Journal of Chromatography A, 1218 (2011) 1966–1973

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Deformations of overloaded bands under pH-stable conditions in reversed phase chromatography

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ARTICLE INFO

Article history:
Available online 15 September 2010

Keywords:
Hybrid silica column
Polymeric column
Adsorption isotherm
Alkaline conditions
pH stable conditions
Band distortions
Reversed phase
Preparative chromatography
Overloaded bands
Sample pH

ABSTRACT

It has recently been demonstrated, using mathematical models, how peculiar overloaded band profiles of basic compounds are due to the local pH in the column when using low capacity buffers. In this study, overloaded peak shapes resulting after injection of carefully pH matched samples close to the pK_a of the chosen solute are investigated primarily on two columns; one hybrid silica C18 column (Kromasil Eternity) and one purely polymeric column (PLRP-S), the latter lacking C18 ligands. It was found that distorted peaks of the basic test compound appear even though there is no difference in pH between the injected sample solution and the eluent; the previous explanation to why these effects occur is based on a pH mismatch. Thus, the unusual band shapes are not due to an *initial* pH difference. Furthermore, it was observed that the effect does not appear on polymeric columns without C18 ligands, but only on columns with C18 ligands, independently of the base matrix (silica, hybrid silica, polymeric).

1. Introduction

Many important pharmaceuticals have basic amino functions. The issue of pH when such compounds are analyzed is therefore a delicate matter in liquid chromatography. Depending on where along the pH-scale experiments are conducted, the compound will be present in charged form, uncharged form, or as a combination of charged and uncharged, a fact that explicitly affects chromatographic performance. It is logical that the dissolving of protolytic compounds in the eluent may lead to pH differences between sample and bulk-eluent. It is thus important to consider which protolytic form of the compound that is used [1].

The addition of organic modifiers to the eluent will change the p*K*a of the solute and the apparent pH of the eluent. These changes are solute and buffer system dependent. A rule of thumb is that the pK_a of acids increases and the pK_a of bases decreases upon addition of modifier to the eluent [2]. These changes can lead to confusing results if the eluent pH with modifier present is not properly monitored; see Figure 1 in Ref. [3].

Numerous studies on the behavior of ionizable compounds in reversed phase liquid chromatography (RPLC) have been conducted through the years, partly because a vast amount of pharmaceutical substances belong to this category. Traditional reversed phase silica C18 materials have however the drawback of limited pH-tolerance in the alkaline end of the pH-scale, i.e. at high pH (>8) the silica starts to dissolve. For the separation of basic compounds this has in many cases limited the studies to a pH-range below the pK_a of the base, thus having a fully or partially positively charged solute that can interact with negatively charged residual silanols on the silica surface. The result is tailing peaks and suboptimal performance [4], a problem that however has been drastically decreased with the modern C18 phases of today. During this time there have been other materials without residual silanols available for high pH reversed phase separations, such as polymeric materials. The polymeric materials suffer however from other disadvantages than silica, such as shrinking/swelling and a generally lower chromatographic performance than silica materials [5]. As of today, alkaline stable hybrid organic/silica phases are available on the market. These phases have pH stability up to 12 which generally allows separation of uncharged amines at high pH.

In preparative applications when dealing with high concentration samples of protolytes there is a great risk of a difference in pH between sample and eluent, particularly if one dissolves the "wrong" protolytic form in the eluent. It is crucial to keep track of this possible difference, since strong deformations of the bands can be seen if the pH difference is large [1]. It has also been shown in studies of adsorption isotherms that matched and mismatched samples will result in different parameters [6]. The appearance of

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doi:10.1016/j.chroma.2010.09.002

