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Hydrophobic and cation exchange mechanisms in the retention of basic compounds in a polymeric column

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Abstract

A cation exchange retention mechanism concomitant with the well-known hydrophobic partition mechanism in a polymeric column has been observed and investigated. This exchange process is attributed to ionization of some acidic sites present in the polymer column at basic mobile phase pH values. Several drugs of different basicity have been chromatographed on a polymeric PLRP-S column with methanol-water and acetonitrile-water mobile phases. The cation exchange between the protonated basic drug and the buffer cations (Na^+, K^+ and $BuNH_4^+$) is observed at the pH range where the protonated drug and the ionized sites of the column coexist. This process produces a shift of the retention versus pH plot of the base to pH values lower than those expected from the pK_a of the base as well as a maximum in the plot at basic pH values. These effects are more pronounced for acetonitrile-water mobile phases.

Keywords: Polymeric columns; Retention mechanisms; Stationary phases, LC; Cation exchange; Basic compounds

1. Introduction

Basic compounds make up a very significant part of chemicals with pharmaceutical or biomedical interest and, therefore, methods and procedures for their analysis have driven forward significant research efforts, mainly in the field of liquid chromatography. However, the RP-HPLC analysis of these substances presents, very often, practical difficulties due to low peak efficiencies, tailed or asymmetric peaks and retention times that are dependent on the mass of injected analyte. This poor chromatographic performance has been attributed to a number of concurrent retention mechanisms with different mass transfer kinetics, in addition to the expected hydrophobic interactions. There are, mainly, hydrogen bonding, π–π interactions, ion exchange, ion pair formation and salting out [1,2].

McCalley and co-workers [3–5] have widely studied the chromatographic behavior of basic compounds using a variety of stationary phases and stressing attention to those that allow the use of highly basic buffered eluents. In a recent paper [3], the authors have studied the retention process of basic compounds on silica-free polymeric columns to assess, by comparison, the role of silanols in separations on silica-based alkyl stationary phases. Using polymeric columns, they performed two kinds of experiments at low, intermediate and high pH: (a) measurements of the variation of retention factor of several basic compounds with the buffer cation concentration and (b) measurements of the change of peak shape with the load of basic analyte. The results of the first experiments clearly showed that cation exchange sites existed on a polymeric phase at intermediate and high pH, probably due to reagents used in the polymer preparation process. Thus, no variation of analyte retention with buffer concentration was observed using acidic and highly basic buffered mobile phases but a noticeable decrease in retention factor with the increase of buffer cation concentration was noticed for all the studied bases when neutral mobile phases (pH = 7) were used. This behaviour was attributed to the competition between the buffer cation and the protonated analyte that reduced the ion exchange and, consequently, the analyte retention. Therefore, it was concluded that retention of basic analytes is mainly a hydrophobic

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process at low pH (basic solutes charged but acidic column sites uncharged) and high pH (basic solutes uncharged but acidic column sites charged), whereas cation exchange contributes additionally to retention at neutral pH (strongly basic solutes charged, acidic column sites charged). The second set of experiments pointed out that, at low pH, column overload caused by ionic repulsion, i.e. mutual ionic repulsion of protonated bases held on the hydrophobic surface of the stationary phase, probably accounts for the majority of the increased band tailing and reduces efficiency when the sample mass is increased. A slow kinetics cation exchange at neutral pH was also confirmed. Thus, when saturation of exchanging sites occurs, a greater proportion of the sample is retained on hydrophobic sites by means of a fast kinetic process, which explains the apparent improvement in efficiency and also the large concomitant decrease in retention parameters with sample load. The experimental results achieved by McCalley are consistent with those described by Neue et al. [6] referred to the retention via ion exchange mechanism of cationic analytes on silica-based columns with ionized silanol groups.

Chromatographic behavior of ionizable compounds, both neutral and cationic acids, has been widely studied. Equations and procedures to describe retention versus pH of the mobile phase have been proposed and tested with a number of chemicals. However, most chromatographic models have been built on the basis that retention is a purely hydrophobic process [7]. Therefore, it seems necessary to test the suitability of these popular models with polymeric columns despite the concomitant cation exchange described above. This is because these materials are able to work with highly basic mobile phases where common silica-based materials are degraded, and they are often used for analysis of basic compounds [1,2].

In this paper, the chromatographic behavior of a series of basic drugs on both, polymeric (PLRP-S) and C_{18} RPLC (MS XTerra^{TM}) columns, has been systematically investigated. The compounds selected show a wide range of aqueous pK_a values that allows us to verify accurately the exchange process on the polymeric column in the neighborhood of the drug pK_a value, when it may have the maximum effect. MS XTerra^{TM} C_{18} stationary phase has been chosen because only a very small contribution of ionic exchange to retention occurs on this material [6], which does not present any evidence of residual ionized silanols [8]. Thus, the comparison of the retention behavior of selected basic drugs in both stationary phases allows the accurate study of cation exchange on the polymeric column in a wide range of intermediate pH values.

IUPAC recommendations for pH quantity have been used in this paper [9]. Each pH (and pK) symbol is preceded by a subscript (w and s, water and organic or hydroorganic solvent, respectively) that accounts for the solvent in which the ionic activity coefficient, γ, is referred to unity at infinite dilution (the solvent in which the electrode system is calibrated) and a superscript (w or s) that points out the medium in that the measurements are being made. Thus, ${}^{s}_{w}$pH is the pH expressed in the in the "intersolvental" or "absolute" pH scale, ${}^{s}_{s}$pH is the pH in the own pH scale of the organic or hydroorganic solvent and, consequently, ${}^{w}_{w}$pH is the notation adopted for the pH in water.

