PAMPA-P16[h] permeability	0.49	0.54	0.59

System[i]		LMELC	LMEEKC	LLEKC
RT	*Rana* tadpoles	**0.19**	**0.20**	**0.14**
FM	Fathead minnow (*Pimephalespromelas*)	**0.23**	**0.09**	**0.20**
GP	Guppy (*Poeciliareticulata*)	**0.23**	**0.14**	**0.15**
BG	Bluegill (*Lepomismacrochirus*)	0.37	**0.20**	0.26
GO	Golden orfe (*Leuciscusidusmelanotus*)	0.45	0.36	0.35
GF	Goldfish (*Carassiusauratus*)	0.59	0.52	0.64
MK48	Medaka high-eyes (*Oryziaslatipes*)	0.37	0.30	**0.21**
MK96	Medaka high-eyes (*Oryziaslatipes*)	0.46	0.38	0.35
DM24	*Daphnia magna*	0.29	**0.16**	0.27
DM48	*Daphnia magna*	**0.23**	**0.13**	**0.22**
CD	*Ceriodaphniadubia*	**0.22**	**0.20**	0.30
DP	*Daphnia pulex*	0.32	**0.20**	0.29
TP	*Tetrahymena pyriformis*	0.25	**0.15**	**0.22**
SA	*Spirostomumambiguum*	0.35	**0.21**	0.32
ES	*Entosiphonsulcantum*	0.47	0.35	0.35
UP	*Uronemaparduczi*	0.70	0.66	0.59
CP	*Chilomonas paramecium*	0.43	0.38	0.32
PP	*Pseudomonas putida*	0.32	0.33	0.30
PG	*Porphyromonasgingivalis*	0.39	0.34	**0.22**
SR	*Selenomonasartemidis*	0.39	0.26	**0.20**
SS	*Streptococcus sobrinus*	0.39	0.34	**0.24**
AP	Alga cell permeation	0.95	1.07	1.08
SWP	Soil-water sorption	0.26	0.25	0.26

571 [a] Systems of pharmaceutical interest; [b] PAMPA: parallel artificial membrane permeability assays / BBB:
572 blood brain barrier; [c] Po: intrinsic permeability; [d] DS: double-sink permeability measurement; [e] HDM: n-
573 hexadecane PAMPA model; [f] DOPC: dioleyoylphosphatidylcholine in n-dodecane PAMPA model; [g] COS:
574 cosolvent PAMPA method; [h] P16: hexadecane membrane system, [i] Systems of environmental interest (the
575 toxicity to several aquatic species, the alga-cell permeation and the soil-water sorption)
576

577 **Table 6**

578 Contributions that determine the overall variance ($SD_{corr\ cal}^2$) in the correlations between skin partition

579 data and chromatographic data of the lecithin-based systems

580

	q_{cal}	p_{cal}	$(p_{cal}SD_{chrom})^2$	n_{chrom}	SD_d^2	$SD_{corr\ cal}^2$
Skin partition (SD_{bio}^2= 0.047, n_{bio}=45)						
LMELC	0.261	0.850	0.004	40	0.028	0.079
LMEEKC	1.747	0.563	0.005	47	0.009	0.062
LLEKC	1.505	0.687	0.007	46	0.013	0.067

581

582 **Table 7**

583 Experimental log K_{SC} values from the literature [22] and experimental log k values

584 measured in this work by LLEKC and LMEEKC for different solutes

Solute	log K_{SC}	log k_{LLEKC}	log k_{LMEEKC}
2-Nitro-*p*-phenylenediamine	0.57	-0.89	-1.25
Cortexolone	0.86	0.13	-0.54
Cortisone acetate	0.80	0.29	-0.72
Diazepam	1.25	0.64	0.07
Estrone	1.13	1.05	0.42
Hydroxyprogesterone	1.08	0.86	-0.10
m-Cresol	1.06	-0.20	-0.61
Nicotinamide	0.07	-	-1.32
o-Phenylenediamine	0.37	-1.18	-1.26
p-Bromophenol	1.46	0.10	0.11
Testosterone	1.40	0.45	-0.11
Progesterone	1.75	1.40	0.57
p-Chlorophenol	1.34	0.10	-0.04
2-Naphthol	1.55	0.73	0.30
Benzene	1.48	-0.10	-0.41
Corticosterone	0.74	0.10	-0.61
Cortisone	0.50	-0.38	-1.03
Estradiol	1.13	1.15	0.37
Estriol	0.86	-	-0.74
Hydrocortisone	0.44	-0.31	-0.90
p-Cresol	1.06	0.78	-0.56
Phenol	0.76	-0.51	-0.92
Pregnenolone	1.70	-1.09	-
Resorcinol	0.29	-0.56	-1.11
Thymol	1.89	0.99	0.44

