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Preliminary communication

Estimation of the effect of the acidosis and alkalosis on the anesthetic potency of local anesthetics by biopartitioning micellar chromatography and micellar electrokinetic chromatography

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Abstract

Local anesthetics are hydrophobic compounds and weak bases with protonation constants ranged between 7.5 and 8.8. These drugs block reversibly nerve conduction near their site of application or injection and thus produce temporary loss of feeling or sensation in a limited area of the body. The efficacy of anesthetic blockade of local anesthetics depends on the charged/uncharged form ratio and the hydrophobicity of the compounds. In addition their toxicological effects have been reported to be highly dependent on the physiological pH. Biopartitioning micellar chromatography (BMC) and micellar electrokinetic chromatography (MEKC), that use micellar solutions as mobile phases, have proven to be useful for describing the biological behavior of different kind of compounds. In this paper, relationships between the retention data in BMC and MEKC using Brij35 as surfactant (at pH 7.4) and some pharmacodynamic parameters of local anesthetics are obtained. These models are compared with those obtained using an immobilized artificial column (IAM). Finally, the effect of the corporal pH in situations of acidosis and alkalosis on the pharmacological and toxicological properties of local anesthetics is studied using the retention of compounds in BMC at different mobile phase pH values.

Keywords: Retention–activity relationships; Biopartitioning micellar chromatography; Micellar electrokinetic chromatography; Immobilized artificial chromatography; Local anesthetics

1. Introduction

Local anesthetics are drugs that block reversibly nerve conduction when they are applied locally to nerve tissue at appropriate concentrations. They act on any part of the nervous system and on every type of nerve fiber. In contact with a nerve trunk, these anesthetics can cause both sensory and motor paralysis in the innervated area. Nearly all local anesthetics act by reducing the tendency of voltage-dependent sodium channels to activate. They are commonly used not only in the peripheral nervous system but also for spinal anesthesia. Adverse effects of local anesthetics usually result from high plasma concentrations of drug, initially produce central nervous system (CNS) stimulatory effects followed by CNS depression and adverse cardiovascular effects, caused by excessive dosage, excessive rate of injection, slow metabolic degradation or injection into highly vascular tissue.

Determination of the structural features that control local anesthetic activity is particularly difficult, because this is an activity possessed by many molecules. However, there are some common structural features in the local anesthetic molecules [1]. Local anesthetics are hydrophobic compounds and weak bases with protonation constants ranged between 7.5 and 8.8, which permits them to exist in both ionized and non-ionized forms at physiologic pH 7.4. In the body, it is the uncharged form that penetrates the lipid membrane of the axon, but it is the charged molecule that binds inside the channel to produce the anesthetic action. Thus, the

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charged/uncharged form ratio and the hydrophobicity of the compound are limiting factors of the efficacy of anesthetic blockade. On the other hand, the pharmacological and toxicological effects of local anesthetics have been reported to be highly dependent on the physiological pH.

The corporal pH is regulated by lungs and kidneys. Variation in the pH alters the degree of ionization of proteins and many drugs. As most ionized substances do not cross cell membranes readily, alterations in pH affect both cellular function and the potency of many pharmaceutical agents. Relative acidity of tissues for example in the vicinity of an abscess is recognized to reduce the efficacy of local anesthetic solutions. Conversely relative alkalinity enhances the uptake of local anesthetic solutions by the increase of the uncharged fraction of compounds.

The dynamic pharmacokinetic/pharmacodynamic processes of drug action are considered to have much in common with the processes that are the basis of chromatographic separations of drugs. In adequate experimental conditions, the same basic properties (hydrophobic, electronic and steric) determine the behavior of chemical compounds in both the biological and chromatographic environment. In addition, none of the essential chromatographic or pharmacokinetic/pharmacodynamic processes except metabolism implies the breaking or the formation of bonds in the drug [2]. Therefore, chromatography can be used as a powerful technique for estimating physicochemical parameters and biological activities. In addition, chromatographic techniques are dynamic systems that permit the strict control of experimental conditions and the obtaining of very reproducible retention data.

The application of chromatographic parameters in structure–activity relationships give rise to a new field, the quantitative retention–activity relationships (QRAR) [3–6]. In order to emulate the biological barriers, different chromatographic strategies have been developed. For instance, phospholipids have been covalently immobilized to silica propyl amide particles (IAM chromatography) [7]. Immobilized liposomes, proteoliposomes and biomembrane vesicles have been proposed as stationary phases for chromatographic analysis of membrane–solute interactions [8].

