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Quantitative retention–structure and retention–activity relationships of barbiturates by micellar liquid chromatography

M. Cuenca-Benito, S. Sagrado, R.M. Villanueva-Camañas, M.J. Medina-Hernández*

Departamento de Química Analítica, Universidad de Valencia, C/Vicente Andrés Estellés s/n, E-46100 Burjassot, Valencia, Spain

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Abstract

Studies on the structural requirements of chromatographic surfaces to emulate in vitro the partitioning process in biomembranes are of great interest. The use of micellar mobile phases in RPLC modifies the hydrophobicity of the stationary phase and provides hydrophobic and electrostatic sites of interaction as a consequence of the adsorption of surfactant monomers to the chromatographic surface. Modified stationary phases in MLC could be structurally similar to biomembranes, but thorough studies are necessary to confirm this. In this paper we focus our attention on barbiturates. The influence of the nature and concentration of the surfactant (Brij 35, SDS and CTAB) and the mobile phase pH on the retention of 13 barbiturates in modified C_{18} stationary phases is studied. Quantitative structure–retention and structure–activity relationships for the barbiturates with different surfactants are proposed and compared with those obtained using hydro–organic mobile phases.

Keywords: Structure–retention relationships; Structure–activity relationships; Barbiturates

1. Introduction

The partitioning of a solute into lipid bilayers and biological membranes is the basis for drug and metabolite uptake, passive transport across membrane and bioaccumulation [1–4]. Of the other descriptors used in quantitative structure–activity (QSAR) studies, the hydrophobic parameter log *P*, the partition coefficient in the biphasic octanol–water solvent system, is most often used. However, the organic solvent–aqueous partitioning systems are good models for solute–membrane partitioning only when polar group interactions between the solute and the phospholipid bilayer are minimal or absent [5].

Reversed-phase liquid chromatography (RPLC) using octadecyl silica (ODS) as a stationary phase has been extensively used to estimate the hydrophobicity of compounds [6–9]. It has been argued [7,9] that the stationary phase–mobile phase systems adequately model the bio-partitioning process because the chemically bonded stationary phase resembles the hydrocarbon chains of the membranes much more than octanol. However, the use of ODS stationary phases presents two main limitations: (i) the interactions between solutes and the polar lipid head groups are not modeled and (ii) the density of the alkyl chains of almost all the commercial C_{18} columns is lower than the phospholipid bilayers (typically 5 $\mu mol/m^2$) [9–12].

It seems necessary to include polar groups in the chromatographic surfaces in order to emulate in vitro the partitioning process in the biomembranes. Re-

*Corresponding author.

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cently, Pidgeon and co-workers [13–15] developed the so-called immobilized artificial membranes (IAMs). The IAMs are chromatographic surfaces synthesized by covalently immobilizing phospholipids [single- or mixed-phosphatidylcholine (PC) ligands] to silica propyl amide particles. The ability of chromatographic surfaces containing interfacial polar groups, i.e., PC, OH and OCH_3, to mimic the biological membranes was studied and compared with the standard ODS column [14]. The authors concluded that differences in the interfacial polar functional groups do not eliminate the ability of the surface to predict drug–membrane interactions, but slightly better results were obtained using a PC column.

Another approach to studying the solute–membrane interactions is to obtain liposome partition coefficients from liposome suspensions of phospholipids [16]. However, although liposomes can model both polar and non-polar solute–membrane interactions, the method is time consuming and experimentally laborious.

Micellar liquid chromatography (MLC) is a mode of RPLC which uses a surfactant solution above the critical micellar concentration (cmc) as mobile phase [17,18]. The use of micellar solutions produces the adsorption of surfactant monomers to the stationary phase and increases the thickness of the stationary phase. The adsorbed amount of surfactant from micellar mobile phase is in the 4–5 $\mu mol/m^2$ range, and remains constant for surfactant concentration higher than the cmc [19–21]. Lavine and co-workers [22,23] studied the interactions of three ionic surfactants [sodium dodecyl sulfate (SDS), cetyltrimethylammonium bromide (CTAB) and DTAB] with the C_{18} and C_8 alkyl-bonded phases. The authors indicated that surfactant adsorption produces distinct changes in the selectivity of stationary phases because of the different nature of the surfactant monomer-bonded phase association. The hydrophobic adsorption of SDS surfactant monomers to the stationary phase leads to the formation of an anionic hydrophilic surface layer, the stationary phase becomes more hydrophilic. In contrast, for cationic surfactants (i.e., CTAB or DTAB) not only hydrophobic adsorption but also silanophilic adsorption takes place, leading to an increase in the hydrophobicity of the stationary phase and the formation of a cationic hydrophilic surface layer.

There are a number of similarities between the mobile phase–modified stationary phase in MLC and the membrane–water interface. The stationary phase modified by the adsorption of surfactant resembles structurally the ordered array of the membranous hydrocarbon chains. In addition, the hydrophilic/hydrophobic character of surfactants adsorbed could be expected to resemble the polar membrane regions. In consequence, the stationary phase provides both hydrophobic and electrostatic sites of interaction. Successful applications of MLC in quantitative retention–activity relationships have been reported previously [24–26]. Extensive studies are needed to establish the experimental conditions that allow mimicking of the biopartitioning of compounds into membranes. In this paper we focus our attention on barbiturates.

