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Micellar electrokinetic chromatography with bile salts for predicting ecotoxicity of aromatic compounds☆

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Abstract

The retention factors of several aromatic compounds were obtained by micellar electrokinetic chromatography (MEKC) using cholate, taurocholate, deoxycholate and deoxytaurocholate as micellar systems. The possibility of using these retention factors to describe and predict several ecotoxicological activities of different aromatic compounds was evaluated. Adequate correlations retention–ecotoxicity (log LC_{50} in fish and daphnia, log EC_{50} in green algae and daphnia, chronic values in fish and green algae, bioconcentration factor, and soil sorption coefficient) were obtained for the micellar systems studied. The predictive ability of the models obtained for these micellar systems was compared. Predicted values concur with the experimental log LC_{50} in Bluegill, Rainbow trout, Fathead minnows and Daphnia Magna values for the compounds studied. The results obtained indicated the usefulness of the MEKC systems investigated for the rapid ecotoxicity assessment of aromatic compounds.

Keywords: Polycyclic aromatic hydrocarbon; Micellar electrokinetic chromatography; Ecotoxicity

1. Introduction

As a result of various human activities, several kinds of organic pollutants are released into the environment. Among these compounds, aromatic chemicals, such as polycyclic aromatic hydrocarbons (PAHs) and their derivatives, are of special concern due to their toxic, carcinogenic, and mutagenic potential, and their ability to be absorbed in sediments and to bioaccumulate in living organisms [1,2].

To ascertain the potential hazard of compounds for the ecosystem, several toxicity bioassays were used. These tests are based on the study on the acute and chronic toxic effect of natural or synthetic pollutants on aquatic organisms (algae, fish and invertebrates). Toxic effects are measured as the concentration of chemical required to cause a lethal or effective response to 50% of a population of organisms (LC_{50}, EC_{50}, etc.). In addition, the bioconcentration factor (ratio between the chemical concentration in an organism and the total chemical concentration in the water) [3] and soil sorption coefficient (weight ratio between the amount of chemical absorbed per unit of organic carbon in the soil or sediment and the chemical concentration in water) [4] are important parameters in the evaluation of the ecotoxicological behaviour of xenobiotics. In recent years, an effort has been made concerning the development of alternative methods to the in vivo tests used for assessing the potential hazard of chemicals [2].

For a substance to cause a biological response when administered to an aquatic organism, a number of processes

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must occur, namely, absorption, transport, and distribution in the biological system. These processes are determined by the partitioning behaviour of the compound between the lipidic and the aqueous phase, which depends on molecular properties such as hydrophobicity, polarity, ionization degree, size, and molecular shape. In this context, quantitative structure–activity relationships (QSAR), requiring structural and/or empirical descriptors of compounds as predictor variables, were used to estimate and predict the toxicity for different organisms [5–12] and to estimate bioaccumulation [13–16] and soil sorption potential of chemicals [17–19].

Chromatographic and electrophoretic techniques are powerful tools for the measurement of physicochemical parameters. The retention of a compound in reversed-phase high-performance liquid chromatography (RP-HPLC) obtained under appropriate experimental conditions using stationary phases that emulate biological barriers can be used to measure the xenobiotic biopartitioning. The application of chromatographic parameters in structure–activity relationships gives rise to a new field, that is, quantitative retention–activity relationships (QRARs). QRARs have been successfully applied to describe the biological activity of different kinds of drugs [20–24] while only few applications for ecotoxicity predictions have been reported [11,25–27].

Micellar systems have been proposed as in vitro systems to emulate the biological partitioning of drugs due to their amphiphilic and anisotropic properties. Different QRAR models to describe pharmacodynamic, pharmacokinetic, and ecotoxicological parameters of a wide range of xenobiotics have been proposed. In these models, the retention factors of compounds in biopartitioning micellar chromatography (BMC), and micellar electrokinetic chromatography (MEKC), which depend on the electronic, steric, and hydrophobic properties of compounds, are used as dependent variables.

In a previous paper, the retention data of 66 compounds (phenols, phenylureas, phenoxy acids, and aromatic compounds) in biopartitioning micellar chromatography, were employed to obtain different QRAR models for the evaluation of different ecotoxicological parameters [28].

MEKC is a capillary electrophoresis technique in which surfactants at concentrations above their critical micellar concentration are added to the separation buffer. The applicability of this technique to describe the biological behaviour of xenobiotics was demonstrated by some researchers [21,29–32]. In a previous paper, the retention factors of a group of phenoxy-acids obtained in BMC and MEKC systems using Brij35 as surfactant were correlated with different ecotoxicity parameters. In both cases, appropriate QRAR models were obtained [33].

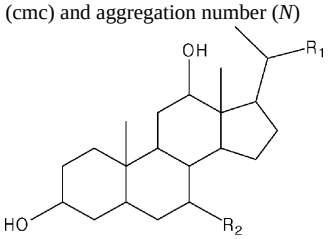
In this paper, the ability of different bile salt micellar systems, cholate, deoxycholate, taurocholate, and deoxytaurocholate to describe the ecotoxicity parameters of a set of 24 aromatic pollutants is studied and compared. Different QRAR models are obtained and the predictive ability of the models is evaluated.

2. Experimental

2.1. Reagents and standards

All reagents employed were of analytical grade. Bile salts sodium cholate (SC), sodium deoxycholate (SDC), sodium taurocholate (STC) and sodium taurodeoxycholate (STDC) (Table 1) were purchased from Sigma (St. Louis, MO, USA) and were used as received. Water used throughout the investigation was purified through a Milli-Q system from Millipore (Bedford, MA, USA). All other reagents used for the preparation of buffer solutions were of analytical grade and used without further purification. Urea from Fluka (Buchs, Switzerland); sodium hydroxide, and dimethylformamide (DMF) from Merck (Darmstadt, Germany); and 2-(*N*-cyclohexylamino)ethanesulphonic acid (CHES) from Sigma. All solutions were filtered through 0.45 μm pore

Table 1
CAS number, structure, logarithm of the octanol–water partition coefficient for non-ionic form of bile salt, molecular mass, critical micellar concentration (cmc) and aggregation number (*N*)



General structure

CAS	Bile salt	R_1	R_2	log P[a]	MW	cmc	N
81-25-4	Cholate (SC)	$CH_2CH_2CO_2^-$	OH	3.52	408.58	0.013	3
83-44-3	Deoxycholate (SDC)	$CH_2CH_2CO_2^-$	H	5.06	392.58	0.013	3
81-24-3	Taurocholate (STC)	$CH_2CH_2CONHCH_2CH_2SO_3^-$	OH	0.01	515.72	0.004	14
1180-95-6	Taurodeoxycholate (STDC)	$CH_2CH_2CONHCH_2CH_2SO_3^-$	H	1.55	499.71	0.009	11

[a] Value estimate using Kowwin software.