2. Experimental

2.1. Chemicals

Trazodone hydrochloride, trimipramine maleate, imipramine hydrochloride, nortriptyline hydrochloride and maprotiline hydrochloride were purchased from Sigma (Poole, UK). Methanol and acetonitrile were HPLC grade from Merck and water was purified by the Milli-Q plus system from Millipore. Other chemicals were reagent grade or better and were obtained from Fluka, Aldrich or Merck.

2.2. Apparatus

pH measurements were taken with a Ross combination electrode Orion 8102 (glass electrode and a reference electrode with a 3.0 M KCl solution in water as a salt bridge) in a Crison micropH 2002 potentiometer with a precision of ±0.1 mV (±0.002 pH units).

Retention data were taken on a 15 cm × 4.6 mm i.d. Polymer Labs PLRP-S 100 Å column (15–20 μm) with a flow rate of 1–2 ml min^{-1} and on a 15 cm × 4.6 mm i.d. MS XTerra^{TM} C_{18} 125 Å column (5 μm) with a flow rate 1 ml min^{-1}. The pH stability ranges claimed by the manufacturers were "all pH range" and 1–12, respectively. A Shimadzu (Kyoto, Japan) HPLC arrangement comprising a pump model LC-10ATvp and a variable wavelength UV-VIS detector, model SPD-10Avp, set at 254 and 200 nm, was used.

2.3. Procedure

Aqueous buffers (10^{-2} M) were prepared as follows: phosphoric acid; citric acid/potassium dihydrogen citrate; potassium dihydrogen citrate/sodium citrate; acetic acid/sodium acetate; sodium dihydrogenphosphate/disodium hydrogenphosphate; potassium dihydrogenphosphate/disodium hydrogenphosphate; sodium phosphate and butylammonium/butylamine. Mobile phases were prepared by mixing the aqueous buffers with methanol at 80% or with acetonitrile at 60% of organic solvent in volume. The mobile phase ${}^{s}_{w}$pH was measured with the electrode system calibrated with the usual aqueous standard reference buffers of potassium hydrogenphthalate (${}^{w}_{w}$pH = 4.00) and potassium dihydrogenphosphate/disodium hydrogenphosphate (${}^{s}_{w}$pH = 7.02). ${}^{s}_{w}$pH and chromatographic data were taken in triplicate at 25 ± 0.1 °C. The chromatographic column was thermostated with a water jacket. Sample solutions were of 100 ppm (or 500 ppm in some instances). Injection volume was 20 μl.

3. Results and discussion

The structures of the substances chosen for this work are given in Fig. 1. A systematic study of retention of these compounds versus ${}^{s}_{w}$pH has been carried out using the polymeric stationary phase and buffered mobile phases containing 80% in volume of methanol or 60% of acetonitrile. The variation of drugs retention with ${}^{s}_{w}$pH is given in Fig. 2 that shows nice S-shaped curves when methanol is the organic modifier. However, results obtained for mobile phases containing acetonitrile show lower retention for points buffered with butylammonium, $BuNH_3^+/BuNH_2$, with respect to others with the same ${}^{s}_{w}$pH but buffered by phosphate salts, and a maximum at basic ${}^{s}_{w}$pH values. With the exclusion of these outlier points, experimental retention volumes for each compound and organic modifier have been fitted to the following general equation:

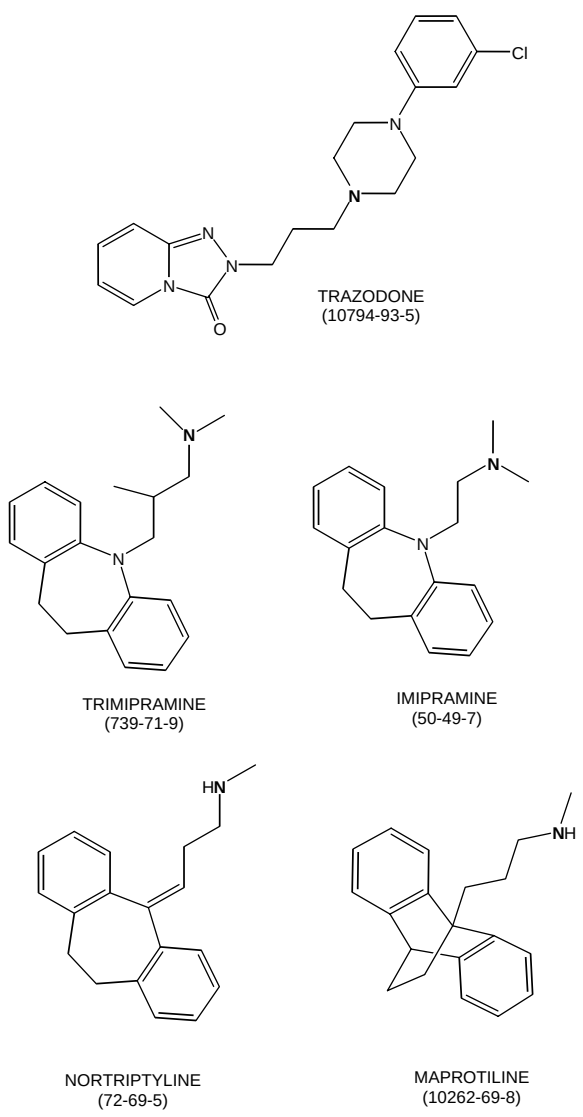

Fig. 1. Studied drugs.

$$V_R = \frac{V_{R(HB^+)} + V_{R(B)} 10^{({}^{s}_{w}\mathrm{pH} - {}^{s}_{w}\mathrm{p}K'_a)}}{1 + 10^{({}^{s}_{w}\mathrm{pH} - {}^{s}_{w}\mathrm{p}K'_a)}} \quad (1)$$

where V_R refers to the retention volume of the species in the subscript and ${}^{s}_{w}\mathrm{p}K'_a$ stands for the concentration acidic dissociation constant expressed in the intersolvental pH scale [7] (assuming there is no ionic exchange equilibria). Adjusted parameters of Eq. (1) for each compound and organic modifier are given in Table 1. Standard deviation values associated to each compound and chromatographic system show the consistency of the experimental data with the used model.