Our research group has demonstrated that the chromatographic system comprising a reversed stationary phase and saline solutions of Brij35 micelles as mobile phase can be used as a drug biopartitioning system [9–11]. We have named this methodology biopartitioning micellar chromatography (BMC) [12,13].

The success of QRAR models based on BMC could be attributed to the similarities between BMC systems and biological barriers and extracellular fluids [14,15]. Thus, the stationary phase modified by the hydrophobic adsorption of surfactant monomers (polyoxyethylene-23 lauryl ether monomers) resembles structurally the ordered array of the membrane hydrocarbon chains, the dual hydrophilic/hydrophobic character and the H-bonding groups of the adsorbed surfactant can provide different interaction types similar to the ones between the membranes components (phospholipids and proteins) and the compounds transported by the biological fluids. On the other hand, the saline micellar mobile phases present characteristics similar to the extracellular fluids. Extracellular fluids are basically composed by water, salts, glucose, proteins and lipids. The last ones are amphifilic molecules with aliphatic chains and polar heads that form micellar aggregates in aqueous solution if their concentration is over the critical micellar concentration (cmc $< 10^{-6}$ M) [16]. This methodology has been applied for describing and predicting the biological activity of different pharmacological kinds of drugs such as barbiturates, opiods, non-steroidal antiflammatories, antidepressants, antihistamines [17–23], permeability across intestinal barriers [13], skin [24] and cornea [25].

Successful applications in this field of micellar electrokinetic chromatography (MEKC), that use surfactant solutions above the critical micellar concentration (cmc) as electrolytic solutions, have also been reported [21,26–30].

In this paper, quantitative relationships between the retention data in BMC and MEKC using Brij35 as surfactant (at pH 7.4) and some pharmacodynamic parameters of local anesthetics are obtained. These models are compared with those obtained using an immobilized artificial column (IAM). Finally, the effect of the acidosis and alkalosis on the pharmacological and toxicological properties of local anesthetics is studied using their retention in BMC at different mobile phase pH values.

2. Experimental

2.1. Instrumental and measurement

A Hewlett–Packard HP 1100 chromatograph with an isocratic pump, an UV–visible detector, a column thermostat and an HP Vectra computer (Amsterdam, The Netherlands) equipped with HP-Chemstation software (A.07.01 [682] ©HP 1999) were used. The solutions were injected into the chromatograph through a Rheodyne valve (Cotati, CA, USA), with a 20 μl loop. Kromasil octadecyl-silane C_{18} columns of 5 μm particle size (150 × 4.6 mm inner diameter (i.d.)) and a guard column of similar characteristics (35 × 4.6 mm i.d.; Scharlau, Barcelona, Spain) were used. The mobile phase flow rate was 1.0 ml min^{-1}. The detection was performed in UV at 220 nm. All the assays were carried out at 36.5 °C.

MEKC was performed using a HP^{3D} CE system (Hewlett–Packard, Waldbronn, Germany) equipped with an on-column diode array detector (DAD) and a HP^{3D} CE Chemstation software. A 50 μm i.d. and 375 μm outer diameter (o.d.) fused-silica capillary with an effective length of 50 cm (58.5 cm total length) was employed (Polymicro Technologies, Phoenix, AZ, USA). Injection was performed hydrodynamically at 30 mbar for 2 s. The detection wavelength was 220 nm, and the applied voltage was 25 kV. The capillary temperature was set at 36.5 °C.

A Crison Micro pH 2000 pHmeter from Crison Instruments (Alella, Barcelona, Spain) was employed to adjust the pH of the separation buffers.

2.2. Reagents and standards

Mobile phases in BMC and electrolytic solutions in MEKC were constituted by aqueous solutions of polyoxyethylene(23) lauryl ether (Brij35, Acros Chimica, Geel, Belgium), 40 and 10 mM, respectively. In both cases, the pH was adjusted to the desired value (6.5–8.0) with 0.05 M phosphate buffer, that was prepared by dissolving the appropriate amounts of disodium hydrogen phosphate and sodium dihydrogen phosphate (analytical reagent, Panreac, Barcelona, Spain).