Barbiturates are used principally as hypnotics in the short-term treatment of insomnia, and pre-operatively to relieve anxiety and provide sedation [27]. Despite the widespread use of barbiturates and their potential for abuse, little is known about their neurochemical mechanisms of action. Barbiturates are capable of producing all levels of central nervous system (CNS) depression, from mild sedation and hypnosis to deep coma and death. The degree of depression depends upon the dosage, route of administration and pharmacokinetics of the particular barbiturate.

Hansch and Anderson [28] compared the ability of various barbiturates to induce some biological responses and they concluded that the hydrophobic character of the compounds but not their structures or acid properties could be related to their biological activities. However, there are differences among barbiturates that cannot be explained by differences in hydrophobicity. In fact, additional studies have shown that the potency of barbiturates also depends on the absorption, distribution, metabolism and degree of ionisation [29]. Specific drug–receptor interaction may also be involved in barbiturate action [30].

In this paper, quantitative retention–structure relationships of barbiturates are established. The influence of the nature (anionic, cationic and non-ionic) and surfactant concentration on the retention of barbiturates is studied. Finally, quantitative relationships between the retention of barbiturates and some hypnotic activities are examined.

2. Experimental

2.1. Instrumental and measurement

A Hewlett-Packard HP 1100 chromatograph with an isocratic pump, a UV–visible detector and an HP Vectra computer was used (Palo Alto, CA, USA). Data acquisition and processing were performed on an HP Vectra XM computer (Amsterdam, The Netherlands) equipped with HP-ChemStation software (A0402, 1996). The solutions were injected into the chromatograph through a Rheodyne valve (Cotati, CA, USA) with a 20-μl loop. Three independent Spherisorb octadecyl–silane ODS-2 C_{18} columns (5 μm, 120×4 mm) and the corresponding guard columns of similar characteristics (35×4 mm) (Scharlau, Barcelona, Spain) were used. The mobile phase flow-rate was 1 ml min^{-1}. The detection was performed in UV at 254 nm. All the assays were carried out at room temperature. The k values determined in this study were averages of at least triplicate determinations. The dead time value (average t_m=0.83 min) was determined for each injection as the first perturbation in the chromatogram.

2.2. Reagents and standard

Mobile phases were prepared by aqueous solutions of polyoxyethylene(23) lauryl ether (Brij 35, Acros, Geel, Belgium); SDS (Merck, Darmstadt, Germany) and CTAB (Acros). The pH of the micellar eluent was adjusted with 0.05 *M* phosphate buffer, prepared with disodium hydrogenphosphate and potassium dihydrogenphosphate (analytical reagent, Panreac, Barcelona, Spain). In order to reproduce the osmotic pressure of biological fluids, NaCl (9.20 g/l) (purism, Panreac) was added to the micellar mobile phase.

Amobarbital, aprobarbital, barbital, butalbital, butabarbital, butethal, 5,5-diallylbarbituric acid, hexobarbital, mephobarbital and pentobarbital (Sigma, St. Louis, MO, USA) were tested. Several Spanish pharmaceutical laboratories kindly donated: phenobarbital (Bayer, Barcelona), secobarbital and bralobarbital (UCB, Barcelona), pentobarbital (B. Braun Medical), butalbital (Sandoz, Barcelona).

Stock standard solutions of barbiturates were prepared by dissolving 10 mg of the compound in 10 ml of phosphate buffer. Working solutions were prepared by dilution of the stock standard solutions using a phosphate buffer solution. The solutions were stored in the refrigerator at 4°C.

Barnstead E-pure, deionized water (Sybron, Boston, MA, USA) was used throughout. The mobile phase and the solutions injected into the chromatograph were vacuum-filtered through 0.45-μm and 0.22-μm Nylon membranes, respectively (Micron Separations, Westboro, MA, USA).

2.3. Software and data processing

The log P values for the non-ionic forms of the barbiturates, and the protonation constants of the compounds were taken from the literature [31]. Excell 7.0 from Microsoft Office software was used to perform the statistical analysis of the multiple linear regression (MLR).

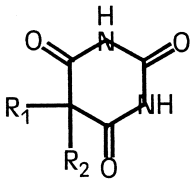
3. Results and discussion

3.1. Retention behaviour of barbiturates

Table 1 shows the structure, the logarithm of the protonation constants (log K) and the log P values for the non-ionic forms of the barbiturates studied. The basic structure common to these drugs is barbituric acid, a substance that has no CNS activity. Replacing the two hydrogens at position 5 with alkyl, alkenyl and/or aryl groups produces compounds with CNS activity. Other variations in this structure include the replacement of the hydrogen at the N1 position with a methyl group. The protonation constants of selected barbiturates ranged from 7.4 (phenobarbital) to 8.2 (hexobarbital). At physiological pH, 7.4, all compounds are negatively charged but the degree of ionization varies from one compound to another from 0.5 for phenobarbital to 0.14 for hexobarbital in aqueous solution.