To test the suitability of Eq. (1) for the chromatographic systems under study, ${}^{s}_{w}\mathrm{p}K_a$ values in 80 and 60% in volume of methanol and acetonitrile hydroorganic solutions, respectively, have been calculated as follows: ${}^{w}_{w}\mathrm{p}K_a$ of drugs, determined potentiometrically in previous work [10], have been converted in ${}^{s}_{s}\mathrm{p}K_a$, being s the mentioned hydroorganic solution, through:

$${}^{s}_{s}\mathrm{p}K_a = a_v \, {}^{w}_{w}\mathrm{p}K_a + b_v \quad (2)$$

where the values of the constant parameters a_v and b_v are those derived for amines (0.96 and −0.60 for 80% of methanol and 1.07 and −1.03 for 60% of acetonitrile hydroorganic solutions, respectively) [7,11,12]. In its turn, ${}^{s}_{s}\mathrm{p}K_a$ values have been converted to ${}^{s}_{w}\mathrm{p}K_a$, in order to compare them with those derived directly by fitting the experimental retention data to Eq. (1). The used expression has been:

$${}^{s}_{w}\mathrm{p}K_a = {}^{s}_{s}\mathrm{p}K_a + \delta \quad (3)$$

where δ is a constant which depends only on the nature and content of organic modifier in the mobile phase (0.08 for 80% of methanol and −0.46 for 60% of acetonitrile, respectively) [13–15]. Despite the calculated ${}^{s}_{w}\mathrm{p}K_a$ are the thermodynamic ones ($I = 0$), they can be compared directly with those derived from chromatographic measurements since the ionic strength of mobile phases is low, lesser than 0.012 M, and the activity coefficient correction is of the same order than the experimental error. All these pK values, as well as the potentiometric ${}^{w}_{w}\mathrm{p}K_a$, which have been taken as the reference values, are given in Table 2. With the exception of trazodone, it can be observed that ${}^{s}_{w}\mathrm{p}K_a$ values derived from chromatographic measurements in the polymeric column are systematically lower than the ones expected from the aqueous ${}^{w}_{w}\mathrm{p}K_a$ and the lower the acidity of the protonated drug the higher the observed difference.

The statement of McCalley and co-workers [3] about a cation exchange process which shows kinetics significantly slower than that of hydrophobic mass transfer and contributes to global retention in silica-free polymeric columns [3,5] allows us to explain the experimental results. To test the exchange process on our polymeric column, samples of 100 and 500 ppm of drug have been injected in the same chromatographic conditions at intermediate ${}^{s}_{w}$pH values. As expected, in all instances the most concentrated sample has