Table 2
Number of compound (*N*), CAS number, log *P* and pK_a for the aromatic compounds assayed

N	CAS	Compound	log *P*[a]	pK_a
1	71-43-2	Benzene	1.99	–
2	55-21-0	Benzamide	0.74	–
3	108-88-3	Toluene	2.54	–
4	100-47-0	Benzonitrile	1.54	–
5	98-95-3	Nitrobenzene	1.81	–
6	60-12-8	2-Phenylethanol	1.57	–
7	108-90-7	Chlorobenzene	2.64	–
8	140-29-4	Phenylacetonitrile	1.56	–
9	91-20-3	Naphthalene	3.17	–
10	129-00-0	Pyrene	4.93	–
11	85-01-8	Phenanthrene	4.35	–
12	243-17-4	2,3-Benzofluorene	5.19	–
13	86-73-7	Fluorene	4.02	–
14	206-44-0	Fluoranthene	4.93	–
15	208-96-8	Acenaphthylene	3.94	–
16	83-32-9	Acenaphthene	4.15	–
17	120-12-7	Anthracene	4.35	–
18	50-32-8	Benzo[a]pyrene	6.11	–
19	119-61-9	Benzophenone	3.15	–
20	89-61-2	2,5-Dichloronitrobenzene	3.1	–
21	108-95-2	Phenol	1.51	9.99
22	108-68-9	3,5-Dimethylphenol	2.61	10.2
23	90-15-3	1-Naphthol	2.69	9.34
24	135-19-3	2-Naphthol	2.69	9.51

[a] Value estimate using Kowwin software.

size disposable nylon filters from Scientific Resources Inc. (Eatontown, NJ, USA). The solutes studied, also of analytical-reagent grade, are listed in Table 2, together with their corresponding identification number and names.

2.2. Instruments and measurements

A programmable injector (model Prince) with a high voltage power supply (30 kV) and an UV detector (model Lambda 1000), all purchased from Lauer Labs (Emmen, The Netherlands), were used as capillary electrophoretic system. Injections were made by pressure (20 mbar for 0.02 min), and detection wavelength was set at 215 nm. All measurements were carried out at room temperature, and electropherograms were recorded with an acquisition data system Model Star 4.5 from Varian Associates (Sugar Land, TX, USA). Separations were performed on fused-silica capillaries (i.d. 50 μm, o.d. 365 μm) purchased from Composite Metal Services Ltd. (Worcester, UK). Capillaries had a total length of 65 cm and an injection-to-detection window length of 50 cm. Separation voltage was 15 kV. A 654 pH-meter from Metrohm (Herisau, Switzerland) and an ultrasonic bath Transsonic 460 (Elma, Germany) were employed to prepare the electrolytic solutions.

2.3. Procedure

A 100 mM CHES stock solution was prepared and pH was adjusted to 9 with a pH-meter using 0.1 M sodium hydroxide. Buffers were prepared by dissolving an appropriate amount of bile salt and urea (2 M) in CHES stock buffer solution. The final concentration was obtained by adding Milli-Q water up to the required volume. Finally, electrolytic solutions were degassed in a ultrasonic bath. The bile salts concentration in the buffers ranged from 0.075 to 0.175 M (five concentrations for each buffer). Standard solutions of solutes (approximately 10 mg/mL) were prepared by dissolving the solutes in the appropriate amount of DMF. All solutes analyzed were injected as mixtures containing the maximum number of solutes that could be separated in each of the measuring conditions. Peaks of solutes in the mixtures were identified by comparing their migration times with those of individual standards injected under the same conditions. The final concentration of the solutes ranged from 0.1 to 0.5 mg/mL, depending on their nature. DMF and Sudan III were used as electroosmotic flow and micelle migration markers, respectively.

The capillary was rinsed every morning with 0.1 M NaOH for 5 min, Milli-Q water for 5 min, separation buffer for 5 min, and then the voltage was applied for 5 min. Prior to each injection, in order to maintain good peak shapes and reproducible retention data, a washing routine for the capillary had to be used. This washing was the following: Milli-Q water for 2 min, 0.1 M sodium hydroxide for 2 min, Milli-Q water for 2 min, and the desired separation buffer for 2 min. In addition, to achieve good baselines, the buffer in the reservoirs had to be replaced after a few analyses. In all the experiments, the running voltage was 15 kV. At the end of the day, the capillary was rinsed with 0.1 M NaOH for 5 min, then Milli-Q water for 5 min, and stored in water overnight.

2.4. Software and data processing

Microsoft® Excel 2000 software and home-made Matlab subroutines (Matlab Ver. 5.3.0.10183 (R11), The Mathwoks I., Natick, MA, USA) were used to perform the statistical analysis of the linear regression. The Unscrambler® version 7.01 by CAMO was used to perform multivariate analysis.

Ecotoxicity parameter data were taken from the EPI Suite software of Syracuse Research Corporation [34]. This software integrates several programs based on QSAR models. In this paper, the following programs were used: (i) ECOSAR Class Program (ECOWIN version 0.99e) to estimate the ecotoxicity parameters LC_{50}, EC_{50}, and Chronic values for various aquatic organisms (fish, daphnia and algae); (ii) BCFWIN version 2.14 to assess the bioconcentration factor (BCF) in aquatic organisms; (iii) PCKOCWIN version 1.66, to ascertain the soil sorption coefficient (K_{oc}) of chemicals; and (iv) KOWWIN version 1.66 was used to estimate the octanol–water partition coefficient (log *P*).