Local anesthetics were obtained from several sources: lidocaine, procaine, and propanocaine were kindly donated by Seid S.A. (Barcelona, Spain); mepivacaine, prilocaine, and bupivacaine were kindly donated by Laboratorios Inibsa S.A. (Barcelona, Spain); and tetracaine and dibucaine were purchased from Sigma (St. Louis, MO, USA).

Stock standard solutions of local anesthetics of 1000 mg l^{-1} were prepared using methanol (HPLC grade, Labscan Ltd., Dublin, Ireland) as solvent. Working solutions were obtained by dilution of the stock standard solutions in the corresponding separation buffer. Solutions were stored at 4 °C.

Water used to prepare solutions was purified through a Barnstead E-Pure (Sybron, Boston, MA, USA). Mobile phases were vacuum-filtered through 0.45 μm nylon membranes (Micron Separations, Westboro, MA, USA). All solutions used in MEKC and injected into the chromatograph were filtered through 0.45 μm pore size disposable nylon filters (Micron Separations).

In order to obtain good peak shapes and reproducible migration data, in MEKC, the capillary was conditioned at the beginning of the day, prior to each injection and the end of the day. In all cases, the conditioning run included the following steps: (1) 2 min rinse with deionized water; (2) 1 min rinse with 0.1 M sodium hydroxide; and (3) 2 min rinse deionized water at 1000 mbar. Before sample injection, the capillary was also rinsed with the running buffer for 5 min.

2.3. Software and data processing

The retention data in BMC were calculated as retention factors, $k_{BMC} = (t_r - t_0)/t_0$, where t_r is the retention time of the test compound and t_0 corresponds to column dead time.

In MEKC, for a charged analyte and using a neutral pseudostationary phase, the retention factors k_{MEKC} were determined experimentally using Eq. (1) [31]

$$k_{MEKC} = \frac{\mu_{eff}^{z}}{\mu_{eff}^{m}} - 1 \quad (1)$$

where μ_{eff}^{z} and μ_{eff}^{m} represent the effective mobility of the compound in CZE and MEKC, respectively. The values of mobility μ_{eff}^{z} and μ_{eff}^{m} were determined experimentally, in the absence or in the presence of the neutral surfactant, using Eq. (2)

$$\mu_{eff} = \frac{Ll}{V}\left(\frac{1}{t_r} - \frac{1}{t_0}\right) \quad (2)$$

where L and l are the total capillary length and the length from the inlet to the detector, respectively, V the applied voltage, t_r the analyte migration time and t_0 is the retention time of an unretained solute (methanol) moving at the EOF rate. k is directly related to the affinity of the solute to the pseudostationary phase of Brij35, therefore k reflects the differences between solute–micelle interactions.

The k values determined in this study were averages of triplicate measurements. Microsoft® Excel 2000 and Statgraphics version 2.1 were used to perform the statistical analysis of the regressions.

3. Results and discussion

3.1. Quantitative retention–activity relationships (QRAR)

Table 1 shows the structure, protonation constants and the logarithm of octanol–water partition coefficients, log P, of the local anesthetics studied. As can be observed, they are basic compounds with log P values ranged between 1.65 and 4.20, at physiological pH they are partially charged.

Table 2 shows the retention factors of local anesthetics studied obtained in BMC using 0.04 M Brij35 mobile phases at different pH values in the range 6.5–8 and the retention factors in MEKC using 10 mM Brij35 solutions at pH 7.4 as electrolytic solution. In the same table the literature retention factors of the compounds in IAM chromatography [32] have been also included. As can be expected the most hydrophobic compounds present large retention factors in both BMC and MEKC. This retention behavior indicates that hydrophobicity plays an important role in both solute–stationary phase and solute–micelle interactions.

Table 3 shows the pharmacodynamic parameters of local anesthetics found in literature. Local anesthetics have been arranged in retention order at pH 7.4. From this table, important qualitative information could be derived. As can be seen, equianesthetic concentration (concentration of compound that produces an effect similar to a reference concentration of cocaine) decreases as local anesthetic retention increases. Qualitative relationships between the pharmacodynamic parameters duration of anesthetic action and toxicity and the retention are also observed; the most retained compounds are the most toxic, and have a longer duration of action. These relationships can be expected because the same molecular features of drugs (hydrophobic, electronic and steric properties) that determine their biological behavior also control the BMC and MEKC retention.