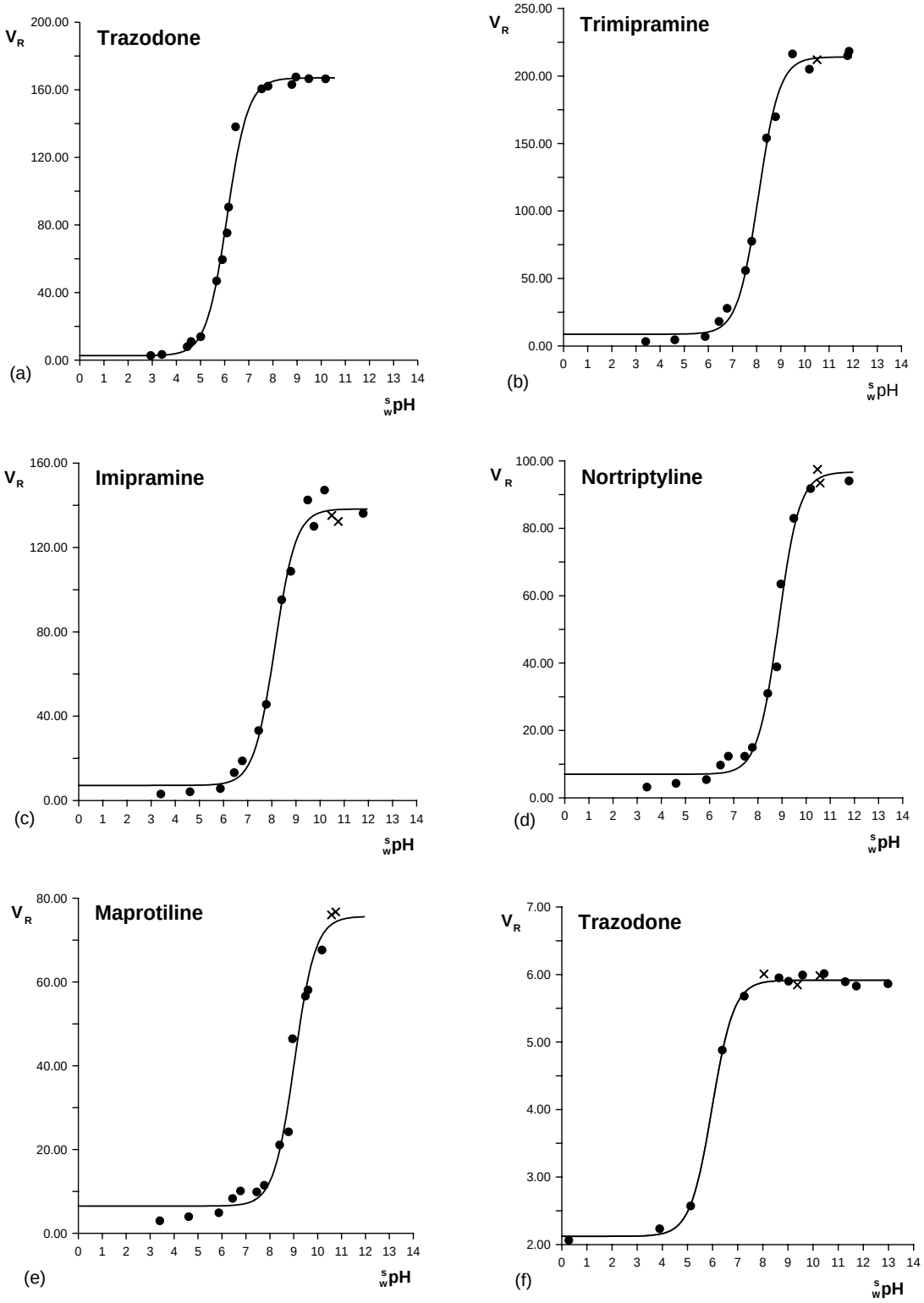

Fig. 2. Variation of retention volume, V_R, with ${}^{s}_{w}$pH for the studied drugs. Stationary phase: Polymeric PLRP-S column. Mobile phases: (a–e) methanol/aqueous buffer (80% in volume of methanol) (f–j) Acetonitrile/aqueous buffer (60% in volume of acetonitrile). Solid lines calculated by means of Eq. (1). (●) Points buffered by acetate, phosphate or citrate systems. (×) Points buffered by butylammonium/butylamine.

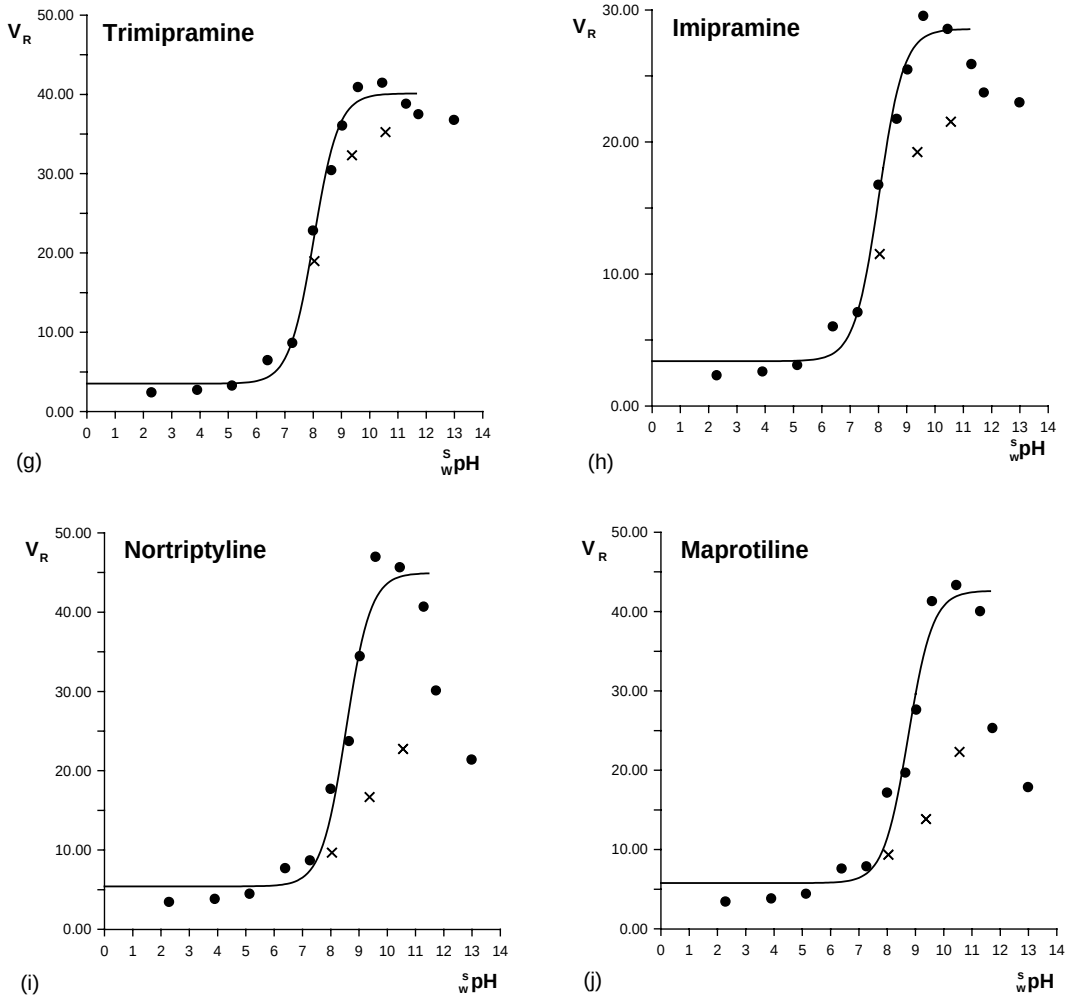

Fig. 2. (*Continued*).