585

586 -: Compounds not detected in the chromatographic system

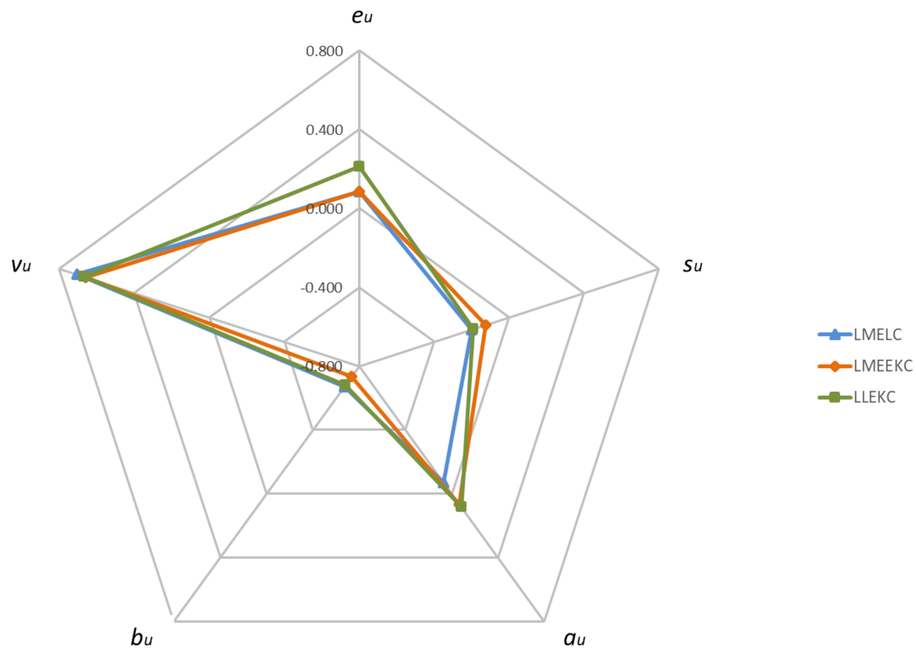
587

588 **FIGURES**

589 **Figure 1**

590 Comparison of the normalized SPM properties of the three lecithin-based systems

591 (LMELC: ▲; LMEEKC: ◊; LLEKC: ■).