Table 1
Structures, logarithm of the protonation constants (log *K*) and logarithm of the partition coefficient in the biphasic octanol–water system (log *P*) for the non-ionic forms of the local anesthetics studied [1]

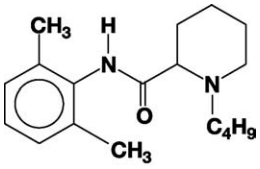
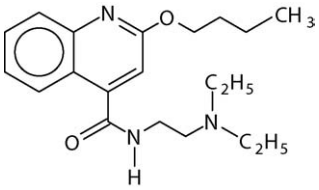
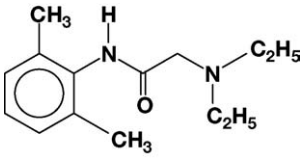
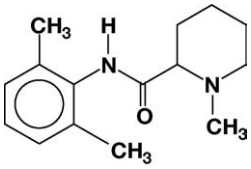
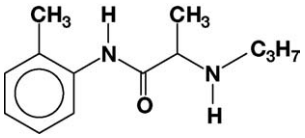
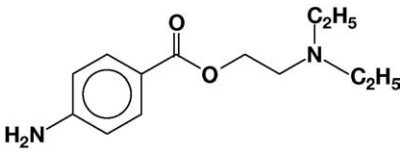
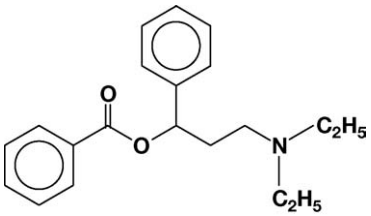
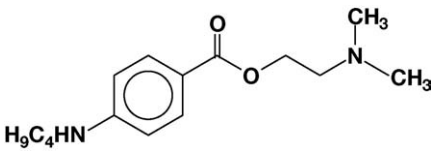
Compound	Number	Structure	log *K*	log *P*
Bupivacaine	1		8.10	3.38
Dibucaine	2		8.85	4.40
Lidocaine	3		7.9	2.26
Mepivacaine	4		7.7	1.75
Prilocaine	5		7.89	1.65
Procaine	6		8.80	2.24
Propanocaine	7		7.53	4.20
Tetracaine	8		8.6	3.73

Table 2
Retention data of local anesthetics obtained in BMC, MEKC and IAM at several pH values

Compound	$k_{BMC} \pm s$						$k_{MEKC} \pm s$	$\log k_w^{IAM,a}$
	pH 6.5	pH 6.8	pH 7	pH 7.4	pH 7.7	pH 8.0	pH 7.4	pH 7.4
Bupivacaine	14.7 ± 0.6	28.08 ± 0.15	40.1 ± 1.1	55.4 ± 0.4	63.5 ± 0.4	67.4 ± 1.6	0.96 ± 0.08	1.45
Dibucaine	48 ± 3	61.9 ± 1.3	84 ± 5	110 ± 3	124 ± 5	127 ± 5	2.843 ± 0.007	–
Lidocaine	6.4 ± 1.1	10.17 ± 0.14	15.4 ± 0.2	23.3 ± 0.4	28.2 ± 0.3	30.6 ± 0.8	0.469 ± 0.018	0.75
Mepivacaine	3.26 ± 0.09	4.26 ± 0.04	6.02 ± 0.12	9.20 ± 0.17	11.33 ± 0.13	12.6 ± 0.3	0.268 ± 0.005	0.77
Prilocaine	5.18 ± 0.14	6.92 ± 0.03	9.91 ± 0.04	15.46 ± 0.09	18.2 ± 0.2	20.7 ± 1.1	0.217 ± 0.007	0.62
Procaine	2.04 ± 0.06	2.63 ± 0.02	3.79 ± 0.02	7.0 ± 0.2	9.21 ± 0.13	12.5 ± 0.5	0.124 ± 0.011	0.39
Propanocaine	46.8 ± 0.5	65.02 ± 0.11	91 ± 3	143.3 ± 1.6	166 ± 6	167 ± 7	1.335 ± 0.006	–
Tetracaine	34.8 ± 0.5	46.6 ± 0.2	61.0 ± 0.6	81.6 ± 1.5	89.1 ± 1.1	93 ± 3	1.26 ± 0.03	1.75

[a] Data from Ref. [32]. Log k_w: extrapolated logarithm of retention factor to 100% aqueous mobile phase.