shown the lowest V_R value. In addition, peaks obtained using different buffered mobile phases are quite symmetric in acidic media whereas exponentially tailed shape peaks are obtained in basic mobile phases, as shown in Fig. 3 for nortryptiline. This tailed shape has been attributed to the existence of two different types of retention sites having different equilibrium isotherms and different rates of mass transfer kinetics [3,5,16–18]. Even when the experimental conditions are such that both mechanisms operate linearly, tailing can be observed if the mass transfer kinetics is much lower on one type of sites than on the other. The most pronounced and typical peak tailing occurs when the slow type of adsorption sites, exchanging sites in this case, give a smaller contribution to the retention than the fast type, hydrophobic sites in this instance, and when the rate constant of mass transfer for the slow sites is between 20 and 2000 times smaller than that of the fast sites [16]. The experimental results clearly show the presence of some residual ion-exchanging sites in the organic polymer under study. Thus, the retention due to cation exchange mechanism should be much lower than that caused by hydrophobic interactions. This results in the tailed shape of peaks at intermediate ${}^{s}_{w}$pH values.

Cations present in the mobile phases buffered by acetate, phosphate or citrate systems are Na^+, K^+, or a mixture of both. Since the experimental V_R fit well the expected curve shape, it can be concluded that global effects of ion exchange on the retention are similar for both cations. Thermodynamic effects of ion exchange are ruled by the selectivity coefficient of protonated drug, BH^+, in reference to the buffer cation, A^+, present in the exchanging sites, according to:

$$\text{Polymer-G}^- A^+ + \text{BH}^+ \leftrightarrow \text{Polymer-G}^- \text{BH}^+ + A^+$$

$$K^{\text{B}^+}_{\text{A}^+} = \frac{[\text{BH}^+]_\text{S}}{[A^+]_\text{S}} \frac{[A^+]_\text{M}}{[\text{BH}^+]_\text{M}} \quad (4)$$

where G^- is the anionic group on the polymer surface, $K^{\text{B}^+}_{\text{A}^+}$ the selectivity coefficient and subscripts M and S refer to the mobile and stationary phases, respectively. If we assume that $K^{\text{B}^+}_{\text{A}^+}$ values, being A^+ Na^+ or K^+, are different, the experimental fact that a unique retention curve is obtained for each drug leads us to admit that the effect of the kinetics of ion exchange on drug retention is much more significant than the thermodynamic one and similar for both cations, Na^+ and K^+. The well shaped curves obtained, Fig. 2, sug-

Table 1
Retention parameters of drugs according to Eq. (1) (values ± standard deviation)

	80% MeOH						60% MeCN			
	C_{18} (MS XTerra™) column				Polymeric (PLRP-S) column			Polymeric (PLRP-S) column		
	$V_{R(BH+)}$	$V_{R(B)}$	$^{s}_{w}pK_a$	S.D.	$V_{R(BH+)}$	$V_{R(B)}$	$^{s}_{w}pK_a$	S.D.	$V_{R(BH+)}$	$V_{R(B)}$	$^{s}_{w}pK_a$	S.D.
Trazodone	2.03 ± 0.02	3.47 ± 0.01	6.08 ± 0.04	0.04	2.62 ± 3.36	167.10 ± 2.92	6.10 ± 0.05	3.50	2.12 ± 0.04	5.92 ± 0.02	5.98 ± 0.04	0.07
Trimipramine	2.43 ± 0.08	10.10 ± 0.10	8.06 ± 0.03	0.24	8.72 ± 3.06	214.16 ± 2.96	8.08 ± 0.04	3.30	3.51 ± 0.84	40.11 ± 0.92	8.03 ± 0.08	1.71
Imipramine	2.35 ± 0.05	7.66 ± 0.07	8.23 ± 0.03	0.16	7.20 ± 2.60	138.30 ± 2.38	8.12 ± 0.06	2.85	3.40 ± 0.71	28.56 ± 0.93	8.02 ± 0.10	1.45
Nortriptyline	2.46 ± 0.04	6.35 ± 0.06	9.20 ± 0.04	0.12	7.00 ± 1.68	96.72 ± 2.20	8.86 ± 0.06	2.11	5.42 ± 1.50	44.95 ± 2.08	8.54 ± 0.13	2.20
Maprotiline	2.38 ± 0.04	6.19 ± 0.08	9.53 ± 0.05	0.13	6.48 ± 1.56	75.66 ± 2.52	9.04 ± 0.08	2.02	5.77 ± 1.39	42.63 ± 2.09	8.74 ± 0.13	2.09

S.D.: overall standard variation of the fit.

