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Effect of ionization and the nature of the mobile phase in quantitative structure-retention relationship studies

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Abstract

The octanol–water distribution constant, commonly called partition coefficient, $P_{o/w}$, is a parameter often retained as a measure of the hydrophobicity of a molecule. $\log P_{o/w}$, for a given molecule, can be conveniently evaluated constructing correlation lines between standard retention factor logarithms ($\log k$) in reversed-phase liquid chromatography (RPLC) and standard $\log P_{o/w}$ values. Many compounds of pharmaceutical interest can be quite hydrophobic and have, simultaneously, basic nitrogen atoms or acidic sulfur containing groups in their structure. This renders them ionizable. The hydrophobicity of the molecular drug form ($P_{o/w}$ value) is completely different from its ionic form ($\log P_{o/w}^{+\text{ or }-}$ value). The actual hydrophobicity of such ionizable molecule depends on the pH. It can be represented by an apparent P_{app} value that takes into account the amount of compound in its molecular and ionic state combining the $P_{o/w}$ and $P_{o/w}^{+\text{ or }-}$ values. In this work, $\log k$ in RPLC for ionizable as well as non-ionizable pharmaceutical compounds with different therapeutic properties (10 β-blockers, seven tricyclic antidepressants (TA), eight steroids and 12 sulfonamides) were correlated with $\log P_{o/w}$. Similar correlations were done between $\log k$ and the corrected $\log P_{app}$ values at pH 3. Aqueous-organic mobile phases containing acetonitrile (conventional RPLC) and micellar-organic mobile phases (micellar liquid chromatography, MLC), prepared with the anionic surfactant sodium dodecyl sulfate and the organic solvents acetonitrile, propanol or pentanol, were also used to elute the compounds. All mobile phases were buffered at pH 3. Using conventional retention RPLC data, the correlation of $\log k$ with $\log P_{o/w}$ was satisfactory for steroids because they cannot ionize. For ionizable β-blockers and TAs, the use of $\log P_{app}$ values improved the quality of the correlations, but yielded similar results for sulfonamides. In MLC, since an electrostatic interaction is added to hydrophobic forces, poorer correlations were obtained in all cases. The retention data obtained in RPLC also seems to correlate better with the biological activity of the drugs.

Keywords: Hydrophobicity; Ionizable compounds; Micellar liquid chromatography; Octanol–water partition coefficient

1. Introduction

The hydrophobic character of a solute is the main responsible for its affinity for biological membranes. For decades, solute hydrophobicity is estimated using the solute octanol–water distribution constant (expressed as $\log P_{o/w}$) [1–3]. Quantification of hydrophobicity is mandatory in various fields such as pharmacology, toxicology, environmental chemistry and food chemistry. Fast and reliable measurements are required to determine $\log P_{o/w}$, and different alternatives to the classical shake-flask method have been proposed [4–7]. Nevertheless, correlation between retention data in reversed-phase liquid chromatography (RPLC) and $\log P_{o/w}$ is a popular and frequent procedure [8–12]. This approach results in quantitative structure retention relationships (QSRRs).

Chromatographic methods are simple. The chromatographic retention factor, k, is, by definition, related to the solute distribution between the mobile and the stationary phases. Furthermore, RPLC does not need ultra pure compounds like the shake-flask method does. Potential impurities can be separated from the main component without affecting

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the results. Also, fast and reproducible data are obtained not knowing exactly the solute concentration.

However, inconsistencies have been found in the log k versus log $P_{o/w}$ correlations. The relevance of the octanol–water system as a good hydrophobicity reference for biological and environmental use was questioned [13]. Also, the polarity of a solute should not be dissociated from its ionizable character. To mimic the biological partitioning better, new and different column packing, such as immobilized artificial membrane (IAM) or immobilized octanol columns were introduced and compared to the classical C_8 or C_{18} stationary phases [14–19].

It is common in active drugs to find on the same molecule quite hydrophobic sites and a strong basic ionizable group. Usually, acidic mobile phases are used to avoid excessive retention [20–23]. In all cases, the chromatographic retention factors depend strongly on the predominant ionic/molecular form of the solute. In consequence, non-linear relationships between log k and molecular log $P_{o/w}$ are not surprising. Corrections taking into account the possible total or partial ionization of the solute (use of the apparent values of $P_{o/w}$, P_{app}) should be introduced.