The presence of an organised medium modifies the acid–base constants, log K, of the solubilized systems. This modification can be explained by the differences between the properties of the bulk solution and the micellar environment and by the electrostatic attractions and repulsions between the species involved and the micelle when both are charged. When cationic surfactants are used, a decrease of 0.5 to 3.0 in the log K value occurs. In contrast, for

Table 1
Structure, logarithm of protonation constant (log K) and log P values



General Structure

Compound	R_1	R_2	Log P	Log K[a]
Barbital	$-CH_2CH_3$	$-CH_2CH_3$	0.68	7.97
Diallyl barbituric	$-CH_2CH=CH_2$	$-CH_2CH=CH_2$	1.17	7.77
Aprobarbital	$-CH_2CH=CH_2$	$-CH(CH_3)_2$	1.27	7.99
Bralobarbital	$-CH_2CH=CH_2$	$-CH_2CBr=CH_2$	1.37	7.70
Phenobarbital	$-CH_2CH_3$	$-C_6H_5$	1.42	7.4
Hexobarbital[b]	$-CH_3$	$-C_6H_9$	1.49	8.2
Butabarbital	$-CH_2CH_3$	$-CH(CH_3)CH_2CH_3$	1.56	7.9
Butethal	$-CH_2CH_3$	$-(CH_2)_3CH_3$	1.65	7.5
Butalbital	$-CH_2CH=CH_2$	$-CH_2CH(CH_3)_2$	1.70	7.9
Mephobarbital[b]	$-CH_2CH_3$	$-C_6H_5$	1.85	7.8
Secobarbital	$-CH_2CH=CH_2$	$-CH(CH_3)(CH_2)_2CH_3$	1.97	7.90
Amobarbital	$-CH_2CH_3$	$-(CH_2)_2CH(CH_3)_2$	2.07	7.8
Pentobarbital	$-CH_2CH_3$	$-CH(CH_3)(CH_2)_2CH_3$	2.07	8.0

[a] Protonation constant of compounds in aqueous medium.
[b] With a methylene group ($-CH_3$) in N_1.

anionic surfactants, there is an increase of 0.5 to 3.0 in the log K value [32]. The log K values of phenobarbital, mephobarbital, butethal and 5,5-diallyl barbituric acid in 0.1 M SDS and 0.05 M CTAB solutions were determined potentiometrically. As can be expected, the log K values of these barbiturates increased in the presence of SDS micelles (7.6, 8.3, 7.8 and 8.11, respectively) and decreased in the presence of CTAB micelles (6.5, 7.3, 7.2 and 7.1, respectively) with respect to the

Table 2
Retention factors of the barbiturates in different mobile phases

Compound	0.15 M SDS		0.02 M Brij 35		0.05 M CTAB	
	pH 3.5	pH 7.4	pH 3.5	pH 7.4	pH 3.5	pH 7.4
Barbital	6.0	3.0	3.7	3.4	2.8	3.6
Diallyl	10.4	5.4	10.8	8.2	5.5	7.4
Aprobarbital	11.6	8.2	13.5	12.9	6.9	9.2
Bralobarbital	13.0	6.5	18.8	18.1	7.9	12.0
Phenobarbital	13.7	6.7	19.5	13.2	9.2	12.4
Hexobarbital	14.8	19.3	16.4	17.2	14.4	18.9
Butabarbital	16.1	10.4	17.4	18.2	8.3	10.1
Butetal	17.8	10.9	19.2	19.3	8.7	11.1
Butalbital	18.8	11.5	23.1	21.0	9.3	12.5
Mephobarbital	22.2	18.2	42.7	26.4	17.2	24.3
Secobarbital	25.5	18.1	40.4	40.0	13.5	18.6
Amobarbital	28.5	15.4	34.0	29.8	11.6	15.3
Pentobarbital	28.5	16.4	53.2	53.0	16.2	36.1

corresponding log K values in aqueous media (see Table 1).

In order to study the influence of the nature of the surfactant and mobile phase pH on the retention of barbiturates, the retention of compounds was measured using mobiles phases containing a non-ionic surfactant, Brij 35, an anionic surfactant, SDS, and a cationic surfactant, CTAB. The mobile phase pH was adjusted to 7.4 and 3.5. Table 2 shows the effect of the mobile phase pH on the retention behaviour of barbiturates. As can be observed, when SDS was used as the eluent the retention factor of the compounds decreased as the mobile phase pH increased. At pH 3.5 the predominant form of the compound is non-ionic, while at pH 7.4 the compounds are partially ionized and, consequently, less retained due to the electrostatic repulsion between the charged compounds and the surfactant monomers absorbed into the stationary phase. In contrast, when CTAB was used as the eluent the retention factors of barbiturates increase as the mobile phase pH increases due to the electrostatic attractions between