2.5. Predictive ability of the QRAR models

To evaluate the predictive ability of the models, the fit error (the root mean square error of calibration (RMSEC)), the predicted error based on cross-validation (root mean square

error of cross-validation (RMSECV)) parameter that includes both interpolation and extrapolation information [35] and the RMSECVi parameter [36] for measuring only the interpolation information were compared. From a qualitative point of view, the lower the differences between RMSEC, RMSECV, and RMSECVi parameters, the greater the robustness of the QRAR model obtained.

2.6. Calculation of retention factors

Taking into account that the aromatic compounds studied in this work are neutral solutes at working pH (see Section 3.1), and that bile salts aggregates are anionic micelles (Table 1), the equation derived by Terabe et al. [37] was used to calculate MEKC retention factors:

$$k' = \frac{t_r - t_0}{t_0(1 - t_r/t_{mc})} \quad (1)$$

where k' is the retention factor, t_r the migration time of the neutral solute, t_0 the migration time of non-interacting solute moving at the electroosmotic flow and t_{mc} the migration time of a solute entirely concentrated in the micelles.

3. Results and discussion

3.1. Retention behaviour of aromatic compounds

The aromatic compounds included in this study (Table 2) comprise a wide range of hydrophobicity (log P values ranged from 0.74 for benzamide to 6.11 for benzo[a]pyrene) and they are neutral at the working pH. Phenol, 3,5-dimethylphenol, 1-naphthol, and 2-naphthol have pK_a values in aqueous media higher than 9.3 and, therefore, in this media, they could be partially ionized at the working pH. However, the presence of an organized medium modifies the acid–base constants of the solubilized compounds. This modification can be explained by the electrostatic attractions and repulsions between the species involved and the micelles when both are charged. When anionic surfactants are used, an increase of 0.5–3.0 in the pK_a values occurs [38]. Therefore, for the above-mentioned compounds, it can be assumed that their ionization degrees at pH 9 in a bile-salt micellar media are negligible.

To study the retention behaviour of aromatic compounds in these micellar systems, five different concentrations of each bile salt were employed (0.075, 0.100, 0.125, 0.150 and 0.175 M). Fig. 1 shows the effect of the SC, STC, SDC, and STDC micellar concentration on the retention factors of phenylacetonitrile (Fig. 1A) and benzene (Fig. 1B). As it could be expected, the retention of compounds depends on the nature of the compound and the nature and concentration of the bile salt. In all cases, compound retention factors increase as micellar concentration increases.

For a given micellar system, retention increases as hydrophobicity of compounds increases. In Table 3, the statistical parameters obtained for the log k–log P relationships at the lowest and highest concentration of bile salt assayed (0.075 and 0.175 M) are shown. As it can be observed in this table, statistically significant models ($P < 0.0001$) and adequate correlations ($r^2 > 0.89$, $F > 160$ and S.E. < 0.263) were obtained in all cases.

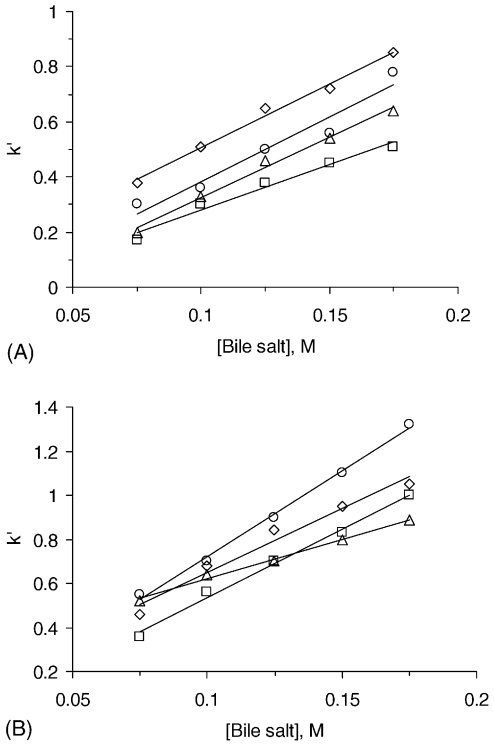


Fig. 1. Effect of the micellar concentration on the retention in MEKC of: (A) phenylacetonitrile and (B) benzene. (○) Deoxycholate; (□) cholate; (◊) taurodeoxycholate; (△) taurocholate.

Statistical comparisons of the slope and intercept values obtained for a given bile salt at 0.075–0.175 M (see Table 3) were performed. To compare the slope values, the adequate hypothesis t-test was used [39]. A similar test was applied to compare the intercept values. The results showed that the increase of bile salt concentration did not significantly affect slope values (SC: t_{cal} = 0.19; SDC: t_{cal} = 0.983; STC: t_{cal} = 0.322; STDC: t_{cal} = 1.753; $t_{0.025, n>40}$ = 2.327), indicating that the slope (sensitivity) depends on the nature of the micelle and not on the micelle concentration. In this sense, the highest slope values were obtained for the deoxy forms of bile salts, STDC, and SDC. On the other hand, the intercept values obtained for a given bile salt at different micellar concentrations were in all cases statistically different (SC: t_{cal} = 7.230; SDC: t_{cal} = 8.374; STC: t_{cal} = 7.41; STDC: t_{cal} = 5.371; $t_{0.025, n>40}$ = 2.327).