In a previous work, the log k factors, obtained at different pH for a group of strong or weakly acidic diuretics, were correlated with the respective molecular log $P_{o/w}$ and the ionization corrected log P_{app} [24]. The relevance of such correction was obvious. In the present study, the results are extended to a wider and more heterogeneous set of compounds showing different acid–base properties: basic (tricyclic antidepressants (TA) and β-blockers), neutral (steroids) and amphoteric (sulfonamides). Retention data obtained in RPLC with aqueous-organic mobile phases are correlated with uncorrected log $P_{o/w}$ and compared to the same correlations done with log P_{app}. Solute partition coefficients were measured by countercurrent chromatography (CCC) or taken from the literature. CCC allows accurate and direct $P_{o/w}$ and P_{app} measurements [25]. Several authors claimed that micellar liquid chromatography (MLC) provides an alternative model to classical RPLC to estimate the hydrophobicity of compounds [26–31]. Then, retention data obtained with micellar mobile phases containing the surfactant sodium dodecyl sulfate (SDS) were also correlated with log $P_{o/w}$ and log P_{app}. The results obtained with both chromatographic modes are compared. MLC is also considered to mimic biological partition processes better than octanol–water partition or classical reversed-phase partition [32–33]. This aspect is also investigated.

2. Experimental

2.1. Reagents

Several families of compounds were studied:

(a) Ten β-blockers: acebutolol (Italfármaco, Alcobendas, Madrid, Spain), alprenolol, sotalol (Sigma, St. Quentin-Fallavier, France), propranolol (ICI-Farma, Madrid), carteolol (Mikel-Otsuka, Barcelona, Spain), labetalol (Glaxo, Tres Cantos, Madrid), metoprolol, oxprenolol (Ciba–Geigy, Barcelona), nadolol (Squibb, Esplugues de Llobregat, Barcelona), and timolol (Merck, Sharp & Dohme, Madrid).
(b) Seven tricyclic antidepressants (TAs): amitryptiline, clomipramine, doxepin, imipramine, nortryptiline, maprotiline and trimipramine, all from Sigma.
(c) Eight steroids: clostebol acetate (Sigma), medroxiprogesterone acetate (Cusi, Barcelona), dydrogesterone (Kalifarma, Barcelona), methyltestosterone (Sigma), nandrolone (Fher, Barcelona), progesterone (Seid, Barcelona), testosterone propionate and testosterone (Shering-Ploug, Madrid).
(d) Twelve sulfonamides: sulfacetamide, sulfachlorpyridazine, sulfadiazine, sulfamerazine, sulfamethazine, sulfamethizol, sulfamethoxazol, sulfanilamide, sulfapyridine, sulfaquinoxaline, sulfathiazol and sulfisoxazol, all from Sigma.

Stock standard solutions containing ∼100 μg ml^{-1} were prepared for all drugs. The compounds were dissolved in methanol (Prolabo, Paris, France). Working standard solutions were diluted with water in the aqueous-organic mode or with 0.1 M sodium dodecyl sulfate (Merck, Darmstad, Germany) in the micellar mode, respectively. All solutions were kept in the dark at 4 °C and remained stable during at least 3 months. Occasionally, ammonium nitrate was used as a dead volume marker.

Aqueous-organic mobile phases were prepared with 50% (v/v) or less acetonitrile (except for steroids, see compositions in Tables). Micellar mobile phases, prepared with the anionic surfactant SDS, contained 6% (v/v) or less acetonitrile for sulfonamides, 15% (v/v) or less propanol for β-blockers, and 7% (v/v) or less pentanol for TAs and steroids. All the organic solvents were purchased from Scharlab (Barcelona). Nanopure water (Barnstead, Sybron, Boston, MA, USA) was used throughout. All mobile phases were prepared with water buffered at pH 3 with 0.01 M NaH_2PO_4 and HCl (both from Panreac, Barcelona). The organic modifier was added to the buffered water. Working solutions and mobile phases were filtered through Nylon membranes of 0.45 μm and 47 mm diameter (Micron Separations, Westboro, MA, USA).

2.2. Columns

To ensure that the method could be used with different stationary phases, four different columns were used with different mobile phases:

(a) RPLC mode: an endcapped XTerra C_{18} column (150 mm × 4.6 mm i.d., Waters, MA, USA) was used for β-blockers and TAs, an ODS–Hypersil column C_{18} (5 μm, 100 mm × 4.6 mm i.d., Agilent, Waldbronn, Germany) separated the sulfonamides, and an ODS–2 C_{18}

column (5 μm, 125 mm × 4.6 mm i.d., Scharlab) was used for steroids.

(b) Micellar-organic mode: another ODS–2 C_{18} column (5 μm, 125 mm × 4.6 mm i.d., Scharlab) separated the β-blockers and an Eclipse XDB–C_8 column (5 μm, 150 mm × 4.6 mm i.d., Agilent, Palo Alto, CA, USA) was used with TAs.

All columns were connected to 30 mm guard columns packed with the corresponding stationary phase.