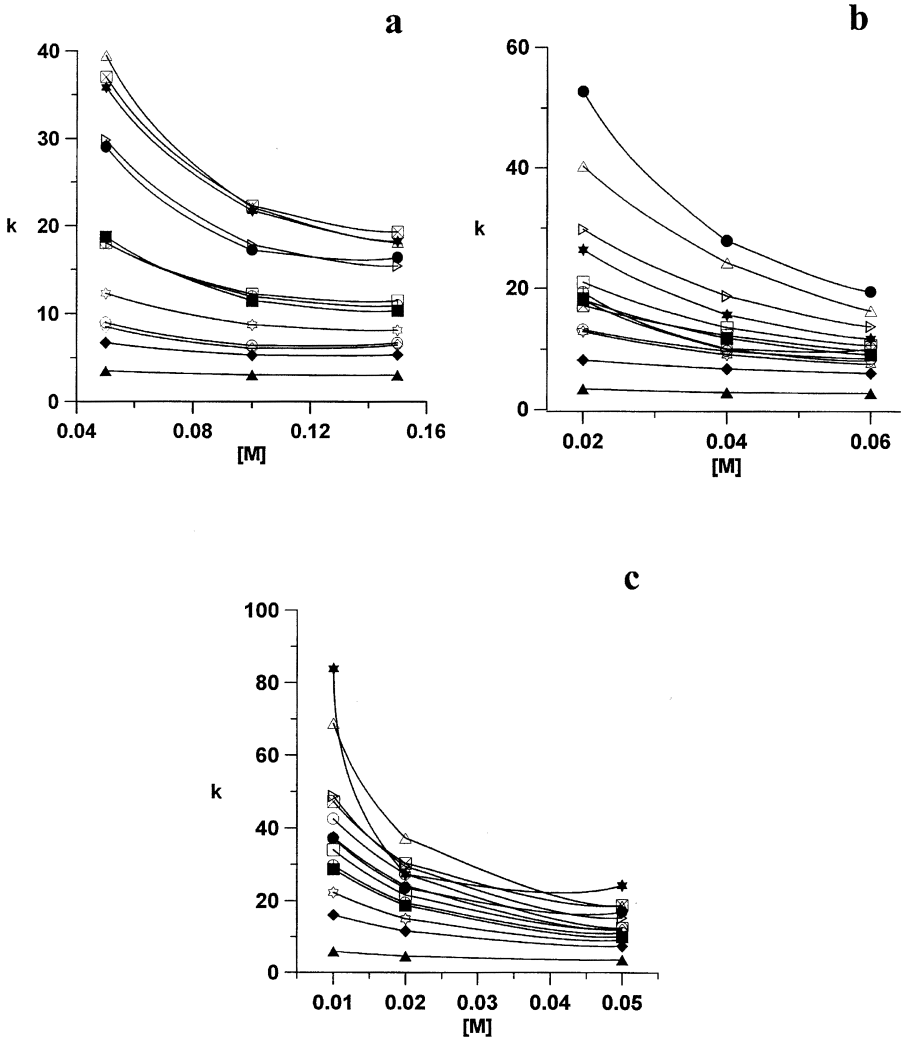


Fig. 1. Effect of surfactant concentration in mobile phase on the retention of barbiturates: (a) SDS, (b) Brij 35, (c) CTAB. Barbital (▲), diallyl (♦), bralobarbital (◇), phenobarbital (○), aprobarbital (☆), butalbital (□), butethal (⊗), butabarbital (■), pentobarbital (●), amobarbital (▷), mephobarbital (★), hexobarbital (⊠), secobarbital (△).

the negatively charged compounds and the modified stationary phase. When a non-ionic surfactant such as Brij 35 was used as micellar mobile phase the hydrophobicity of stationary phase increased, but remains uncharged. Consequently, as can be observed in Table 2, the retention of barbiturates, except mephobarbital and phenobarbital, was generally independent of the mobile phase pH.

Fig. 1 shows the effect of the Brij 35, SDS and CTAB concentration in the mobile phase on the retention of barbiturates. In all cases, the mobile phase pH was adjusted to 7.4 to obtain experimental conditions as close as possible to the physiological pH. For the highly hydrophobic compounds studied (secobarbital, amobarbital and pentobarbital), large changes in the retention were obtained upon increasing the surfactant concentration in the mobile phase, while for the slightly hydrophobic compounds (barbital and diallyl barbituric acid) the retention was scarcely modified. This behaviour indicates, as expected, that the eluent strength of the surfactant increases as the hydrophobicity of the compounds increases.

The use of SDS as micellar mobile phase provided higher retention values of compounds than those obtained with similar micellar concentrations of Brij 35 and CTAB (for example, the retention factors obtained for secobarbital were 39.5, 16.3 and 15.3 for 0.05 *M* SDS, 0.06 *M* Brij 35 and 0.05 *M* CTAB mobile phases, respectively). For barbiturates, the eluent strength of CTAB was larger than that corresponding to Brij 35 and SDS.

The retention factors at zero micellar concentration, k_m, and the solute–micelle association constants, K_{AM}, of barbiturates at pH 7.4 in pure micellar mobile phases were calculated by adjusting the pairs of data (k, $[M]$) to the following equation:

$$\frac{1}{k} = \frac{1}{k_m} + \frac{K_{AM}}{k_m}[M] \quad (1)$$

where k is the capacity factor and $[M]$ is the total concentration of surfactant in the mobile phase minus the critical concentration (cmc). Table 3 shows the k_m and K_{AM} values obtained for the barbiturates studied eluted with Brij 35, SDS and CTAB. The fact that the results were in concordance with those obtained by applying two other regression models [33] increases the reliability of the estimates. As can be expected, the k_m and K_{AM} values increased as the hydrophobic character of the compounds increased.

The k_m and K_{AM} values of the compounds depend on the physicochemical properties of the solutes. In consequence, if the solute properties contribute in a similar way to the k_m and K_{AM} values, a linear relationship between log k_m and log K_{AM} should exist. When Brij 35 and SDS were used, adequate correlations were obtained ($r^2 = 0.96$ and 0.94, respectively), indicating the existence of structural similarities between compounds. However, for

Table 3
k_m and K_{AM} values for the studied barbiturates eluted with different surfactants

	SDS		Brij 35		CTAB	
	k_m	K_{AM}	k_m	K_{AM}	k_m	K_{AM}
Barbital	3.7±0.3	1.6±1.0	3.8±0.4	7±3	6.5±0.7	18±6
Diallyl barbituric	7±1	3±2	9.9±0.5	11±2	21±2	38±8
Aprobarbital	17±4	8±4	20±2	28±6	31±4	51±13
Brallobarbital	9±2	3±3[a]	40±20	70±40	72±2	103±4
Phenobarbital	10±3	3.4±3.7[a]	18±2	20±5	91±14	130±30
Hexobarbital	60±20	15±9	27.9±0.8	32±2	67±13	50±20
Butabarbital	30±10	12±8	36±3	50±6	48±4	79±11
Butetal	25±7	9±5	24±17	20±30[a]	46±6	65±14
Butalbital	23±6	7±4	45.7±0.5	59±1	53±9	70±20
Mephobarbital	60±20	17±8	70±9	85±15	60±80[a]	20±100[a]
Secobarbital	80±40	25±16	130±30	120±30	160±50	160±60
Amobarbital	50±20	16±9	70±2	70±3	93±15	100±20
Pentobarbital	40±20	10±9	380±90	320±80	44±11	30±20

[a] Statistically non-significant.