3.2. Retention–ecotoxicity relationships. Exploratory data analysis

Table 4 shows the values of the toxicity parameters extracted from ECOSAR programs and those of the biocon-

Table 3
Statistical analysis of the log k–log P relationship for two concentration levels of the bile salts assayed, log $k = a + b$ (log P)

Bile salt	[Bile salt] [M]	n	$a \pm$ ts[a] (P-value)[b]	$b \pm$ ts (P-value)	r^{2c} (r^2_{adj})[d]	F^e (P-value)	S.E.[f]
SC	0.075	23	−1.3 ± 0.2 (<0.0001)	0.49 ± 0.07 (<0.0001)	0.91 (0.91)	220 (<0.0001)	0.217
SDC		20	−1.4 ± 0.3 (<0.0001)	0.63 ± 0.10 (<0.0001)	0.91 (0.90)	180 (<0.0001)	0.263
STC			−1.1 ± 0.2 (<0.0001)	0.48 ± 0.06 (<0.0001)	0.93 (0.93)	280 (<0.0001)	0.188
STDC		23	−1.2 ± 0.2 (<0.0001)	0.55 ± 0.07 (<0.0001)	0.93 (0.93)	285 (<0.0001)	0.209
SC	0.175	23	−0.9 ± 0.3 (<0.0001)	0.47 ± 0.08 (<0.0001)	0.88 (0.87)	150 (<0.0001)	0.232
SDC		22	−0.9 ± 0.3 (<0.0001)	0.57 ± 0.09 (<0.0001)	0.89 (0.88)	161 (<0.0001)	0.256
STC		22	−0.78 ± 0.19 (<0.0001)	0.49 ± 0.06 (<0.0001)	0.94 (0.93)	301 (<0.0001)	0.159
STDC		16	−0.9 ± 0.3 (<0.0001)	0.62 ± 0.12 (<0.0001)	0.90 (0.89)	126 (<0.0001)	0.212

[a] ts: confidence interval at 95%.
[b] P-value: measure of significance of a model derived from ANOVA.
[c] r^2: correlation coefficient.
[d] r^2_{adj}: correlation coefficient adjusted for degrees of freedom.
[e] F: residual to modelled variance ratio.
[f] S.E.: standard error of the estimate.

centration factor and soil sorption coefficient extracted from BCFWIN and PCKOCWIN for each aromatic compound studied.

In order to establish the relationships among variables, principal component analysis (PCA) was applied to the eight ecotoxicity parameters: log LC_{50} for fish and daphnia, log EC_{50} for daphnia and green algae, and log ChV for fish and green algae, log BCF, and log K_{oc}. In the variable set, the logarithm of the retention factors of chemicals obtained using 0.075 M micellar solutions of the different bile salts studied

Table 4
Ecotoxicity parameters for aromatic compounds

N	log LC_{50} (fish)[a]	log LC_{50} (fish)[b]	log LC_{50} (daph)[c]	log EC_{50} (GA)[d]	log ChV (fish)[e]	log EC_{50} (daph)[f]	log ChV (GA)[g]	log LC_{50} (fish,sw)[h]	log BCF[i]	log K_{oc}[j]
1	1.77	2.03	1.80	1.60	0.88	0.51	0.60	1.13	0.94	2.22
2	3.14	3.31	3.13	2.89	2.16	1.60	1.58	2.23	0.50	1.71
3	1.33	1.62	1.37	1.18	0.47	0.19	0.32	0.80	1.40	2.43
4	2.32	2.54	2.33	2.12	1.39	0.95	1.00	1.58	0.50	1.99
5	2.14	2.38	2.16	1.95	1.24	0.84	0.91	1.46	0.72	2.28
6	2.36	2.59	2.38	2.16	1.44	1.01	1.06	1.63	−0.30	1.46
7	1.32	1.62	1.37	1.18	0.47	0.20	0.34	0.81	1.49	2.43
8	2.35	2.58	2.37	2.15	1.43	1.00	1.04	1.62	0.50	2.26
9	0.88	1.22	0.94	0.77	0.07	−0.12	0.06	0.48	1.84	3.26
10	−0.58	−0.12	−0.46	−0.59	−1.26	−1.19	−0.86	−0.60	3.06	4.84
11	−0.09	0.33	0.01	−0.13	−0.81	−0.83	−0.54	−0.23	2.73	4.32
12	−0.79	−0.32	−0.67	−0.79	−1.46	−1.36	−0.99	−0.76	3.74	5.10
13	0.19	0.59	0.28	0.13	−0.56	−0.62	−0.36	−0.02	2.52	4.05
14	−0.58	−0.12	−0.46	−0.59	−1.26	−1.19	−0.86	−0.60	3.27	4.85
15	0.23	0.62	0.32	0.16	−0.53	−0.60	−0.35	0.00	2.33	3.79
16	0.04	0.44	0.13	−0.02	−0.70	−0.75	−0.48	−0.15	2.32	3.79
17	−0.09	0.33	0.01	−0.13	−0.81	−0.83	−0.54	−0.23	2.73	4.31
18	−1.59	−1.05	−1.44	−1.54	−2.22	−1.96	−1.51	−1.37	4.02	5.90
19	1.05	1.39	1.11	0.94	0.24	0.04	0.23	0.65	0.91	3.03
20	1.12	1.45	1.18	1.01	0.31	0.10	0.28	0.71	1.68	2.71
21	1.44	n.a.	0.91	2.10	0.63	n.a.	0.98	n.a.	0.42	2.43
22	0.88	n.a.	0.57	1.24	0.05	n.a.	0.40	n.a.	1.11	2.85
23	0.90	n.a.	0.61	1.24	0.08	n.a.	0.42	n.a.	1.49	3.48
24	0.90	n.a.	0.61	1.24	0.08	n.a.	0.42	n.a.	1.38	3.47

[a] Logarithm of LC_{50} (mg l^{-1}) in fish after 96 h.
[b] Logarithm of LC_{50} (mg l^{-1}) in fish after 14 days.
[c] Logarithm of LC_{50} (mg l^{-1}) in daphnia after 48 h.
[d] Logarithm of EC_{50} (mg l^{-1}) in green algae after 96 h.
[e] Logarithm of ChV (mg l^{-1}) in fish after 30 days.
[f] Logarithm of EC_{50} (mg l^{-1}) in daphnia after 16 days.
[g] Logarithm of ChV (mg l^{-1}) in green algae after 96 h.
[h] Logarithm of LC_{50} (mg l^{-1}) in fish (saltwater) after 96 h.
[i] Logarithm of bioconcentration factor.
[j] Logarithm of the soil sorption coefficient ((mg adsorbed/kg organic carbon)/(mg/l)).