Table 3
Pharmacodynamic data of local anesthetics reported in the literature

Compound	Potency[a]	Max. single dose[b] (mg kg^{-1})	Equianest. conc.[c]	Duration action	Qualitative toxicity[d]	Relative toxic dose[e]	Min. toxic dose[f] (mg kg^{-1})
Procaine	1	12	2	Short	1	1	19.2
Mepivacaine	2	4.5	1	Medium	4	1.5	9.8
Prilocaine	3	8	1	Medium	2	1.5	–
Lidocaine	4	4.5	1	Medium	3	2	6.4
Bupivacaine	16	3	0.25	Length	5	4	1.6
Tetracaine	16	3	0.25	Length	6	10	2.5
Dibucaine	–	1	0.25	Length	7	–	–

[a] Relative anesthetic potency (procaine). Data from Ref. [34].
[b] Maximum single dose in adults. Data from Ref. [36].
[c] Equianesthetic concentration. Data from Ref. [37].
[d] Data from Ref. [33].
[e] Relative toxic dose (procaine = 1). Data from Ref. [35].
[f] Minimum intravenous toxic dose. Data from Ref. [38].

In order to obtain predictive and interpretative models, retention factors in BMC and MEKC, obtained using 40 and 10 mM Brij35 solutions at pH 7.4 as mobile phase and electrolytic solution, respectively, and the corresponding pharmacodynamic parameters were adjusted to potential models of the type.

$$y = a + bx^c + dx^e + \ldots \quad (3)$$

Where y is the biological activity considered, x the physicochemical property and a, b, c, d, ... fitting parameters. Linear and polynomial models can be considered as particular cases of Eq. (3). These empirical relationships are usually obtained in univariate QSAR and QRAR analysis and indicates that there is a range of values of the considered physicochemical property where this biological activity is optimal (maximum or minimum).

The QRAR models obtained using BMC and MEKC were compared with those obtained using the retention data in immobilized artificial membranes (IAM's) reported in literature [32] (Fig. 1). As can be observed similar trends were obtained in all cases. Table 4 shows the statistical analysis and the predictive features of the corresponding models obtained. In all cases, the coefficients and models were statistically significant at the 95% confidence level (P values < 0.05), except for the minimum intravenous dose in BMC, maximum single dose in IAM and relative toxic dose in MEKC. Mepivacaine was excluded of the model corresponding to maximum single dose because it shows an anomalous behavior in BMC, MEKC and IAM models. In addition, the r^2 and S.E. statistics of the models that use micellar systems (BMC and MEKC) were in general better than the corresponding to IAM chromatography. Therefore, from a practical and economic point of view, micellar systems are preferable to describe the pharmacodynamic parameters of local anesthetics studied.

3.2. Effect of acidosis and alkalosis on some local anesthetics' properties

Since the charged/uncharged form ratio of local anesthetics is a limiting factor of the efficacy of anesthetic blockade, pharmacological and toxicological effects of these compounds are highly dependent on the physiological pH. The QRAR models previously obtained in BMC have been used to study the effect of the acidosis and alkalosis on the pharmacological and toxicological properties of local anesthetics. For this purpose, retention in BMC at different mobile phase pH values (from 6.5 to 8, Table 2) of each local anesthetic was interpolated in the QRAR models obtained at pH 7.4 to describe the anesthetic potency and maximum dose in adults.