CTAB the correlation was not adequate ($r^2 = 0.56$). Hexobarbital, mephobarbital and pentobarbital showed more affinity for the modified stationary phase than for the micellar mobile phase. This behaviour could be explained by the fact that the localisation of these solutes in the CTAB micelle is different, probably because of steric factors.

3.2. Retention–structure relationships

The possibility of predicting the retention behaviour of compounds from the physicochemical properties and experimental conditions is an interesting aspect of quantitative structure–retention (QSRR) studies. Prior to the study of the regression models, an exploratory data analysis was carried out. Principal component analysis (PCA) was applied to the retention data of thirteen barbiturates obtained with different concentrations of SDS (variables 1–3), Brij 35 (variables 4–6) and CTAB (variables 7–9) and several molecular descriptors [34], in order to establish the relationships between variables. The molecular descriptors used are log P (variable 10) as the hydrophobic parameter, polarizability (variable 15) and the molar fraction of the charged form of the compounds (δ, variable 11) as electronic parameters, and molar refractivity (variable 12), molar volume (variable 13) and parachor (variable 14) as steric descriptors. Because the variables are in different scales, the data were autoscaled before applying the PCA model. Table 4 shows the explained variance corresponding to each principal component. Two

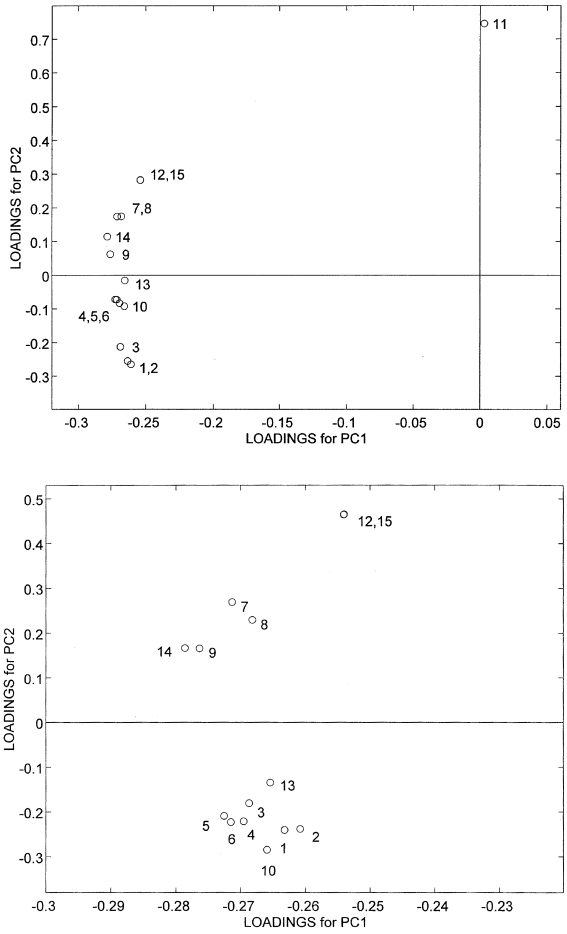


Fig. 2. Loading plots corresponding to the PCA analysis. (Numbers correspond to variables, see Section 3.2).

Table 4
Explained variance corresponding to each principal component

No. PCs	Eigen value	% Variance explained	% Accumulated explained variance
1	12.4	82.46	82.46
2	1.62	10.80	93.26
3	0.629	4.19	97.45
4	0.179	1.19	98.65
5	$8.07 \cdot 10^{-2}$	0.54	99.18
6	$7.33 \cdot 10^{-2}$	0.49	99.67
7	$2.68 \cdot 10^{-2}$	0.18	99.85
8	$1.32 \cdot 10^{-2}$	0.09	99.94
9	$6.39 \cdot 10^{-3}$	0.04	99.98
10	$1.90 \cdot 10^{-3}$	0.01	99.99

principal components (PCs) explain more than 93.26% of the variance. The use of the first four latent variables accounts for more than 98% of the variance in the data. The PCA model analyzed was obtained with four PCs. Both the qualitative (score plot) and quantitative (confidence limits for the Hotelling T^2 and Q statistics; 95% confidence level) studies of the object outliers revealed the absence of out-of-control objects [35]. Fig. 2 upper part, shows the loading plot corresponding to the first two principal components. As can be observed, the retention of compounds obtained in Brij 35 and SDS (variables 1–6) is highly correlated with the log P (variable 10) and molar volume (variable 13), whereas the log k values obtained with CTAB (variables 7–9) related to parachor (variable 14). Two groups of uncorrelated variables can be observed, the first one related to the first PC, which includes log P, molar volume, parachor, molar refractivity and polarizability, and the second group related to the second PC constituted by variable δ (variable 11). When the PCA model was repeated without variable 11 (Fig. 2 lower part), few changes were observed in the relative position of variables in the loading plot. This is consistent with the fact that the PCs are orthogonal and therefore uncorrelated. Because principal components are orthogonal, it could be expected that the retention of barbiturates might be explained by means of bivariate models like:

$$\log k = a + b(\text{associated variable to PC1}) + c(\text{associated variable to PC2}) \quad (2)$$