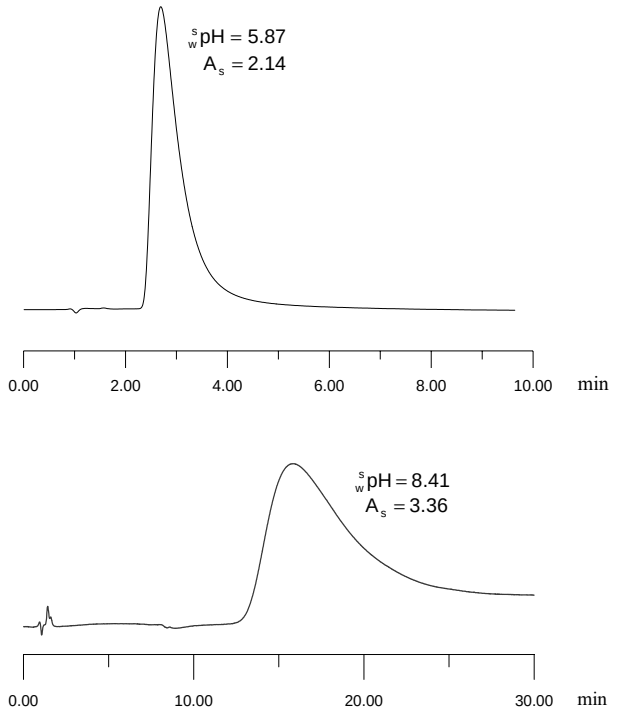

Fig. 3. Peak shapes of nortryptiline obtained in a polymeric PLRP-S column. Mobile phases: methanol/aqueous buffer (80% in volume of methanol). A_s is the symmetry value.

gest that the global cation exchange processes between each protonated drug and either cation, Na^+ or K^+, on the polymer surface are equivalent in terms of retention and this equivalence is independent of the organic modifier present in the mobile phase.

At this point, it seems clear that the cation exchange produces an increase of retention that shifts the retention versus $^{s}_{w}$pH curve towards acidic $^{s}_{w}$pH. The higher the true $^{s}_{w}pK_a$ of the protonated drug, the wider the $^{s}_{w}$pH range in which protonated drug and ionized groups on the polymer surface coexist and cation exchange takes place. Therefore, the stronger the base the greater the difference between chromatographically and potentiometrically derived $^{s}_{w}pK_a$ (Table 2). However, these differences are much lower for mobile phases containing methanol than for those with acetonitrile. Table 3 includes the results obtained for a variety of amino compounds previously studied on the same stationary phase [13,15,21,22]. They clearly confirm the shifts of chromatographic $^{s}_{w}pK_a$ to acidic values for protonated amines with $^{w}_{w}pK_a$ 8.9 or higher. Moreover, values referred to *N*,*N*-dimethylbenzylammonium show that the higher the content of organic modifier, the higher the difference between chromatographic and calculated $^{s}_{w}pK_a$. Thus, a higher concentration of organic modifier favors the relative contribution of ion exchange, compared with hydrophobic interaction, to retention.

Fig. 2 shows a retention decrease for almost all drugs at very high $^{s}_{w}$pH values in acetonitrile mobile phases. The only exception is trazodone, because its $^{s}_{w}pK_a$ is the lowest one

Table 2
Calculated and chromatographic ${}^{S}_{w}pK_a$ values of drugs

	${}^{w}_{w}pK_a$	80% MeOH					60% MeCN		
		${}^{S}_{w}pK_a$calc	C_{18} (MS Xterra™) column		Polymeric (PLRP-S) column		${}^{S}_{w}pK_a$calc	Polymeric (PLRP-S) column	
			${}^{S}_{w}pK_a$chr	$\Delta^{S}_{w}pK_a$	${}^{S}_{w}pK_a$chr	$\Delta^{S}_{w}pK_a$		${}^{S}_{w}pK_a$chr	$\Delta^{S}_{w}pK_a$
Trazodone	6.93	6.13	6.08	0.05	6.10	0.03	5.94	5.98	−0.04
Trimipramine	9.13	8.25	8.06	0.19	8.08	0.17	8.29	8.03	0.26
Imipramine	9.30	8.41	8.23	0.18	8.12	0.29	8.47	8.02	0.45
Nortriptyline	10.14	9.22	9.20	0.02	8.86	0.36	9.37	8.54	0.83
Maprotiline	10.45	9.52	9.53	−0.01	9.04	0.48	9.70	8.74	0.96

$\Delta^{S}_{w}pK_a = {}^{S}_{w}pK_a\text{calc} - {}^{S}_{w}pK_a\text{chr}$.

and it is not ionized at the pH values where the acidic column groups begin to ionize. Therefore, no cation exchange at all takes place for this drug at any mobile phase pH. This means that the retention of trazodone is only caused by hydrophobicity. However, the other drugs, with higher pK values, show a peak superimposed to the usual sigmoidal V_R versus pH plot in the ${}^{s}_{w}$pH range 8–12. The sigmoidal plot is the expected behaviour for the hydrophobic retention mechanism, whereas the superimposed peak must be attributed to the retention by cation exchange in the pH region where partial ionization of drug and column coexist. The presence of the maximum caused by cation exchange retention indicates that this interaction is very strong and it increases retention appreciably even when only small parts of the drug and column are ionized. The effect in retention of the cation exchange is larger for nortriptyline and maprotiline. This indicates that the contribution of cation exchange to total retention is more significant for these two drugs in good agreement with their chemical structure. Thus, whereas the basic sites of imipramine and trimipramine are tertiary amines, nortriptyline and maprotiline are secondary amines, which present less steric hindrance around the nitrogen atom. Thus, their access to exchanging sites is less limited and stronger interactions with the exchanging sites can be achieved [5,23], which involve a higher retention effect.

To study the effect of the buffer cation in the ion exchange mechanism, mobile phases of pH in the pH range of ion exchange buffered by $BuNH_3^+/BuNH_2$ were tested too. The results show that the retention data obtained for methanol/water mobile phases with butylamonium buffers is similar to the retention data obtained with phosphate buffers, which have Na^+ or K^+ as cations (Fig. 2). However, in acetonitrile/water mobile phases the retention of the bases with butylammonium buffer is significantly lower than the retention of the same bases with phosphate buffers of the same ${}^{s}_{w}$pHvalue. This means that the selectivity coefficients for the retention of the protonated base by ion exchange with Na^+ or K^+ or $BuNH_3^+$ (Eq. (4)) are very similar in methanol/water mobile phases, but that the selectivity coefficient for ion exchange of the protonated base by $BuNH_3^+$ in acetonitrile/water is much lower than the ones for ion exchange by Na^+ or K^+.