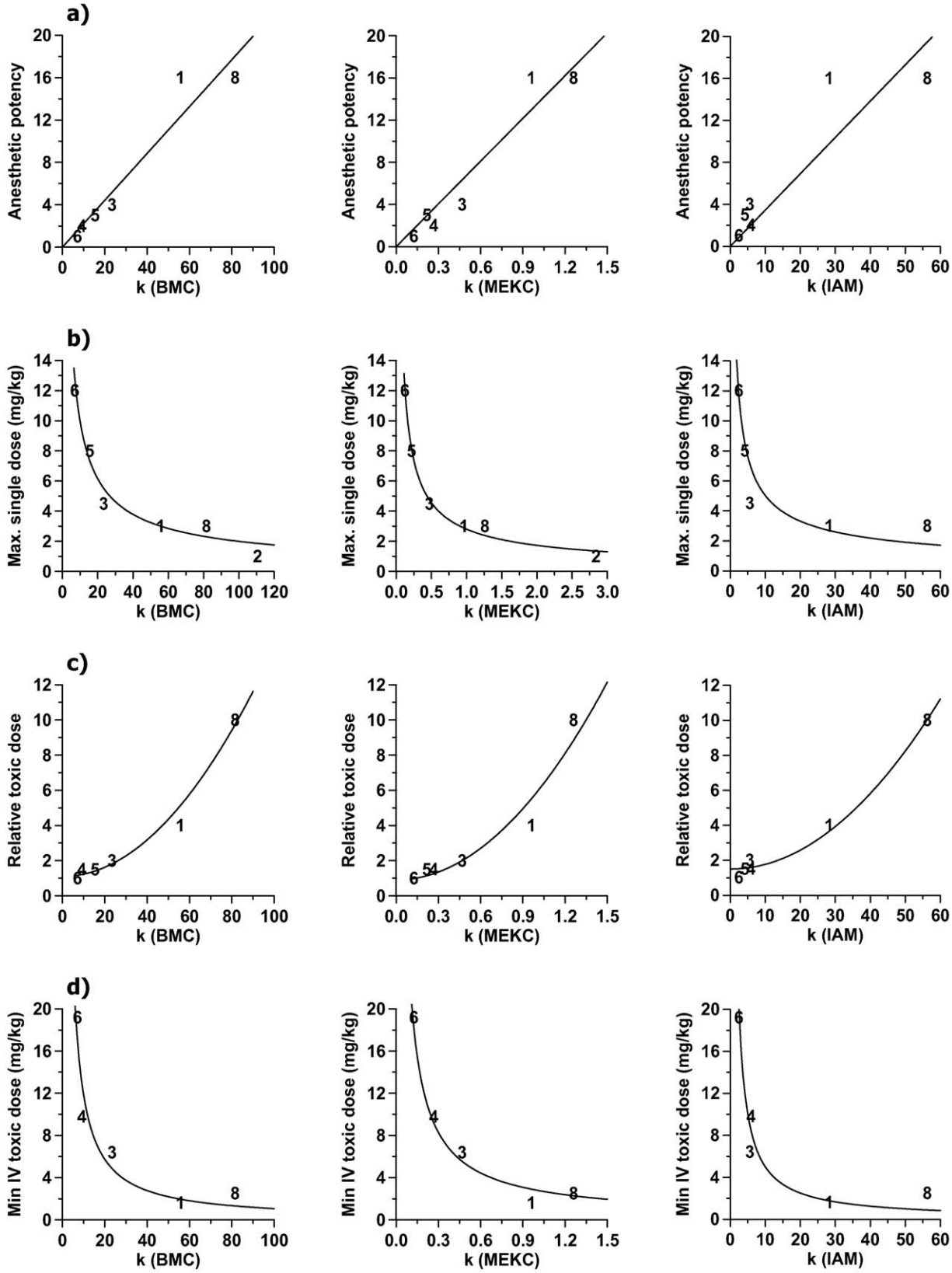


Fig. 1. QRAR models obtained for local anesthetics from the retention data in BMC, MEKC and IAM and some pharmacodynamic parameters: (a) anesthetic potency, (b), maximum single dose in adults (mg kg^{-1}), (c) relative toxic amount (procaine = 1), and (d) minimum intravenous toxic dose (mg kg^{-1}).

Table 4
Statistical analysis of QRAR models for different pharmacodynamic properties of local anesthetics[a]

Activity	Model	Technique (*n*)	$a \pm ts$	$b \pm ts$	$c \pm ts$	R^2	S.E.
Anesthetic potency	$y = a + bx + cx^2$	BMC (6)	–	0.22 ± 0.05	–	0.920	1.990
		MEKC (6)	–	14 ± 3	–	0.920	1.930
		IAM (6)	–	0.3 ± 0.1	–	0.767	3.211
Maximum single dose in adults (mg kg^{-1})	$y = ax^b$	BMC (6)	50 ± 30	–0.7 ± 0.2	–	0.965	0.841
		MEKC (6)	2.8 ± 0.5	–0.7 ± 0.1	–	0.993	0.250
		IAM (5)	20 ± 20*	–0.6 ± 0.6*	–	0.870	1.589
Relative toxic dose (procaine = 1)	$y = a + bx + cx^2$	BMC (6)	1.1 ± 0.8	–	0.0013 ± 0.0003	0.976	0.591
		MEKC (6)	0.9 ± 1.8*	–	5 ± 3	0.936	0.967
		IAM (6)	1.5 ± 0.5	–	0.0027 ± 0.0005	0.990	0.379
Minimum intravenous toxic dose (mg kg^{-1})	$y = ax^b$	BMC (5)	100 ± 300*	–1.1 ± 1.0	–	0.915	2.395
		MEKC (5)	2.8 ± 1.3	–0.9 ± 0.3	–	0.988	0.904
		IAM (5)	50 ± 30	–1.0 ± 0.5	–	0.951	1.807