2.3. Apparatus

An Agilent chromatographic system (Model HP 1050, Palo Alto, CA, USA) with an isocratic pump, an autosampler with 2 ml vials (Series 1100, Model G1313A), and an Agilent UV detector were used. The β-blockers were detected at 225 nm except timolol, which was detected at 300 nm. Wavelengths of 275, 254 and 246 nm were selected for sulfonamides, TAs and steroids, respectively. Data acquisition was made with the Peak-96 software (Agilent, Avondale, PA, USA). Chromatographic runs were carried out at room temperature. The flow-rate was 1.0 ml min^{-1} and the injection volume, 20 μl. Duplicate injections were made.

3. Results and discussion

3.1. QSRR studies with apparent and molecular log $P_{o/w}$

3.1.1. Aqueous-organic mobile phases

The acid–base dissociation constants in aqueous solution (expressed as pK_a), molecular and ionic octanol–water partition coefficients ($\log P_{o/w}$ and $\log P^{+}_{o/w}$) for all the studied compounds are given in Table 1, along with the apparent coefficients at pH 3. The drugs can be classified in three groups: basic (β-blockers and TAs), amphoteric (sulfonamides) and neutral (steroids).

Originally, the $\log P_{o/w}$ of a substance is defined for its molecular form. As the hydrophobicity, the partition characteristics of the ionized form are completely different, hence, a derived parameter, $\log P_{app}$, has to be calculated. For basic molecules, the apparent value of $P_{o/w}$ at different pH values is noted P_{app} and expressed as follows [4]:

$$P_{app} = \frac{P^{0}_{o/w} + P^{+}_{o/w}(h/K_a)}{1 + (h/K_a)} \quad (1)$$

where $P^{0}_{o/w}$ and $P^{+}_{o/w}$ are the octanol–water partition coefficients of the molecular and cationic forms of the solute, respectively, K_a the acid–base dissociation constant and h is the concentration of hydrogen ions. For amphoteric compounds, the apparent partition coefficient is given by:

$$P_{app} = \frac{P^{0}_{o/w} + P^{+}_{o/w}(h/K_{a1}) + P^{-}_{o/w}(K_{a2}/h)}{1 + (h/K_{a1}) + (K_{a2}/h)} \quad (2)$$

being $P^{-}_{o/w}$ the octanol–water partition coefficient of the anionic form of the solute. The equation of retention in CCC is simply:

$$V_R = V_M + P_{app}\, V_S \quad (3)$$

where V_M and V_S are the mobile and stationary phase volumes, respectively, inside the CCC apparatus, and P_{app} is the distribution or partition ratio of the solute. IUPAC recommends to note it as K_D and to call it solute distribution ratio since it is not a constant, but depends on the physicochemical state of the solute [37]. Since the P notation and the "partition coefficient" denomination are tolerated, they will be used in this work because they are most commonly used in the chromatographic and analytical field.

At pH 3, cationic forms of the β-blockers and TAs dominate, and steroids are neutral. The molecular forms of sulfonamides dominate in the whole working pH range of a conventional C_{18} column. Sulfonamides, β-blockers and TAs have polarity, expressed in terms of $\log P_{o/w}$, of $(-0.8 < \log P_{o/w} < 1.5)$, $(-0.8 < \log P_{o/w} < 3.4)$ and $(3.9 < \log P_{o/w} < 5.3)$, respectively. These $\log P_{o/w}$ values, as well as the corresponding $\log P_{app}$ values at different pH, were measured by CCC in previous works [7,36], or in this work by applying Eqs. (1) or (2) and (3). All results are listed in Table 1. It can be clearly seen that the $\log P_{app}$ values for β-blockers and TAs are very different being three to five log units (=orders of magnitude) lower than their molecular corresponding values. There are no such differences for non-ionizable steroids, by definition. Also, at pH 3, the sulfonamides are mainly in their molecular form so that their $\log P_{app}$ value does not differ very much from their molecular $\log P_{o/w}$ value.

In the aqueous-organic mode, mobile phases containing high or moderate amounts of organic solvent were required to avoid extremely long retention times. The acetonitrile contents were in the following ranges: 15–25% for β-blockers, 25–50% for TAs, 10–30% for sulfonamides, and 40–80% for steroids.

Retention data (expressed as $\log k$) obtained for all the compounds with the aqueous-organic mobile phases were plotted against $\log P_{o/w}$ or $\log P_{app}$ in order to examine the quality of the correlations between retention and hydrophobicity. The correlation coefficients are listed in Table 2.