Despite the polarity of both mobile phases expressed as the Dimroth–Reichardt scale [24], E_T^N, is very similar (0.792 and 0.787 for 80% of methanol and 60% acetonitrile mobile phases, respectively [25]) it is reasonable to assume that

Table 3
Calculated and chromatographic ${}^{S}_{w}pK_a$ values of several basic compounds in a polymeric PLRP-S column

	${}^{w}_{w}pK_a$lit	MeOH (%)				MeCN (%)					
		60		80		20		40		60	
		${}^{S}_{w}pK_a$calc	${}^{S}_{w}pK_a$chr	${}^{S}_{w}pK_a$calc	${}^{S}_{w}pK_a$chr	${}^{S}_{w}pK_a$calc	${}^{S}_{w}pK_a$chr	${}^{s}_{w}pK_a$calc	${}^{S}_{w}pK_a$chr	${}^{S}_{w}pK_a$calc	${}^{S}_{w}pK_a$chr
2,6-Dimethylaniline	3.87[a]	3.49	3.56[e]	3.18	3.29[e]	3.55	3.57[f]	3.10	3.22[f]	2.68	2.78[f]
Aniline	4.60[b]	4.20	4.25[e]	3.88	3.91[e]	4.30	4.35[f]	3.87	3.96[f]	3.45	3.57[f]
p-Toluidine	5.08[b]	4.66	4.83[e]	4.34	4.40[e]	4.79	4.83[f]	4.38	4.58[f]	3.96	4.08[f]
Pyridine	5.23[b]	4.09	4.23[e]	7.76	7.75[e]	4.87	4.91[f]	4.47	4.61[f]	3.90	4.03[f]
2,4,6-Trimethylpyridine	7.46[c]	6.22	6.52[e]	5.88	5.87[e]	7.07	7.09[f]	6.68	6.58[f]	6.07	6.11[f]
N,*N*-Dimethylbenzylammonium	8.91[d]	8.37	8.13[d]	8.04	7.44[d]	8.73	8.51[f]	8.40	8.15[f]	8.05	7.68[f]
Trimethylamine	10.78[e]	–	–	–	–	–	–	10.36	9.72[g]	–	–

[a] [19].
[b] [20].
[c] Unpublished value.
[d] [13].
[e] [21].
[f] [22].
[g] [15].