* Statistically non-significant.
[a] Definitions: *n*, number of available data; *ts*, 95% confidence interval for coefficient estimates; R^2, *R*-squared adjusted for degrees of freedom; S.E., standard error of the estimate.

Table 5
Effect of the acidosis and alkalosis on the anesthetic potency for the local anesthetics studied

Anesthetic	pH					
	6.5	6.8	7.0	7.4	7.7	8.0
Bupivacaine	26.4	50.4	72.1	100.0	114.2	122.0
Dibucaine	43.3	55.9	75.8	100.0	112.0	115.0
Lidocaine	27.2	43.5	65.7	100.0	120.8	130.9
Mepivacaine	35.3	46.1	65.2	100.0	122.9	136.4
Prilocaine	33.5	44.7	64.1	100.0	117.9	134.0
Procaine	29.2	37.6	54.0	100.0	131.7	182.2
Propanocaine	32.6	45.4	63.7	100.0	115.6	116.3
Tetracaine	42.6	57.0	74.3	100.0	109.1	113.4

Table 5 shows the pH effect on the anesthetic potency for the local anesthetics studied. The values of potency of local anesthetics at pH 7.4 have been taken as reference and normalized to 100. As can be seen, the anesthetic potency decreases in cases of acidosis while increases in situations of alkalosis. The increase in cases of alkalosis is more pronounced for the procaine, mepivacaine, prilocaine and lidocaine in relative terms. However, the critical situations can be expected for tetracaine and dibucaine, the most potent local anesthetics.

Fig. 2 shows the dose of local anesthetics that have to be administered in situations of acidosis or alkalosis in order to get the same anesthetic effect that at physiologic pH. These values are estimated from the corresponding QRAR model using retention in BMC at different pH values. As can be observed, the dose to be administered increases in cases of acidosis as a consequence of the decrease in the anesthetic potency, while it should be reduced in conditions of alkalosis. However, in these situations toxic effects of anesthetics should also be considered because they depend on both pH and administered dose.

Toxic or adverse reactions occur primarily in the CNS and cardiovascular system (CVS), being able to provoke coma states and even the death. The toxicity of local anesthetics in CNS is related to the amount of administered drug. Therefore, CNS toxicity increases in case of acidosis, because the percentage of non-useful local anesthetic increases to exercise their secondary effects. This effect will become more accused if the dose is increased to exercise the same anesthetic effect, due to the amount of free drug increases in blood torrent.

On the other hand, the toxicity associated to CVS is related to relative potency of the local anesthetics, so the more potent anesthetics (bupivacaine and tetracaine) are the more toxic anesthetics in CVS. Fig. 3 shows the relationship between potency and toxic dose. In the CVS an increase of the anesthetic potency as consequence of alkalosis gives rise a toxicity increase. In this situation, a not controlled alkalosis can cause the individual's death, because the anesthetic potency would increase after administering a normal dose.

4. Conclusions

The approaches proposed in this paper, involving QRAR may be adequate in vitro options to obtain estimations of pharmacodynamic parameters of local anesthetics. The retention factors in MEKC and BMC, using solutions of Brij35 above the cmc as electrolytic solutions and mobile phases, are capable of describing the pharmacodynamic