As expected, for sulfonamides, mainly in their neutral form at pH 3, and steroids, non-ionizable compounds, correlations with $\log P_{o/w}$ were satisfactory or acceptable. The use of apparent values for sulfonamides at this pH does not show significant changes and lead to very similar results. For β-blockers, correlations with $\log P_{o/w}$ were very poor. In this case, regression coefficients improved considerably when the apparent values were used. Fig. 1 shows the value of some of these correlations. However, the correlation coefficient of the $\log k$ versus $\log P_{o/w}$ lines obtained for the TAs compounds are acceptably good considering that these compounds are 99.99% in their cationic form. This means that the molecu-

Table 1
Dissociation constants and hydrophobicity of the studied compounds

Compounds	pK_{a1}[a]	Cation log $P^{+}_{o/w}$	pK_{a2}	Anion log $P^{-}_{o/w}$	Molecular log $P_{o/w}$	log P_{app} at pH 3
β-Blockers[b]						
Acebutolol	9.24	−2.1	–	–	1.83	−2.1
Alprenolol	9.34	−1.4	–	–	3.15	−1.39
Carteolol	9.24	−2.8	–	–	1.49	−2.8
Labetalol	6.20	−1.2	8.8	–	1.06	−1.2
Metoprolol	9.31	−2.1	–	–	1.90	−2.1
Nadolol	9.00	−2.6	–	–	1.00	−2.6
Oxprenolol	9.08	−1.8	–	–	2.30	−1.8
Propranolol	9.25	−1.1	–	–	3.41	−1.09
Sotalol	7.70	−2.9	9.0	–	−0.77	−2.9
Timolol	9.19	−1.8	–	–	1.98	−1.8
TAs[a]						
Amitryptiline	9.4	−0.53	–	–	4.64	−0.53
Clomipramine	9.4	0.47	–	–	5.30	0.47
Doxepin	9.0	−1.73	–	–	3.88	−1.73
Imipramine	9.5	−0.78	–	–	4.41	−0.78
Maprotiline	9.4	−0.66	–	–	4.22	−0.66
Nortryptiline	9.7	−0.70	–	–	4.32	−0.70
Trimipramine	9.5	−0.21	–	–	4.73	−0.21
Steroids[c]						
Clostebol acetate	n.i.	–	–	–	4.94	4.94
Medroxiprogesterone acetate	n.i.	–	–	–	4.22	4.22
Dydrogesterone	n.i.	–	–	–	3.35	3.35
Methyltestosterone	n.i.	–	–	–	3.36	3.36
Nandrolone	n.i.	–	–	–	2.62	2.62
Progesterone	n.i.	–	–	–	3.87	3.87
Testosterone propionate	n.i.	–	–	–	4.69	4.69
Testosterone	n.i.	–	–	–	3.32	3.32
Sulfonamides[d]						
Sulfacetamide	1.82	−1.9	5.85	−2.6	−0.19	−0.22
Sulfachlorpyridazine	1.72	−1.2	6.39	−2.2	0.71	0.69
Sulfadiazine	1.98	−1.6	6.01	−2.1	−0.06	−0.10
Sulfamerazine	2.16	−1.2	6.80	−2.2	0.11	0.05
Sulfamethiazine	2.46	−1.3	7.45	−2.1	0.27	0.16
Sulfamethizole	2.24	−1.2	5.30	−2.6	0.47	0.40
Sulfamethoxazole	1.81	−1.6	5.46	−2.1	0.85	0.82
Sulfanilamide	3.22	−1.1	10.61	−2.9	−0.77	−0.95
Sulfapyridine	2.37	−1.5	7.48	−1.5	0.03	−0.06
Sulfaquinoxaline	2.62	−1.3	6.00	−2.1	1.45	1.30
Sulfathiazole	2.06	−1.5	7.07	−2.2	−0.04	−0.09
Sulfasoxazole	2.15	−1.6	5.00	−2.5	0.81	0.75

Steroids are non-ionizable compounds; n.i.: non-ionizable.
[a] Refs. [34].
[b] Refs. [7].
[c] Refs. [35].
[d] Refs. [36].

lar $P_{o/w}$ value of the TAs compounds is correlated with their cationic $P^{+}_{o/w}$ value since, at pH 3, $P_{app} = P^{+}_{o/w}$. The correlation equation is:

$$\log P^{+}_{o/w} = 1.4 \log P_{o/w} - 6.9 \qquad (n = 7, r^2 = 0.914) \tag{4}$$

Detroyer et al. [6] have pointed out that a great difference between the pH of the experimental system and the pK_a of the basic compounds could lead to erroneous estimations of their log P_{app} from Eq. (1). In this case, molecular values are suggested to give acceptable correlations. However, the use of the corrected P_{app} value that takes into account the possible solute ionization is much better. The β-blockers show a significant degree of ionization at the working pH 3, evidenced by the several unit difference between the log P_{app} values and the log $P_{o/w}$ values (Table 1). Their retention is much better correlated with their P_{app} values than with their molecular $P_{o/w}$ values. The correction could be further improved using the actual dissociation constant values. Indeed, the solute pK_a values are slightly shifted in the mobile phases either by the organic modifier or by the surfactant.