Table 5
Statistical analysis of the QRAR models obtained using SC as micellar system[a]

Ecotoxicity parameter	n	$a \pm$ ts (P-value)	$b \pm$ ts (P-value)	r^2 (r^2_{adj})	F (P-value)	S.E.
log LC_{50} (fish)1	24	1.25 ± 0.19 (<0.0001)	−1.4 ± 0.2 (<0.0001)	0.88 (0.87)	161 (<0.0001)	0.414
log LC_{50} (fish)2	20	1.6 ± 0.2 (<0.0001)	−1.3 ± 0.2 (<0.0001)	0.89 (0.88)	146 (<0.0001)	0.399
log LC_{50} (daph)	24	1.2 ± 0.2 (<0.0001)	−1.3 ± 0.3 (<0.0001)	0.84 (0.83)	113 (<0.0001)	0.464
log EC_{50} (GA)	24	1.22 ± 0.18 (<0.0001)	−1.4 ± 0.2 (<0.0001)	0.88 (0.87)	157 (<0.0001)	0.406
log ChV (fish)	24	0.41 ± 0.17 (0.0001)	−1.3 ± 0.2 (<0.0001)	0.88 (0.87)	157 (<0.0001)	0.385
log EC_{50} (daph)	20	0.20 ± 0.17 (0.0215)	−1.09 ± 0.19 (<0.0001)	0.89 (0.88)	144 (<0.0001)	0.327
log ChV (GA)	24	0.39 ± 0.13 (<0.0001)	−0.96 ± 0.16 (<0.0001)	0.88 (0.87)	154 (<0.0001)	0.283
log LC_{50} (fish,sw)	20	0.81 ± 0.17 (<0.0001)	−1.11 ± 0.19 (<0.0001)	0.89 (0.88)	145 (<0.0001)	0.330
log BCF	24	1.3 ± 0.2 (<0.0001)	1.4 ± 0.3 (<0.0001)	0.84 (0.83)	114 (<0.0001)	0.472
log K_{oc}	24	2.9 ± 0.2 (<0.0001)	1.4 ±0.3 (<0.0001)	0.86 (0.85)	130 (<0.0001)	0.457

Ecotoxicity parameter = $a + b$ (log k).
[a] See Tables 3 and 4 for abbreviations.

Table 6
Statistical analysis of the QRAR models obtained using SDC as micellar system[a]

Ecotoxicity parameter	n	$a \pm$ ts (P-value)	$b \pm$ ts (P-value)	r^2 (r^2_{adj})	F (P-value)	S.E.
log LC_{50} (fish)1	20	1.59 ± 0.19 (<0.0001)	−1.2 ± 0.2 (<0.0001)	0.90 (0.89)	156 (<0.0001)	0.361
log LC_{50} (fish)2	20	1.98 ± 0.17 (<0.0001)	−1.16 ± 0.17 (<0.0001)	0.94 (0.93)	212 (<0.0001)	0.290
log LC_{50} (daph)	20	1.5 ± 0.2 (<0.0001)	−1.1 ± 0.3 (<0.0001)	0.82 (0.81)	85 (<0.0001)	0.462
log EC_{50} (GA)	20	1.56 ± 0.16 (<0.0001)	−1.18 ± 0.18 (<0.0001)	0.91 (0.91)	192 (<0.0001)	0.315
log ChV (fish)	20	0.73 ± 0.17 (<0.0001)	−1.13 ± 0.19 (<0.0001)	0.89 (0.89)	153 (<0.0001)	0.336
log EC_{50} (daph)	20	0.50 ± 0.14 (<0.0001)	−0.94 ± 0.14 (<0.0001)	0.94 (0.93)	210 (<0.0001)	0.237
log ChV (GA)	20	0.63 ± 0.11 (<0.0001)	−0.82 ± 0.12 (<0.0001)	0.91 (0.91)	190 (<0.0001)	0.219
log LC_{50} (fish,sw)	20	1.12 ± 0.14 (<0.0001)	−0.95 ± 0.14 (<0.0001)	0.94 (0.93)	209 (<0.0001)	0.240
log BCF	20	1.0 ± 0.2 (<0.0001)	1.1 ± 0.2 (<0.0001)	0.85 (0.84)	104 (<0.0001)	0.401
log K_{oc}	20	2.6 ± 0.2 (<0.0001)	1.2 ± 0.2 (<0.0001)	0.87 (0.87)	124 (<0.0001)	0.388

Ecotoxicity parameter = $a + b$ (log k).
[a] See Tables 3 and 4 for abbreviations.

Table 7
Statistical analysis of the QRAR models obtained using STC as micellar system[a]

Ecotoxicity parameter	n	$a \pm$ ts (P-value)	$b \pm$ ts (P-value)	r^2 (r^2_{adj})	F (P-value)	S.E
log LC_{50} (fish)1	24	1.55 ± 0.17 (<0.0001)	−1.6 ± 0.2 (<0.0001)	0.91 (0.91)	236 (<0.0001)	0.348
log LC_{50} (fish)2	20	1.87 ± 0.18 (<0.0001)	−1.5 ± 0.2 (<0.0001)	0.93 (0.92)	225 (<0.0001)	0.329
log LC_{50} (daph)	24	1.5 ± 0.2 (<0.0001)	−1.5 ± 0.3 (<0.0001)	0.88 (0.87)	158 (<0.0001)	0.402
log EC_{50} (GA)	24	1.51 ± 0.18 (<0.0001)	−1.6 ± 0.2 (<0.0001)	0.90 (0.90)	204 (<0.0001)	0.361
log ChV (fish)	24	0.69 ± 0.16 (<0.0001)	−1.5 ± 0.2 (<0.0001)	0.91 (0.91)	230 (<0.0001)	0.325
log EC_{50} (daph)	20	0.42 ± 0.15 (<0.0001)	−1.24 ± 0.18 (<0.0001)	0.92 (0.92)	216 (<0.0001)	0.272
log ChV (GA)	24	0.59 ± 0.13 (<0.0001)	−1.09 ± 0.16 (<0.0001)	0.90 (0.89)	193 (<0.0001)	0.256
log LC_{50} (fish,sw)	20	1.03 ± 0.15 (<0.0001)	−1.26 ± 0.18 (<0.0001)	0.92 (0.92)	218 (<0.0001)	0.275
log BCF	24	1.1 ± 0.2 (<0.0001)	1.5 ± 0.3 (<0.0001)	0.83 (0.82)	108 (<0.0001)	0.482
log K_{oc}	24	2.6 ± 0.2 (<0.0001)	1.6 ± 0.3 (<0.0001)	0.89 (0.88)	175 (<0.0001)	0.402

Ecotoxicity parameter = $a + b$ (log k).
[a] See Tables 3 and 4 for abbreviations.