592

593

594

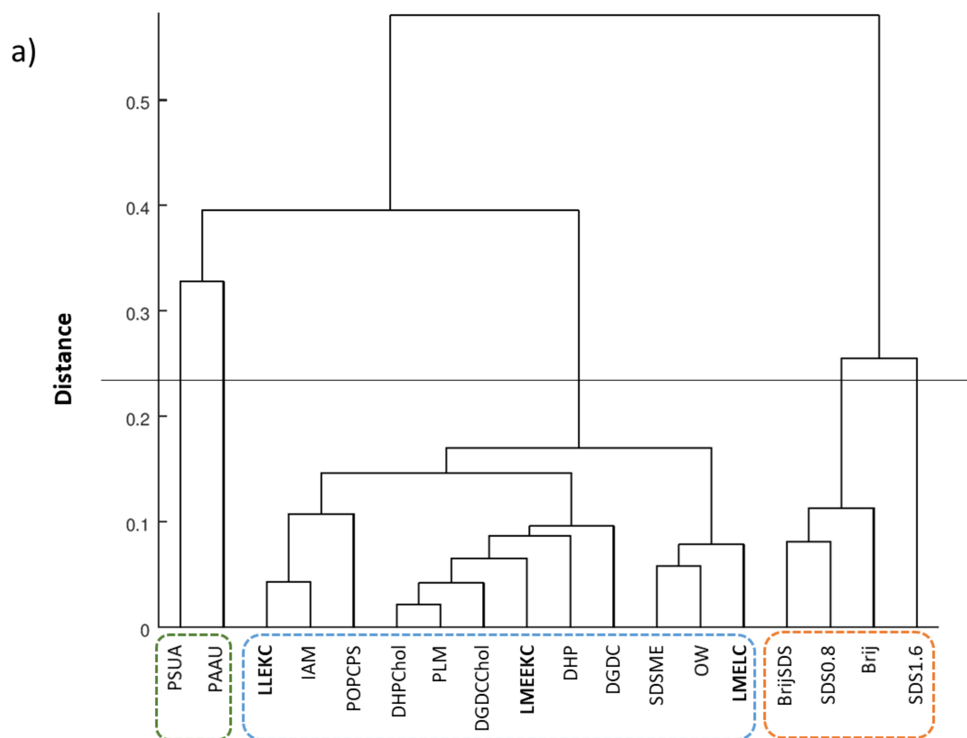
595 **Figure 2**

596 Comparison of the similarity between the three lecithin-based systems and other
597 physicochemical systems

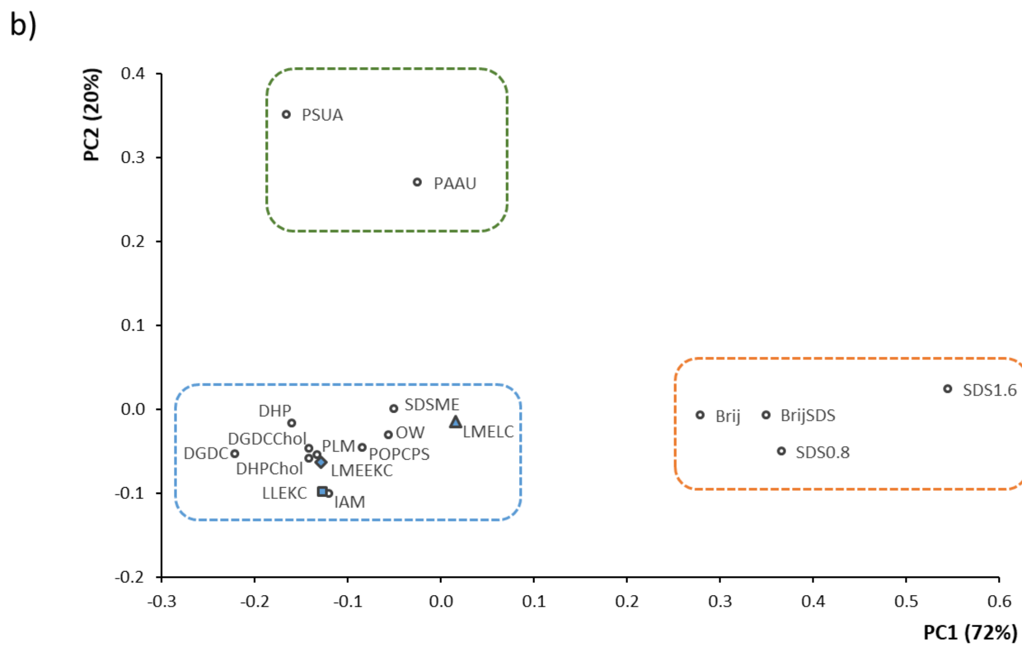
598 a) Dendrogram plot of the selected physicochemical systems, together with the
599 lecithin-based ones.

600 b) PCA scores plot of the physicochemical systems (o) and the lecithin systems
601 evaluated in this work (symbols as in Figure 1).

a)



b)