In Fig. 2, the log k values are plotted versus the corresponding log P_{app} values (log $P_{o/w}$ for non-ionizable steroids) for

Table 2
Correlation coefficients of the log k vs. log $P_{o/w}$ (molecular coefficients) or log P_{app} (apparent partition coefficients) lines in the aqueous-organic mode at pH 3

Compounds	Acetonitrile (%)				
	10	15	20	25	30
Sulfonamides					
log k vs. log $P_{o/w}$	0.993	0.993	0.989	0.977	0.973
log k vs. log P_{app}	0.984	0.984	0.981	0.967	0.964
	40	50	60	80	
Steroids					
log k vs. log $P_{o/w}$	0.866	0.935	0.944	0.867	
	15	17	20	25	
β-Blockers					
log k vs. log $P_{o/w}$	0.789	0.794	0.776	0.776	
log k vs. log P_{app}	0.967	0.964	0.961	0.956	
	25	30	35	40	50
TAs					
log k vs. log $P_{o/w}$	0.929	0.938	0.970	0.963	0.972
log k vs. log P_{app}	0.994	0.997	0.992	0.999	0.992

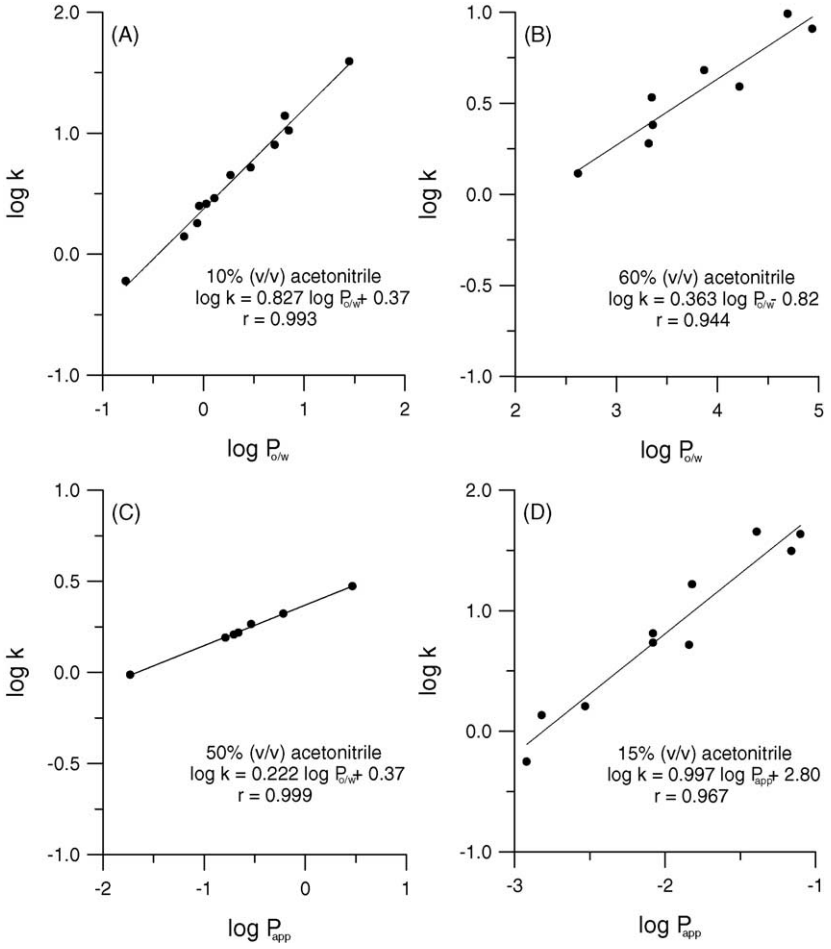

Fig. 1. Examples of regression lines obtained in RPLC with different classes of compounds. (A) Sulfonamides; (B) steroids (both are regression lines obtained with the molecular $P_{o/w}$ coefficient); (C) tricyclic antidepressants and (D) β-blockers (regression lines obtained with the P_{app} coefficient at pH 3).

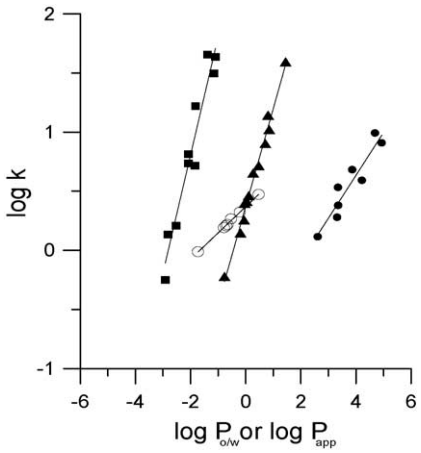

Fig. 2. Plot of log k vs. log P_{app} (or log $P_{o/w}$ for steroids and sulfonamides) in RPLC with acetonitrile/water mobile phases. β-Blockers with 15% acetonitrile (■); sulfonamides with 10% acetonitrile (▲); steroids with 60% acetonitrile (●) and TAs with 40% acetonitrile (○).

all the studied compounds. The lines with the best regression coefficients are shown.