The score plot (not shown) of the first two PCs revealed that the first PC reflects the compounds hydrophobicity; the low hydrophobic compounds show large positive PC1 scores while the high hydrophobic compounds show large negative PC1 scores. On the other hand, the phenobarbital with the largest ionization degree value shows a large positive PC2 score. These results are in agree with the corresponding loading plot. The score and loading plots, corresponding to the third and fourth PCs (not shown) revealed the importance of the compounds phenobarbital and bralobarbital (with high ionization degree values) and hexobarbital (with low ionization degree values) and variable 11, respectively.

In a previous paper a novel retention model for ionic compounds with different degrees of ionization that includes the hydrophobicity of compounds and the molar fraction of the charged form of the compounds was proposed [26].

$$\log k = a\log P + b\delta + c \quad (3)$$

The δ value, the molar fraction of the charged form of the compound, for barbiturates, can be calculated as:

$$\delta = 1/(1 + K[H^+]) \quad (4)$$

where K is the protonation constant in the micellar medium. According to Eq. (2), for pH values far from log K, differences in retention are only due to differences in hydrophobicity.

In order to study how well the model fits the

Table 5
Influence of mobile phase composition on the log k–log P relationships

		log $k = a\log P + b\delta + c$					
Surfactant	[M]	$a \pm ts_a$	$b \pm ts_b$	$c \pm ts_c$	r	S_e	F
Brij 35	0.02	0.73±0.07	−0.13±0.31[a]	0.14±0.14	0.96	0.08	226
Brij 35	0.04	0.62±0.06	−0.06±0.25[a]	0.11±0.11	0.96	0.07	243
Brij 35	0.06	0.52±0.04	−0.003±0.2[a]	0.16±0.09	0.97	0.05	275
SDS	0.05	0.72±0.10	−1.1±0.5	0.37±0.10	0.93	0.12	113
SDS	0.1	0.60±0.09	−1.0±0.4	0.36±0.18	0.92	0.11	104
SDS	0.15	0.51±0.08	−0.7±0.3	0.40±0.15	0.92	0.09	102
CTAB	0.01	0.62±0.13	0.7±0.6	0.37±0.26	0.85	0.15	48
CTAB	0.02	0.50±0.10	0.5±0.5[a]	0.4±0.2	0.85	0.12	48
CTAB	0.05	0.46±0.09	0.1±0.4[a]	0.33±0.19	0.85	0.12	57

[a] Statistically non-significant.

experimental data, the retention of barbiturates obtained for different concentrations of Brij 35, SDS and CTAB in mobile phase, log k, the δ values at physiological pH, and the log P values were adjusted to Eq. (2) by applying multiple linear regression. Table 5 shows the regression statistics obtained. As can be observed, the correlations obtained for SDS and Brij 35 were adequate ($r>0.9$). For Brij 35, the fitting parameters associated with the molar fraction of the charged form of the compounds (b coefficients) were not statistically significant. This may be due to the absence of attractive–repulsive electrostatic interactions between compounds and non-ionic surfactant and the narrow range of the δ values. It was found that for Brij 35 univariate models (log k–log P) gave adequate regression statistics. However, for SDS, the b coefficients were statistically significant and negative, due to the electrostatic repulsion between negatively charged compounds and modified stationary phase.

Poor correlations were obtained for CTAB. Using the retention factors of barbiturates at pH 3.5, the coefficient of correlation was also low ($r<0.9$). These results are in agreement with the PCA results shown above, which indicated low correlations between retention and hydrophobicity for CTAB.

On the other hand, the fitting parameter related to the log P values (a coefficient) for all the surfactants studied decreases as the surfactant concentration in the mobile phase increases, which indicates that the system is less sensitive to hydrophobicity the larger the micellar concentration.

It is also possible to find a global model of the type $\log k = a\log P + b\delta + c[M] + d$, which makes it possible to predict retention in MLC (log k) as a function of the structural parameters (log P and log K) and the experimental conditions (surfactant concentration and mobile phase pH). In addition, it is possible to obtain hydrophobicity predictive models of the type $\log P = a\log k + b\delta + c[M] + d$. Table 6 shows the statistics of MLRs obtained using the retention data of barbiturates at three micellar concentrations together with the log P, δ and [M] values ($n=39$). For Brij 35 and CTAB, δ values were not considered. As can be observed, the best results were obtained when Brij 35 was used as the micellar mobile phase.

3.3. Quantitative retention–activity relationships of barbiturates

The relationships between retention in MLC and some anaesthetic actions of barbiturates, minimum effective hypnotic dose (mol/kg) in rabbits ($-\log C$), molar drug concentration necessary to reduce cell division ($-\log ED$) and molar drug concentration required to reduce 50% inhibition of oxygen on rat brain respiration in vitro ($-\log O$) [12] were examined.