Table 8
Statistical analysis of the QRAR models obtained using STDC as micellar system[a]

Ecotoxicity parameter	n	$a \pm$ ts (P-value)	$b \pm$ ts (P-value)	r^2 (r^2_{adj})	F (P-value)	S.E.
log LC_{50} (fish)1	24	1.6 ± 0.2 (<0.0001)	−1.29 ± 0.19 (<0.0001)	0.90 (0.89)	188 (<0.0001)	0.386
log LC_{50} (fish)2	20	2.0 ± 0.2 (<0.0001)	−1.21 ± 0.19 (<0.0001)	0.91 (0.90)	181 (<0.0001)	0.363
log LC_{50} (daph)	24	1.6 ± 0.2 (<0.0001)	−1.2 ± 0.2 (<0.0001)	0.85 (0.84)	121 (<0.0001)	0.451
log EC_{50} (GA)	24	1.57 ± 0.19 (<0.0001)	−1.25 ± 0.19 (<0.0001)	0.90 (0.89)	197 (<0.0001)	0.367
log ChV (fish)	24	0.74 ± 0.19 (<0.0001)	−1.19 ± 0.18 (<0.0001)	0.89 (0.89)	182 (<0.0001)	0.361
log EC_{50} (daph)	20	0.48 ± 0.17 (<0.0001)	−0.98 ± 0.16 (<0.0001)	0.91 (0.90)	175 (<0.0001)	0.299
log ChV (GA)	24	0.63 ± 0.13 (<0.0001)	−0.86 ± 0.13 (<0.0001)	0.90 (0.89)	189 (<0.0001)	0.259
log LC_{50} (fish,sw)	20	1.10 ± 0.17 (<0.0001)	−1.00 ± 0.16 (<0.0001)	0.91 (0.90)	177 (<0.0001)	0.302
Log BCF	24	1.0 ± 0.2 (<0.0001)	1.2 ± 0.2 (<0.0001)	0.87 (0.87)	150 (<0.0001)	0.419
log K_{oc}	24	2.5 ± 0.2 (<0.0001)	1.3 ± 0.2 (<0.0001)	0.89 (0.88)	176 (<0.0001)	0.400

Ecotoxicity parameter = $a + b$ (log k).
[a] See Tables 3 and 4 for abbreviations.

(STC, STDC, SDC, and SC) and the molecular descriptor, log *P*, were included. In addition, the experimental values of log LC_{50} for three different kinds of fishes (Fathead minnows, Bluegill and Rainbow trout, 96 h of test duration), and the experimental values for log LC_{50} (48 h) for Daphnia Magna, were included as variables [40].

Because the variables are in different scales, the data were auto-scaled before applying the PCA. The PCA results revealed that the Fluoranthene (chemical number 14), had a high leverage in the PCA model with respect to the other objects. So, it was decided to exclude its calculation to prevent its influence on the variable latent structure.

Fig. 2 shows the loading plot corresponding to the first two principal components. The first principal component (PC1) explains 89% of the variance in the data, whereas the use of two principal components increases the percentage to 95%. As it can be seen, there is a high correlation between the retention of chemicals in MEKC (STC, STDC, SDC, and SC) and log *P* (in agreement with previous results), the bioconcentration, and soil sorption of chemicals. On the other hand, there is an inverse correlation between the retention and the toxicity parameters estimated by ECOSAR, and to some extent with the experimental values of log LC_{50} for daphnia and fishes.

PC2 explains the variance in data due to the experimental LC_{50} parameters. This variance is mainly due to the interspecies, laboratory or methodology variability.

3.3. Quantitative retention-ecotoxicity relationships

Once the qualitative relationships among variables was established, the relationships among retention and ecotoxicological parameters were studied. Tables 5–8 show the statistical analysis and the predictive features of the QRAR models obtained using the retention data of compounds in 0.075 M micellar solutions of each bile salt as predictive variable.

As it can be observed in all cases, the adequacy of the linear models to the data was satisfactory, r^2 values ranged between 0.82 and 0.94. In addition, for all models, the *P*-values were less than 0.05, indicating that the relationships between the ecotoxicity parameters and the retention using micellar solutions of bile salts were statistically significant at the 95% confidence level. In all cases, the coefficients (*a* and *b*) were also significant at this confidence level.

In order to compare the ability of the different bile salts studied to describe and predict ecotoxicity parameters, the RMSEC, RMSECV, and RMSECVi values for the developed QRAR models were obtained (Table 9). As it can be observed,

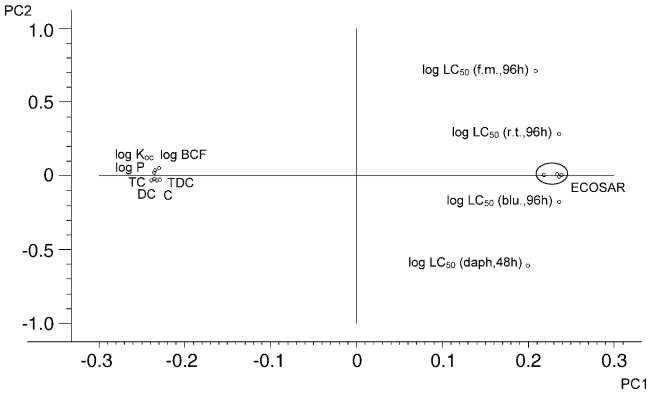


Fig. 2. Loading plot PC1–PC2.