The parameter log $P_{o/w}$, as a measure of the molecule hydrophobicity, can be related to the capability of the molecule to cross a biological membrane. Compounds with high molecular log $P_{o/w}$ will easily cross lipophilic biological membranes. Compounds with low molecular log $P_{o/w}$ value are expected to be polar and stay in aqueous media outside the cells. The ability of a compound to cross the blood–brain barrier (BBB) is difficult to estimate. It is one of the biological processes of fundamental importance in drug discovery and design [5,38–40]. Some of the studied β-blockers are able to cross the blood–brain barrier. They are noted BBB+. They are acebutolol, alprenolol, oxprenolol and propranolol. The six remaining β-blockers, carteolol, nadolol, metoprolol, labetalol, sotalol and timolol, are unable to cross the BBB. They are noted BBB− [38,40]. Considering the correlations obtained between log k and log $P_{o/w}$ or log P_{app} for the BBB+ and BBB− β-blockers, no obvious differences were observed. It seems, however, that the BBB+ molecular $P_{o/w}$ values are significantly higher than the BBB− ones (Table 1). It is recalled that the pH for the blood–brain barrier test is 7.4, not 3 (this study).

3.1.2. Micellar-organic mobile phases

Hybrid micellar mobile phases contain a surfactant and a low amount of an organic solvent. The anionic SDS surfactant and short chain alcohols, such as propanol with β-blockers, pentanol with TAs and steroids, or acetonitrile with sulfonamides, were used in this study. The same set of compounds was used to perform a QSRR study with micellar mobile phases. In MLC with charged surfactants, the solute retention is influenced by the net surface charge of the stationary phase, as well as by the nature of the micelle–solute interaction [41]. For basic compounds, both electrostatic and hydrophobic interactions are thus combined.

Results in MLC are given in Table 3. The first result is that the correlations established in MLC, for either log k versus log $P_{o/w}$ or log k versus log P_{app}, were always poorer than those obtained with aqueous-organic RPLC. This is striking for sulfonamides and steroids with correlation coefficients barely reaching 0.8 in MLC when they were above 0.97 (sulfonamides) or 0.87 (steroids) in classical RPLC. We note that these two families of compounds are in a molecular form at pH 3.

For the compounds mostly in their ionized forms (β-blockers and TAs), the correlation improvements obtained in classical RPLC (Table 2) are fully confirmed by MLC (Table 3). The correlation is still very poor for TAs ($r < 0.8$).

Table 3
Correlation coefficients of the log k vs. log $P_{o/w}$ (molecular coefficients) or log P_{app} (apparent partition coefficients) lines in the micellar-organic mode at pH 3

Compounds	Organic modifier[a] (%)–SDS (M)				
	6–0.025	0–0.025	0–0.125	6–0.125	3–0.075
Sulfonamides					
log k vs. log $P_{o/w}$	0.762	0.756	0.784	0.787	0.784
log k vs. log P_{app}	0.738	0.742	0.772	0.767	0.768
	4–0.10	7–0.10	6–0.12	4–0.20	7–0.20
Steroids					
log k vs. log $P_{o/w}$	0.808	0.805	0.782	0.766	0.796
	5–0.075	15–0.075	10–0.1125	5–0.15	10–0.15
β-Blockers					
log k vs. log $P_{o/w}$	0.789	0.784	0.797	0.781	0.791
log k vs. log P_{app}	0.939	0.929	0.931	0.923	0.937
	2–0.075	6–0.075	4–0.1125	2–0.15	6–0.15
TAs					
log k vs. log $P_{o/w}$	0.590	0.285	0.215	0.577	0.261
log k vs. log P_{app}	0.722	0.538	0.480	0.710	0.518

[a] Sulfonamides (acetonitrile), β-blockers (propanol), and TAs and steroids (pentanol).