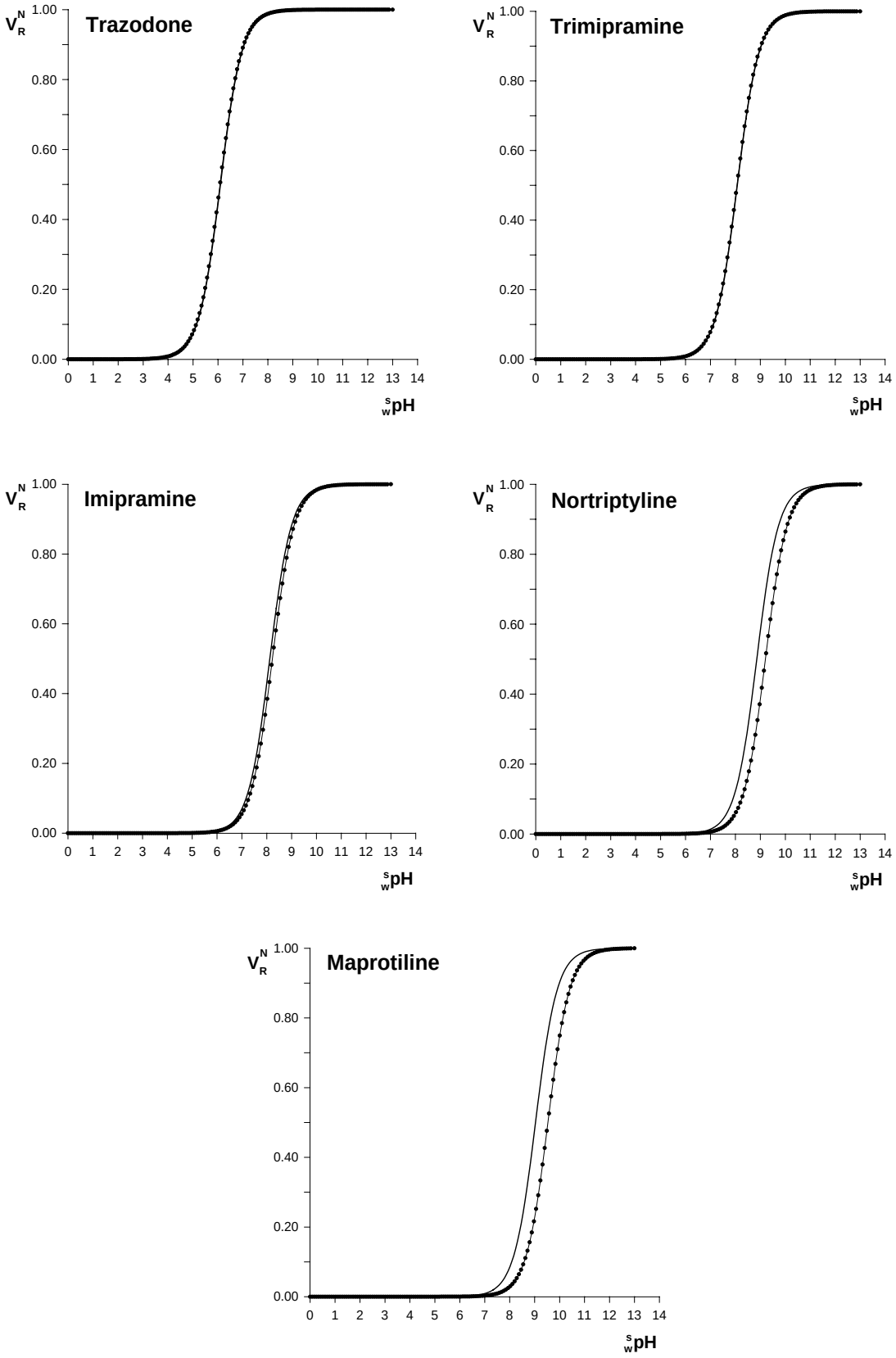

Fig. 4. Variation of normalized retention volume: V_R^N, with ^s_wpH for the studied drugs. Stationary phases: Polymeric PLRP-S (—),MS XTerra^{TM} (●). Mobile phases: methanol/aqueous buffer (80% in volume of methanol).

Na^+ and K^+ are preferentially solvated by water whereas $BuNH_3{}^+$ and protonated drugs prefer the organic solvent. E_T^N parameters for pure water, methanol and acetonitrile are 1.000, 0.762, and 0.460, respectively [24], and these values can be taken as an approximate evaluation of the polarity of the cybotactic region of buffer cations and analytes. Thus, in methanol mobile phases, the cation exchange takes place between ions solvated by methanol (HB^+) and water (Na^+ or K^+) or methanol ($BuNH_3{}^+$) and both solvents show close solvation properties. Therefore, the cation exchange rate should be similar for the three buffering cations and no differences are appreciated in the chromatographic behavior with the buffering chemical. However, acetonitrile shows solvation properties far to those of water, and the exchange between the protonated drug and alkaline cations is more difficult. This origins a slower exchange kinetics and, consequently, a larger effect on retention. When the exchange is between protonated drug and $BuNH_3{}^+$, both preferentially solvated by acetonitrile, the exchange is easier, the rate faster and its effect on retention lower (Fig. 2).

To determine the ${}^{s}_{w}$pH threshold at which column ionization is already noticeable, the drugs were chromatographied using an MS MS XTerra^{TM} C_{18} column and buffered mobile phases which contain 80% in volume of methanol as organic modifier. Obtained peaks are essentially Gaussian and yield shorter V_R values than those obtained with the polymeric stationary phase. Experimental V_R have been also fitted to Eq. (1) and calculated parameters given in Table 1. It can be observed the agreement between derived ${}^{s}_{w}pK_a$ values and the calculated ones showing that no cationic exchange at all takes place on this material, as previously demonstrated [8]. The slightly higher $\Delta^{s}_{w}pK_a$ values calculated for trimipramine and imipramine are attributed to the ${}^{w}_{w}pK_a$ reported, which show a slightly higher variability [10]. Because of the great differences in retention volumes achieved with polymeric and MS XTerra^{TM} columns, V_R obtained in both columns have been normalized according to:

$$V_R^N = \frac{V_R - V_{R(BH^+)}}{V_{R(B)} - V_{R(BH^+)}} \quad (5)$$

and V_R^N values computed for each drug shown in Fig. 4. For all drugs, the normalized curves split at ${}^{s}_{w}$pH 7.0 and this value points out the presence of ionized groups in the polymer surface. Therefore, measurements at ${}^{s}_{w}$pH below this threshold value account only for hydrophobic interactions between the analyte and chromatographic phases, whereas cation exchange should be taken into account at higher ${}^{s}_{w}$pH values in this polymeric material when organic cations are measured.

4. Conclusions

The statement of McCalley about a concomitant cation exchange retention mechanism on a silica-free polymeric column in intermediate and high pH has been tested and confirmed on a polymeric (PLRP-S) column. Thus, the hypothesis of two different mechanisms associated to retention of organic cations, hydrophobic partition and cation exchange at ${}^{s}_{w}$pH higher than a threshold value, allows us to interpret the experimental results. The experiments have been performed using a series of basic drugs with a wide range of ${}^{w}_{w}pK_a$ values and using both, methanol and acetonitrile as organic modifiers of mobile phases. The results clearly show:

(a) an increase in drug retention at ${}^{s}_{w}$pH between 7.0 and 10.5, attributed to a cationic exchange between the protonated drug and the buffer cation present on the ionized sites of the polymeric column;
(b) the experimental V_R values fit the general model derived for pure hydrophobic partition processes but a shift of V_R versus ${}^{s}_{w}$pH curves to acidic ${}^{s}_{w}$pH is clearly detected and, consequently, chromatographically derived ${}^{s}_{w}pK_a$ shift also to values lower than the true ones;
(c) a higher sensitivity to exchange process of chromatographic systems containing acetonitrile as organic modifier. This is shown by the maximum in retention curves at basic ${}^{s}_{w}$pH values;
(d) the concomitant cation exchange retention mechanism on polymeric stationary phases disturbs the ${}^{s}_{w}pK_a$ determination and the retention prediction of basic compounds by means of current chromatographic models because they are built on the basis of pure hydrophobic partition mechanisms.

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