Table 7 shows the statistical parameters of the relationships between the mentioned biological activity of barbiturates found in the bibliography and their retention data (log k) obtained using 0.02 M Brij 35, 0.05 M SDS (Fig. 3) and 0.01 M CTAB as micellar mobile phases. The results were compared with those obtained from the log k_w values, reported in Ref. [12], using a C_{18} column and MeOH as

Table 6
Global predictive models for barbiturates ($n=39$)

Surfactant	a (ts_a)	b (ts_b)	c (ts_c)	d (ts_d)	r	S_e
	$\log k = a\log P + b\delta + c[M] + d$					
Brij 35	0.62 (0.06)	–	−7 (2)	0.39 (0.12)	0.96	0.08
SDS	0.62 (0.10)	−0.9 (0.4)	−2 (0.9)	0.6 (0.2)	0.92	0.11
CTAB	0.53 (0.13)	–	−10 (3)	0.7 (0.2)	0.88	0.14
	$\log P = a\log k + b\delta + c[M] + d$					
Brij 35	1.45 (0.16)	–	10 (3)	−0.4 (0.2)	0.95	0.12
SDS	1.3 (0.2)	3 (1)	3 (1)	−0.4 (0.4)[a]	0.90	0.17
CTAB	1.3 (0.3)	–	13 (5)	−0.4 (0.5)[a]	0.81	0.23

[a] Statistically non-significant.

Table 7
Linear correlation between biological activities (variable 1) and either log k_w value or octanol–water partition coefficient (variable 2) for selected barbiturates

	Variable 1 = *a* Variable 2 + *b*														
	log *k*												log *P*		
	0.05 *M* SDS			0.02 *M* Brij 35			0.01 *M* CTAB			MeOH (log k_w)					
Variable 1	*a* (ts_a)	*b* (ts_b)	*r*	*a* (ts_a)	*b* (ts_b)	*r*	*a* (ts_a)	*b* (ts_b)	*r*	*a* (ts_a)	*b* (ts_b)	*r*	*a* (ts_a)	*b* (ts_b)	*r*
−Log *C*	0.8 (0.3)	2.7 (0.3)	0.92	0.8 (0.2)	2.7 (0.3)	0.93	0.7 (0.5)	2.6 (0.8)	0.74	0.6 (0.2)	2.4 (0.4)	0.92	0.6 (0.2)	2.7 (0.4)	0.88
−Log *DE*	2.1 (0.1)	–[a]	0.94	1.9 (0.2)	–[a]	0.90	1.7 (0.1)	–[a]	0.83	1.11 (0.08)	–[a]	0.96	1.5 (0.2)	–[a]	0.88
−Log *O*	2.1 (0.1)	–[a]	0.99	1.9 (0.2)	–[a]	0.95	1.7 (0.3)	–[a]	0.82	1.11 (0.08)	–[a]	0.97	1.5 (0.2)	–[a]	0.96

[a] Statistically non-significant.

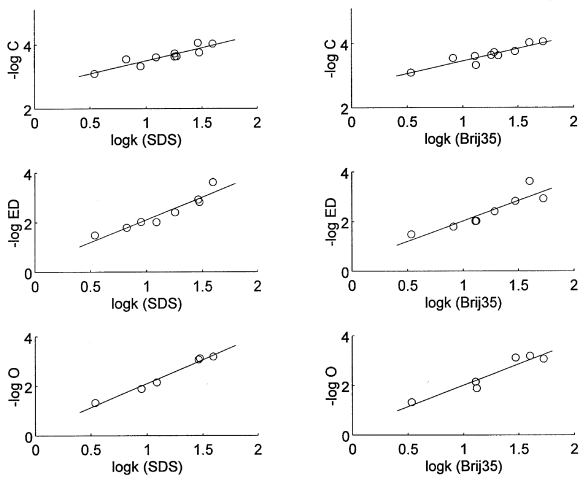


Fig. 3. QRAR models obtained with 0.05 *M* SDS mobile phase (left side) and 0.02 *M* Brij 35 (right side).

eluent and with the log *P* values (classic QSAR model). As can be observed, the QSAR models obtained from chromatographic retention data using SDS, Brij 35 and MeOH as eluents were good. In contrast, the QSAR models obtained for CTAB were not adequate ($0.74<r<0.83$), nor did the QSAR model obtained from log *P* values for log *C* and log *DE* biological activities provide adequate correlation coefficients. However, when the ionization degree, δ, was incorporated into the regression (log[biological activity]$=a\log P+b\delta+c$), the correlation coefficients improved ($r=0.93$, 0.91 and 0.99 for $-\log C$, $-\log DE$ and $-\log O$, respectively), and were similar to those obtained for SDS, Brij 35 and MeOH. This confirms that the ability of barbiturates to induce some biological responses not only depends on the hydrophobic character of the compounds but also on the degree ionization [29].

The results obtained in this work suggest that the retention data obtained with SDS, Brij 35 and MeOH contain enough information to describe some of the biological activities of barbiturates, probably because the information on hydrophobicity and the degree of ionization is already incorporated into the retention data.

It has been suggested that specific drug–receptor interaction must be involved in the action of barbiturates [29,30]. The high correlations obtained with Brij 35, SDS and MeOH and the low correlations obtained with CTAB seem to indicate that attractive electrostatic interactions are not involved in the neurochemical mechanism of barbiturate action. If we take into account the fact that the effect produced for the absorption of SDS, Brij 35 and MeOH on the hydrophobic stationary phase is the introduction of polar groups with a strong hydrogen bond donor character, it would seem that interactions of this type could participate in the neurochemical mechanism of action of these compounds.

4. Conclusions

For barbiturates, the eluent strength of CTAB was greater than that of Brij 35 and SDS. For Brij 35 and SDS, structural similarities between compounds were observed.