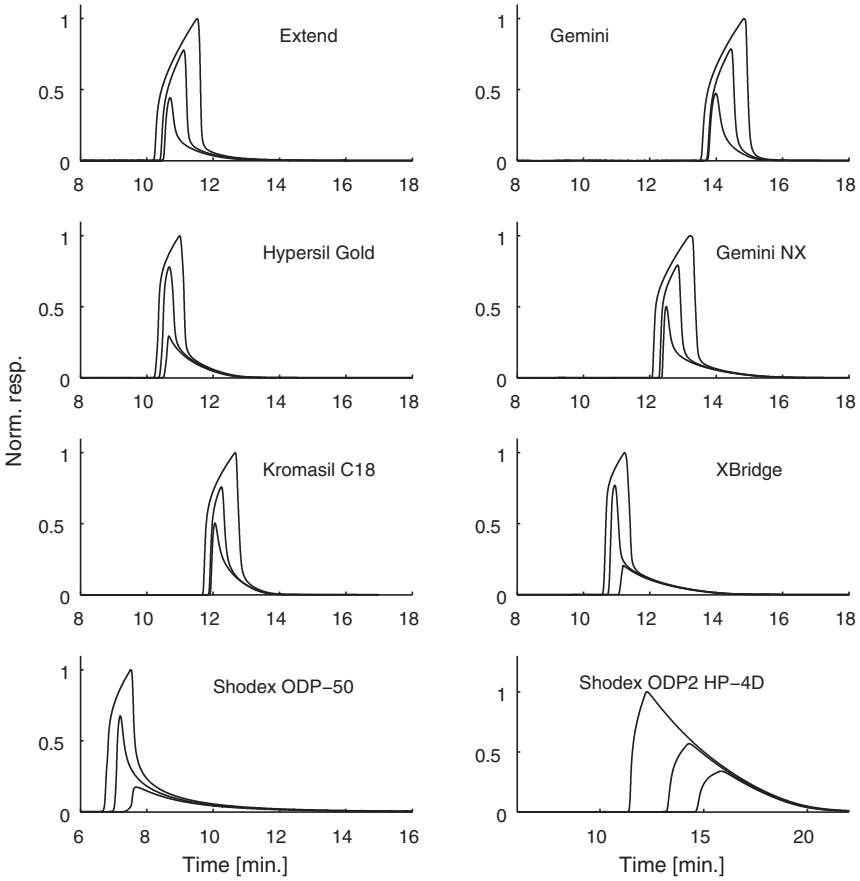

Fig. 1. Overloaded injections of 5, 10 and 20 mM metoprolol in 20 mM $^{w}_{w}$pH 7 phosphate buffer: MeOH on eight different reversed phase columns. For column specifications, see Table 1.

band shapes deviating from type I (i.e. sharp front, diffuse rear) around the pK_a of ionizable compounds has been noticed by several researchers [1,3,7–10]. The change in band shape in this interval, indicating a switch of the adsorption isotherm type, is suggested to appear when the solute concentration is high enough to cause local pH differences between the sample and the eluent [7–10]. This is in many cases a disadvantage which can lead to undesired deformed band shapes [1], but it has also been taken advantage of, e.g. to produce desirable band compressions [11] and to, by preparative HPLC, increase high purity recovery of pharmaceuticals from post-crystallization synthesis waste [12].

Previous studies on different reversed phase materials have shown that type I peaks (sharp front, diffuse rear) appear at $^{w}_{w}$pH 3 and $^{w}_{w}$pH 11 for basic probes [3], but that "deformed" (type II isotherm, initially a sharp and subsequently diffuse front, followed by an initially sharp and subsequently diffuse rear, see Fig. 1) peaks appear at intermediate pH [1,3,8].

It has recently been demonstrated and proved by modeling that peculiar overloaded band profiles of basic compounds are due to the local pH difference between the sample and the eluent in the column when using buffers with low buffer capacity [8,9]. There were however indications that these effects may take place even if the pH is stable throughout the column, which was the scope of this investigation. More in particular, the aim was to make a detailed investigation of the peak shapes of carefully pH-controlled solutions closely around the strange-band-shape region. This was performed with pH-controlled samples of an ionizable basic compound, as well as a neutral compound and a constantly charged quaternary amine as control substances, particularly on one polymeric column with particles made of polystyrene/divinylbenzene (PLRP-S) and one new generation alkaline pH resistant organic hybrid silica C18 column (Kromasil Eternity).

2. Theory

The retention factors of monoprotic bases are strongly affected by the pH, and according to Horváth et al. the retention factor could be described with [13]:

$$k = \frac{k_0 + k_1 \times 10^{(^{S}_{w}\mathrm{p}K_a - ^{S}_{w}\mathrm{pH})}}{1 + 10^{(^{S}_{w}\mathrm{p}K_a - ^{S}_{w}\mathrm{pH})}} \quad (1)$$

where k_0 and k_1 are the retention factors for uncharged respectively charged form. The pH and pK_a values in this context are better described by the $^{S}_{w}$pH/$^{S}_{w}$pK_a scales (pH calibrated in aqueous solution and measured with organic modifier present) than the $^{w}_{w}$pH/$^{w}_{w}$pK_a (pH calibrated and measured in aqueous solution) scales, since the latter can cause poor retention predictions [3].