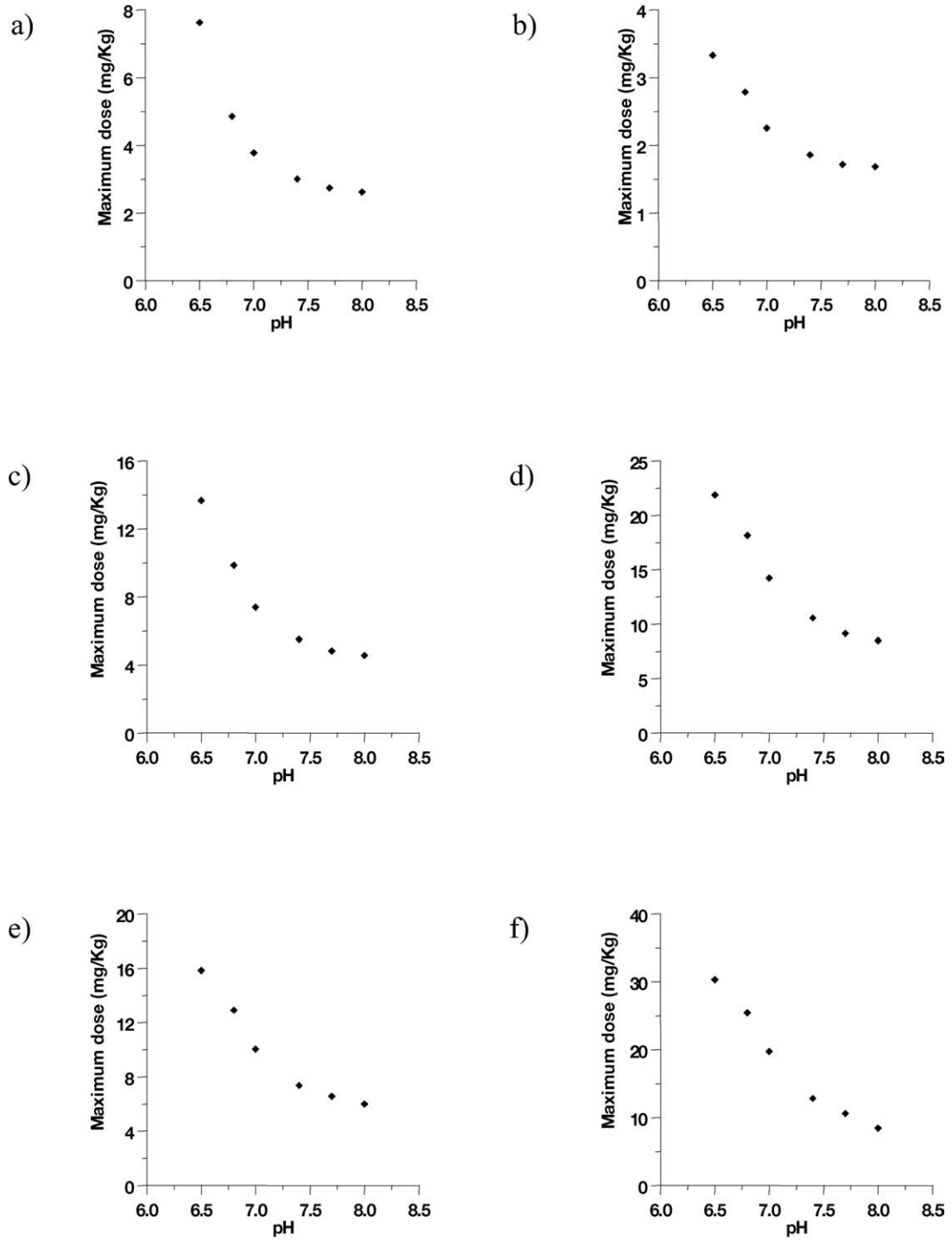


Fig. 2. Estimated maximum single dosages in adults (mg kg^{-1}) of local anesthetics at different pH values: (a) bupivacaine, (b) dibucaine, (c) lidocaine, (d) mepivacaine, (e) prilocaine, (f) procaine, (g) propanocaine and (h) tetracaine.

properties of the local anesthetics. In addition, QRAR models can be used to estimate the effect of acidosis or alkalosis on some properties as potency or maximum dose to be administered. The results obtained can be used as a starting point to design dosage protocols in clinical practice particularly in alkalosis situations, where the use of normal doses can provoke overdose.

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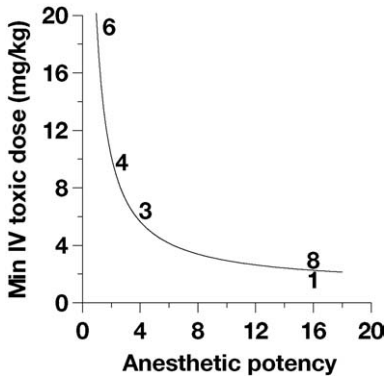

Fig. 3. Relationship between anesthetic potency and maximum intravenous toxic dose (data taken from Table 2).

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