A bivariate retention model, which includes the hydrophobicity and the molar fraction of the charged form of compounds, proved to be valid for barbiturates when SDS was used as surfactant. For Brij 35, univariate log *k*–log *P* models produced adequate results, while for CTAB inadequate results were obtained using both models. Using Brij 35 as surfactant, it is possible to predict the retention of barbiturates as a function of physicochemical parameters and experimental variables and predict the hydrophobicity from retention data and experimental variables.

A single retention parameter, log *k*, obtained at physiological pH is capable of describing some of the biological activities of barbiturates. The QSAR models obtained from chromatographic retention data of barbiturates eluted with SDS, Brij 35, CTAB and MeOH confirmed that the ability of barbiturates to induce some biological responses not only depends on the hydrophobic character of the compounds but also on the degree of ionization.

The high correlations obtained with Brij 35, SDS and MeOH and the common characteristics of these eluents could indicate that specific interactions are involved in the neurochemical action mechanism of barbiturates.

The use of micellar mobile phases in RPLC is a simple alternative for predicting the biological activities of compounds. The system does not require the use of special columns because the stationary

phase modified by adsorption of surfactant provides both hydrophobic and polar action sites like those of biological membranes. In addition, identifying the partitioning mechanism in MLC could help to elucidate the mechanism of action of drugs in the body.

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References

[1] C. Hansch, in: E.J. Aries (Ed.), Drug Design, Vol. 1, Academic Press, New York, 1971.
[2] L. Goldberg (Ed.), Structure–Activity Correlation as a Predictive Tool in Toxicology, Hemisphere, Washington, DC, 1983.
[3] C. Hansch, A. Leo, Substituent Constants for Correlation Analysis in Chemistry and Biology, Wiley-Interscience, New York, 1987.
[4] C. Hansch, T. Fujita, J. Am. Chem. Soc. 86 (1964) 1616.
[5] J.A. Rodgers, A. Wong, Int. J. Pharm. 6 (1980) 339.
[6] R. Kaliszan, Quantitative Structure–Activity Chromatographic Retention Relationships, Wiley, New York, 1987.
[7] J.G. Dorsey, M.G. Khaledi, J. Chromatogr. A 656 (1993) 485.
[8] T. Braumann, J. Chromatogr. 373 (1986) 191.
[9] W.J. Lambert, J. Chromatogr. A 656 (1993) 469–484.
[10] K.A. Dill, J. Naghizadeh, J.A. Marqusee, Anuu. Rev. Phys. Chem. 39 (1988) 425–461.
[11] J.G. Dorsey, R.A. Dill, Chem. Rev. 89 (1989) 331–346.
[12] M.M. Hsieh, J.G. Dorsey, Anal. Chem. 67 (1995) 48.
[13] S. Ong, H. Liu, X. Qui, G. Bhat, C. Pidgeon, Anal. Chem. 67 (1995) 755.
[14] H. Liu, S. Ong, L. Glunz, C. Pidgeon, Anal. Chem. 67 (1995) 3550.
[15] S. Ong, H. Liu, C. Pidgeon, J. Chromatogr. A 728 (1996) 113.
[16] Y.W. Choi, J.A. Rogers, Pharm. Res. 7 (1990) 508.
[17] D.W. Armstrong, F. Nome, Anal. Chem. 53 (1981) 1662.
[18] M. Arunyanart, L. Cline-Love, Anal. Chem. 56 (1984) 1557.
[19] J.G. Dorsey, M.G. Khaledi, J.S. Landy, J.L. Lind, J. Chromatogr. 342 (1984) 130.
[20] A. Berthod, I. Girard, C. Gomet, Anal. Chem. 58 (1986) 1356.
[21] M.J. Medina-Hernández, M.C. García Alvarez-Coque, Analyst 117 (1992) 831.
[22] B.K. Lavine, S. Hendaya, J. Tetreault, Anal. Chem. 66 (1994) 3458.
[23] B.K. Lavine, S. Hendaya, J. Liq. Chromatogr. 20 (1997) 351–376.
[24] E.D. Breyer, J.K. Strasters, M.G. Khaledi, Anal. Chem. 63 (1991) 828.
[25] J.M. Sanchis Mallols, R.M. Villanueva Camañas, S. Sagrado, M.J. Medina-Hernández, Chromatographia 46 (1997) 605.
[26] L. Escuder Gilabert, S. Sagrado, R.M. Villanueva Camañas, M.J. Medina-Hernández, Anal. Chem. 70 (1998) 28.
[27] G.K. McEvoy (Ed.), Drug Information, American Hospital Formulary Service, 1997.
[28] C. Hansh, S.M. Anderson, J. Med. Chem. 10 (1967) 745–753.
[29] K.I. Ho, R.A. Harris, Annu. Rev. Pharmacol. Toxicol. 21 (1981) 83–111.
[30] J. Florez, in: Farmacologia Humana, Masson, Barcelona, 1996.
[31] C. Hansch, in: Comprehensive Medicinal Chemistry, Vol. 6, Pergamon Press, New York, 1990.
[32] O.A. El Seoud, Adv. Colloid. Interface Sci. 30 (1989) 1.
[33] F. Argiles, S. Sagrado, M.J. Medina-Hernández, J. Chromatogr. A 778 (1997) 67.
[34] ACD/logP Method Software (Demo version), Advanced Chemistry Development Inc., Toronto, Canada, 1996.
[35] F. McLenan, B.R. Kowalski, Process Analytical Chemistry, Chapman and Hall, London, 1995.