Table 9
Descriptive and predictive features of the QRAR models obtained using different bile salts, ecotoxicity parameter = $a + b$ (log k)

Ecotoxicity parameter	Bile salt	RMSEC[a]	RMSECV[b]	RMSECVi[c]
log LC_{50} (fish)1	SC	0.3962	0.4536	0.4734
	SDC	0.3424	0.3768	0.3862
	STC	0.3332	0.3606	0.3728
	SDTC	0.3697	0.4321	0.4457
log LC_{50} (fish)2	SC	0.379	0.4397	0.4609
	SDC	0.2712	0.3059	0.2883
	STC	0.3117	0.3413	0.3494
	SDTC	0.3445	0.4112	0.4252
log LC_{50} (daph)	SC	0.4441	0.5013	0.5191
	SDC	0.4379	0.4782	0.4809
	STC	0.3849	0.4152	0.4267
	SDTC	0.4314	0.4916	0.5018
log EC_{50} (GA)	SC	0.3889	0.4403	0.4511
	SDC	0.2986	0.3262	0.3368
	STC	0.3459	0.3722	0.3731
	SDTC	0.3517	0.4088	0.416
log ChV (fish)	SC	0.369	0.4224	0.4406
	SDC	0.319	0.3512	0.359
	STC	0.3109	0.3369	0.3478
	SDTC	0.3454	0.4037	0.4158
log EC_{50} (daph)	SC	0.3101	0.3599	0.3768
	SDC	0.2217	0.2505	0.2372
	STC	0.2578	0.2827	0.2886
	SDTC	0.284	0.3387	0.3488
log ChV (GA)	SC	0.2708	0.3066	0.3121
	SDC	0.2078	0.2273	0.2314
	STC	0.2449	0.2636	0.2614
	SDTC	0.2477	0.2874	0.2897
Log LC_{50} (fish,sw)	SC	0.3129	0.3632	0.3802
	SDC	0.2249	0.2539	0.2404
	STC	0.2607	0.2855	0.2916
	SDTC	0.2866	0.3417	0.352
log BCF	SC	0.4518	0.5055	0.5227
	SDC	0.3805	0.4157	0.4344
	STC	0.4617	0.5007	0.5151
	SDTC	0.4015	0.4526	0.4652
log K_{oc}	SC	0.4377	0.493	0.5116
	SDC	0.3685	0.4126	0.4093
	STC	0.3845	0.4191	0.4337
	SDTC	0.3833	0.4384	0.448

[a] RMSEC: root mean square error of calibration.
[b] RMSECV: root mean square error of cross-validation (leave-one-out).
[c] RMSECVi: root mean square error of cross-validation (leave-one-out) for interpolated data.

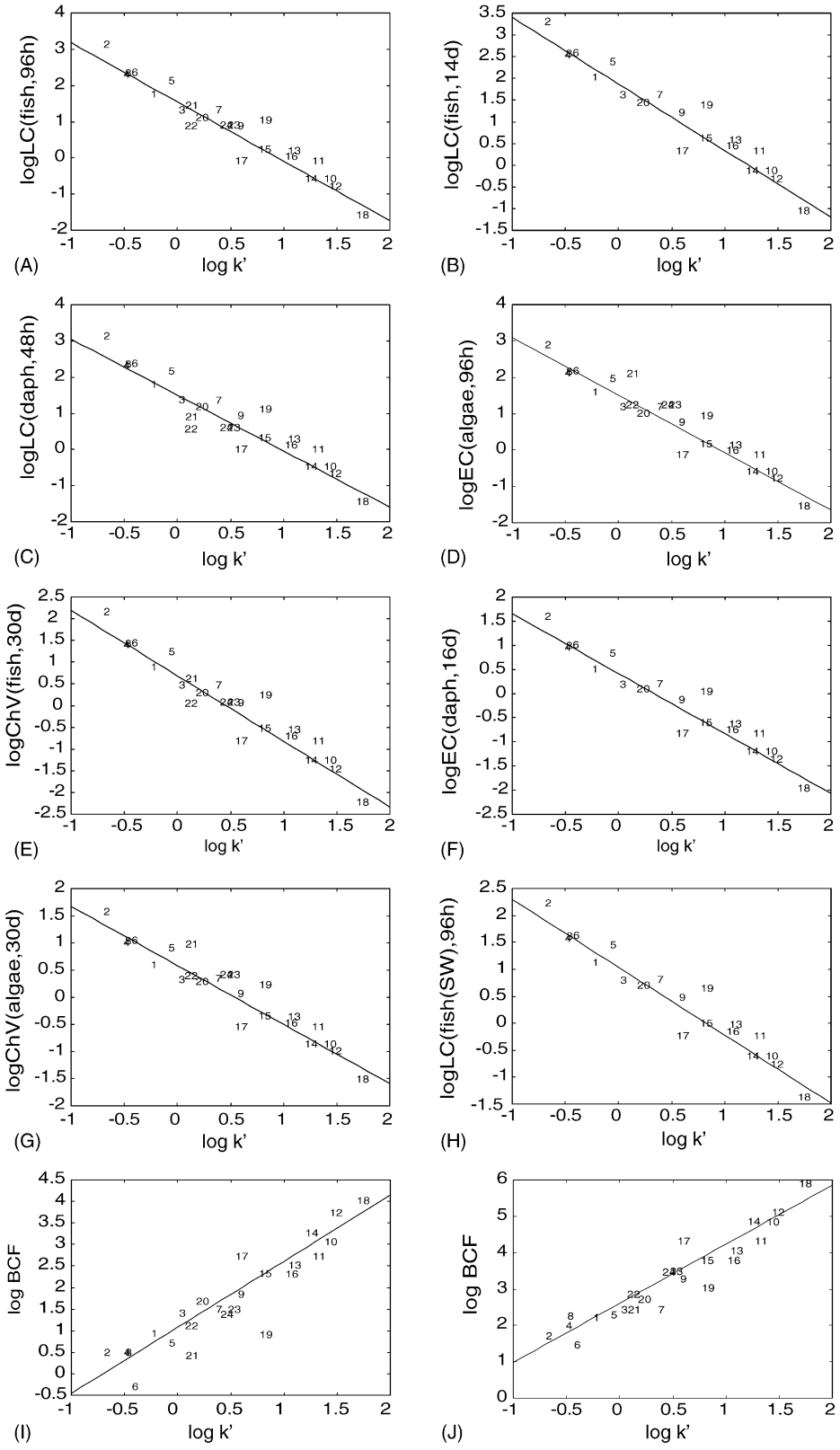


Fig. 3. Toxicity parameters-retention factors relationships for aromatic compounds. 0.075M taurocholate, pH 9. See Table 4 for details.