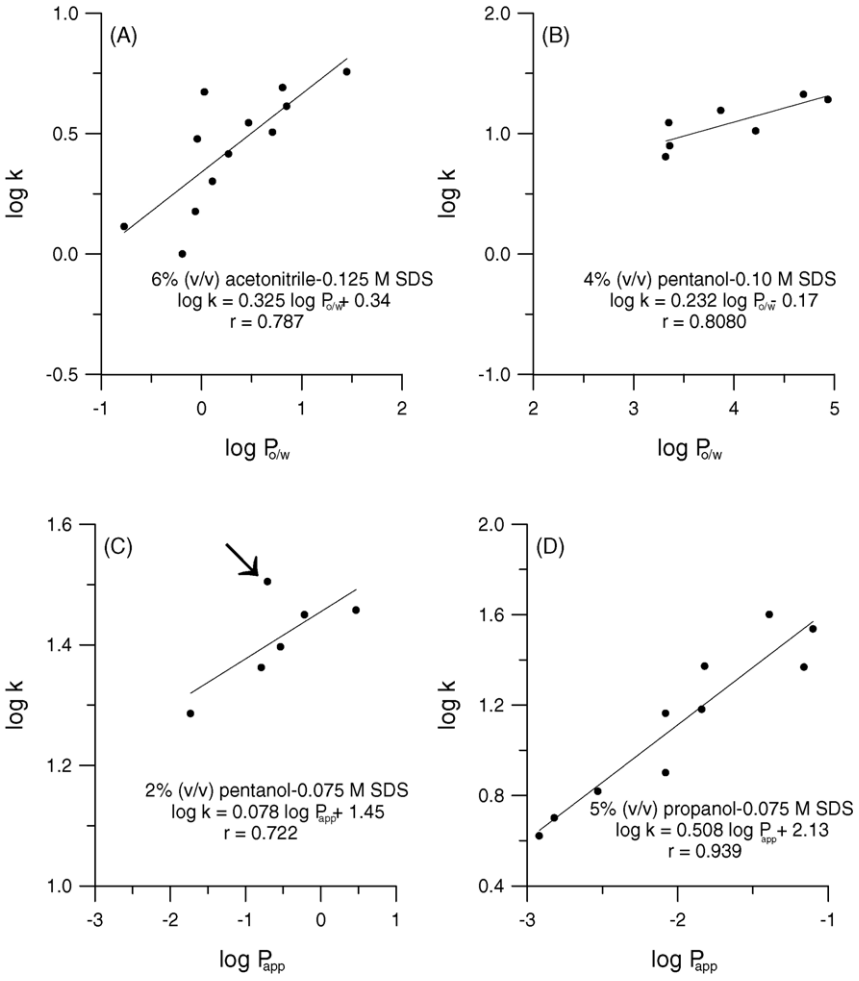

Fig. 3. Plot of $\log k$ vs. P_{app} (or $\log P_{o/w}$ for steroids and sulfonamides) with hybrid micellar mobile phases. (A) Sulfonamides; (B) steroids; (C) tricyclic antidepressants (arrow, nortrypiline); (D) β-blockers.

The correlations made with the actual P_{app} values are much better than those made with the molecular $P_{o/w}$ values ($r < 0.6$). Also, the coefficients obtained for β-blockers in MLC (Table 3) are almost as good as those obtained in classical RPLC (Table 2). Some of the plots obtained in MLC are shown in Fig. 3. It is also shown that the correlation coefficient can be dramatically decreased by a single outlier. The correlation coefficient of the TAs line increases from $r = 0.722$ to 0.912 when the nortrytiline point is removed (arrow in Fig. 3C). Otherwise, it should be noted that the TA maprotiline was highly retained with the two micellar mobile phases containing 2% pentanol and the experimental retention factors with these mobile phases were not available. Also, the steroid nandrolone, which in MLC elutes with the void volume, has not been included in the micellar correlation.

3.2. QSAR studies with several biological data

If there is a direct connection between retention in classical RPLC and the hydrophobicity of the molecule, then there is a correlation between retention and biological properties [42]. It was postulated that the membrane forming characteristics of anionic micelles could mimic physiological processes with phospholipidic cellular membranes. In both cases hydrophobic and electrostatic interactions are present. The literature gives examples in which retention in MLC has been used to predict biological activity [33,43,44]. Similar comparisons have been made concerning solute retention employing aqueous-organic mobile phases (QSAR). Such correlation study is tempted here with β-blockers, steroids and sulfonamides.