602

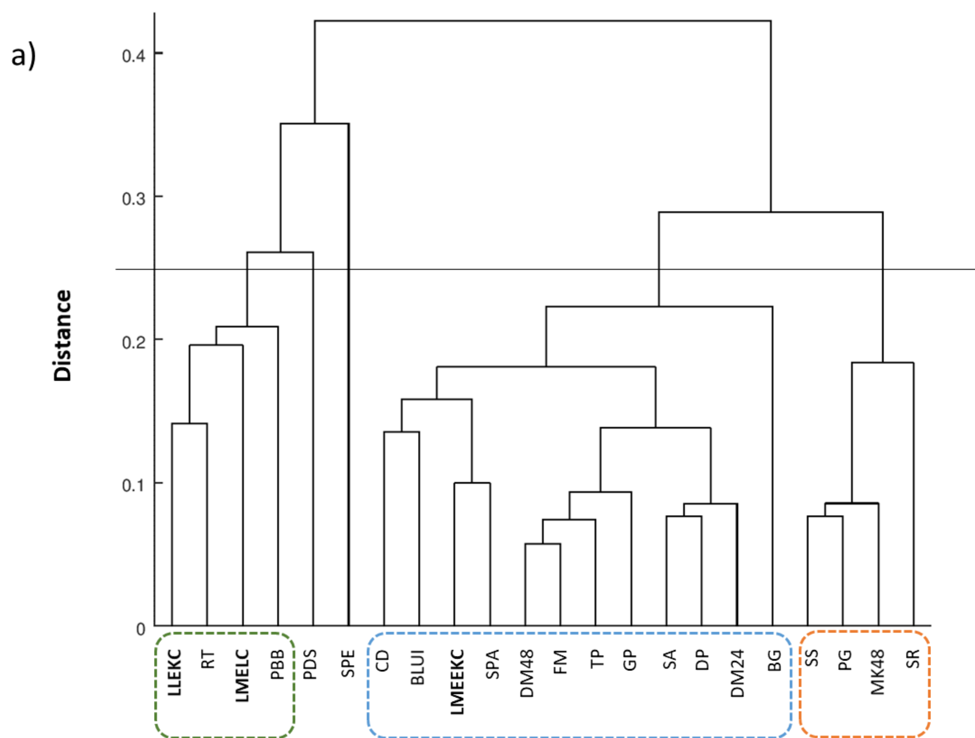
603

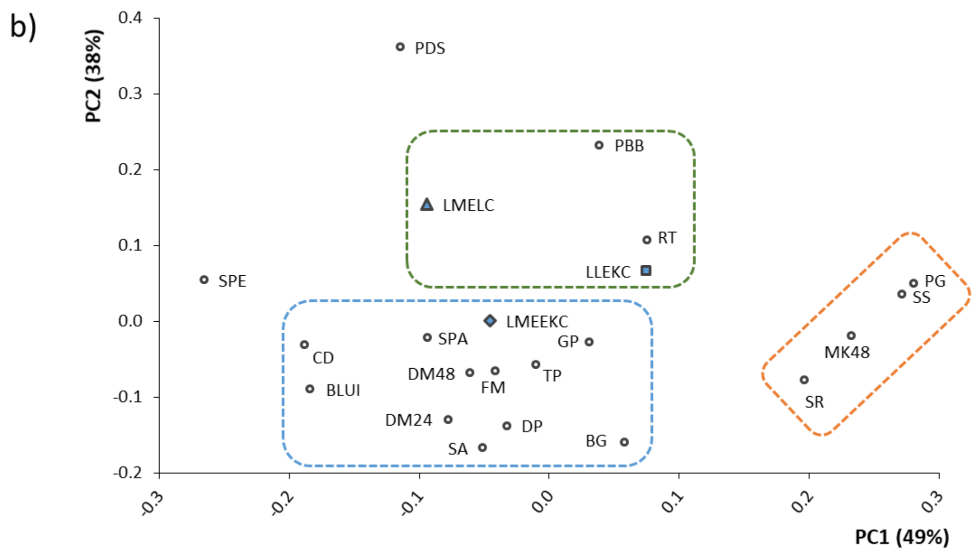
604

605 **Figure 3**

606 Comparison of the similarity between the three lecithin-based systems and biological
607 systems

608 a) Dendrogram plot of the selected biological systems, together with the lecithin-
609 based ones.
610 b) PCA scores plot of the biological systems (o) and the lecithin systems evaluated
611 in this work (symbols as in Figure 1).





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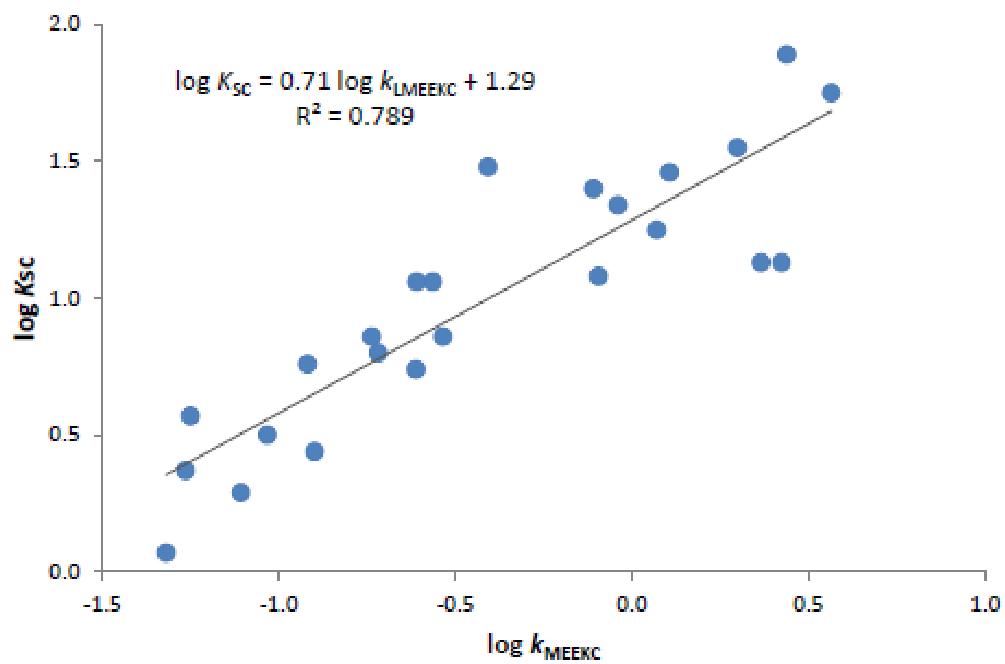
613

614 **Figure 4**

615 Correlation between skin partition and retention in the LMEEKC system. The solid line

616 is the plot of the regression equation.



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