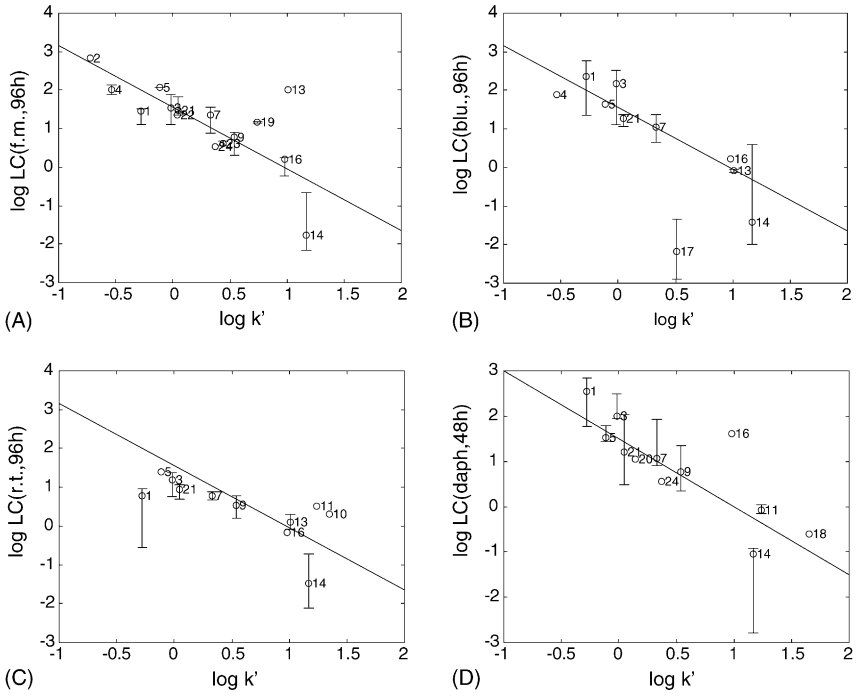


Fig. 4. Comparison between QRAR models proposed and experimental LC_{50} values for: (A) Fathead minnows (96 h); (B) Bluegill (96 h); (C) Rainbow trout (96 h); and (D) Daphnia Magna (48 h).

the RMSEC values were comparable to RMSECV and RMSECVi values suggesting the robustness of the models. In addition, RMSECV and RMSECVi values were also similar, indicating that both interpolations and extrapolations of parameters based on the current QRAR models should be reasonably adequate.

The comparison between the predictive ability of the models (RMSECV) obtained for the different micellar systems studied showed that STC and SDC micellar systems are more adequate in terms of prediction. In addition, the ecotoxicity models obtained using the STC micellar system, were better from a statistical point of view (slope, r^2 values, the F parameter, S.E. and most of cross-validated parameters). Fig. 3 shows the QRAR models obtained using 0.075 M STC as retentive phase.

3.4. Comparison between predicted and experimental data

In the reported experimental ecotoxicologic parameter values for a given compound, in a particular living organism and a given end point, a high variability is observed. This data variability could determine the statistical quality of mathematical models (QSAR, QRAR, etc.) as well as future predictions [40].

The LC_{50} (fish, 96 h) experimental values for three different kinds of fishes (Fathead minnows, Bluegill and Rainbow trout) and the LC_{50} (daphnia, 48 h) experimental values for Daphnia Magna found in literature [41] were compared with the predicted values obtained using the STC-QRAR models.

Fig. 4 shows the QRAR models obtained for each parameter from ECOSAR data (solid line). In the same figure, the experimental values of these parameters for each compound (medians and intervals of variability) have been included. As can be seen, the intervals show a variation around one logarithmic unit, being in the worst of the cases (i.e. fluoranthene $N = 14$) higher than two logarithmic units (see Fig. 4B).

For all the biological systems considered, adequate agreement between the predicted and the experimental values was obtained except for fluoranthene ($N = 14$) as could be expected from the PCA analysis.

Other outliers were detected in specific biological systems, fluorene ($N = 13$, LC_{50} fish Fathead minnows), anthracene ($N = 17$, LC_{50} fish Bluegill), benzene ($N = 1$, LC_{50} fish Rainbow trout), and acenaphthene ($N = 16$, LC_{50} daphnia magna). However, for these compounds the predicted values obtained with the others biological systems are in agreement with the experimental ones. Taking into account that the same property is measured in different biological systems, two possible reasons could explain this behaviour: they are either unreliable values or the compounds operate in these specific organisms by a different mechanism of action. It has been reported that high quality QSAR and QRAR models can only be established for molecules with a common mechanism of toxic action. On the other hand, it has also been reported that, in some cases, a given chemical may exhibit a different mechanism of toxic action based on the biological system used [42–43].

4. Conclusions

The approach proposed in this paper which involves the retention factors of aromatic compounds in MEKC, using the bile salts cholate, taurocholate, taurodeoxycholate, and deoxycholate may be an adequate alternative option to obtain estimation of the toxicity, bioconcentration, and soil sorption potential of this kind of chemicals.

Adequate quantitative retention–activity relationships for ecotoxic parameters were obtained, both in terms of calibration and validation. The comparison between the predictive ability and the statistical parameters of the different QRAR models indicates that the models obtained using the STC micellar systems were the most adequate. The agreement between the QRAR predictions and the experimental values supports the reliability of this in vitro technique for the ecotoxicological assessment of aromatic compounds.

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