The permeation coefficient (K_s) of eight β-blockers through the natural membrane egg phospolipid liposomes (EPL) was correlated with $\log P_{o/w}$ in ref. [7], giving rise to the following model:

$$\log K_s(\text{EPL}) = 0.848 \log P_{o/w} + 0.262 \quad (n = 8, r^2 = 0.98) \qquad (5)$$

The EPL coefficient of the β-blockers considered in this study was then estimated using Eq. (5). These values were

Table 4
Correlation coefficients of the $\log k$ vs. $\log K_s$ (egg phospholipid liposomes) for β-blockers, $\log K_p$ (percutaneous absorption) for steroids and $\log (1/C)$ for sulfonamides in MLC and classical RPLC

Compounds	MLC-propanol (%)–SDS (M)				
	5–0.075	15–0.075	10–0.1125	5–0.15	10–0.15
β-Blockers					
$\log k$ vs. $\log K_s$	0.840	0.823	0.847	0.827	0.841
	HPLC-acetonitrile (%) (v/v)				
	15	17	20	25	
$\log k$ vs. $\log K_s$	0.823	0.830	0.815	0.819	
	MLC-pentanol (%)–SDS (M)				
	4–0.10	7–0.10	6–0.12	4–0.20	7–0.20
Steroids					
$\log k$ vs. $\log K_p$	0.751	0.774	0.750	0.588	0.628
	HPLC-acetonitrile (%)				
	50	60	80		
$\log k$ vs. $\log K_p$	0.815	0.860	0.880		
	MLC-acetonitrile (%)−SDS (M)				
	6–0.025	0–0.025	0–0.125	6–0.125	3–0.075
Sulfonamides					
$\log k$ vs. $\log(1/C)$	0.430	0.498	0.526	0.468	0.496
	HPLC-acetonitrile (%)				
	10	15	20	25	30
$\log k$ vs. $\log(1/C)$	0.704	0.705	0.707	0.686	0.689

correlated with retention in classical and micellar RPLC. From Table 4 (top row), it can be observed that the correlations with the EPL coefficients were very similar for both RPLC and MLC chromatographic modes.

Percutaneous absorption of human skin by chemical compounds is a rate process of considerable importance. The ability of a compound to penetrate the skin can be expressed as K_p in units of cm s^{-1}. Several equations have been proposed to predict K_p values [42]. Recently, Moss and Cronin [45] derived an equation for this parameter analyzing a large set of solutes, including steroids. The model describes skin permeability in terms of $\log P_{o/w}$ and molecular weight (MW):

$$\log K_p = 0.74 \log P_{o/w} - 0.0091\,MW - 2.39 \quad (n = 116, r^2 = 0.82) \qquad (6)$$

Approximated values of permeability for the steroids analyzed in this work were estimated using Eq. (6). The values were correlated with $\log k$ in classical and micellar modes (Table 4, middle row). It seems that classical RPLC retention represented the skin permeability better than micellar retention.

The following QSAR relationship was obtained for a miscellaneous group of drugs containing the sulfonamide group and acting on dog red cell enzyme [46]:

$$\log(1/C) = 0.35(\pm 0.28) \log P_{o/w} - 0.61(\pm 0.19) pK_a + 12.2(\pm 1.5) \quad (n = 7, r^2 = 0.953) \qquad (7)$$

This QSAR was also correlated with the retention of sulfonamides in the aqueous and micellar chromatographic modes although rather poorly. The results are also given in Table 4 (bottom row) and were again better for classical RPLC with r coefficients close to 0.7.

4. Conclusion

Retention data ($\log k$) of 10 β-blockers, 12 sulfonamides, seven TAs and eight steroids showing different acid–base properties were correlated with molecular and apparent octanol–water partition coefficients at pH 3, for aqueous-organic and SDS micellar-organic mobile phases using conventional or end-capped octadecylsilane columns.

For neutral solutes, chromatographic retention correlates well with the molecular coefficient $\log P_{o/w}$, but for ionizable basic compounds, the correction of $\log P_{o/w}$ using the $\log P_{app}$ value, which takes into account the possible partial ionization of the molecule, is necessary. Of course, this correction is less critical for compounds that are mainly in a molecular form such as the sulfonamide family of this study at pH 3. It is confirmed that, for basic compounds also, the $\log P_{app}$ or $\log P_{o/w}$ values are correlated with their ability as a pharmaceutical compound to cross a biological barrier.

It should be observed that, at the moment, all QSRR and QSAR studies just gave an idea of the possible biological activity of a compound. The measurement of solute hydrophobicity was tempted using non-octanol–water based methods. In RPLC, the $\log k_w$ parameter (extrapolated solute retention in pure water mobile phase) and φ_0 (extrapolated mobile phase composition giving a solute retention factor of unity) has been suggested [24,47–51]. In MLC, the use of non-ionic (Brij 35) micellar mobile phase was proposed to better mimic the biological media [29–31]. Unfortunately, none of these methods could improve significantly the quality of the correlations. It should be noted that the achievement of reliable QSRR predictions is such a worthy goal that continuous effort is reported in the literature. Recently, the use of IAM columns has been proposed as more appropriate models for biological partition processes, but different biomimetic chromatographic systems are necessary to characterize the wide variety of biological activity [6,15,18,19,50–53]. Last, it will be always better to use the actual pH and pK_a values in the working medium. Changes of pH and pK_a value due to organic additives and/or micelles are actively studied [54].

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