Eq. (1) can however only describe analytical amounts of solute. For overloaded cases adsorption isotherms in combination with a column model are needed to describe the band shape. Expansion of Eq. (1) to adsorption isotherms has been done (Eq. (2)). The resulting equation has been used to explain similar band deformations for bases as the ones seen in the present study, however in these studies, with sample–eluent pH mismatch [8]:

$$q_T = q_{S,I}\frac{(1-\alpha)b_I C_T}{1+(1-\alpha)b_I C_T} + q_{S,N}\frac{\alpha b_N C_T}{1+\alpha b_N C_T}, \quad (2)$$

where α is the ratio of uncharged solute to the total concentration and C_T is the total concentration, b_I and b_N are the adsorption–desorption equilibrium constants of the ionic and the

Table 1
Column specifications. All columns were of the dimensions 150 mm × 4.6 mm, I.D. with 5 μm particle size.

Column	Manufacturer	Surface chemistry	Phase ratio (F)
Gemini	Phenomenex Inc., USA	Hybrid Silica C18	0.43
Gemini-NX	Phenomenex Inc., USA	Hybrid Silica C18	0.70
Hypersil Gold	Thermo Scientific, USA	Classic Silica C18	0.26
Kromasil Classic	Eka Chemicals, Sweden	Classic Silica C18	0.71
Kromasil Eternity	Eka Chemicals, Sweden	Hybrid Silica C18	0.70
PLRP-S	Polymer laboratories, Varian Inc., USA	Polystyrene/divinylbenzene	0.73
Shodex ODP2 HP-4D	Showa Denko K-K., Japan	Poly(hydroxymethacrylate)	0.80
Shodex ODP-50	Showa Denko K-K., Japan	Polyvinylalcohol with C18	0.74
X-bridge	Waters Corp., USA	Hybrid Silica C18	0.60
Zorbax Extend	Agilent Technologies, USA	Classic Silica C18 with bidentate silane	0.84

neutral species respectively, and $q_{S,I}$ and $q_{S,N}$ are the saturation capacities of the ionic and neutral species.

3. Experimental

3.1. Apparatus

The experiments were performed on an Agilent 1100 and an Agilent 1200 chromatographic system from Agilent Technologies (Palo Alto/Santa Clara, CA, USA), both consisting of a binary pump, an auto sampler and a diode array UV detector. During the experiments the column was mounted into a laboratory assembled column jacket with temperature control from a LAUDA type B circulating water bath adjusted to 25.0 °C (Köningshofen, Germany). pH was measured with an Orion Model 410A pH meter. Evaporation during the preparation of different protolytic species of metoprolol was conducted with a Büchi RE111 rotary evaporator and a 461 water bath (BÜCHI Laboratoriums-technik AG, Switzerland).

3.2. Column properties

Several columns were used in this study all were of the dimensions 150 mm × 4.6 mm packed with 5 μm nominal particle size. Four hybrid columns: Xbridge C18, Gemini C18, Gemini NX C18, Eternity C18 and three C18 silica columns: Kromasil C18, Hypersil Gold C18, Zorbax Extend C18, and three polymeric columns; PLRP-S, Shodex ODP2 HP-4D and Shodex ODP-50 (the latter with C18 chains). The extended analytical and overloaded study was done on the Kromasil Eternity C18 and the PLRP-S column. Column properties from the manufacturers are presented in Table 1.

3.3. Chemicals

De-ionized water (conductivity 18.2 MΩ cm) was used for for preparation of eluents and was delivered from a ZMQS 5000Y Milli-Q Academic water purification system from Millipore (Molsheim, France). The organic modifiers used were methanol (MeOH) and acetonitrile E (ACN) of CHROMASOLV quality. The solutes used were 3-phenyl-1-propanol (PP, 98%), benzyltriethylammonium chloride (BT, 99%) and metoprolol in different protolytic forms, produced from racemic metoprolol tartrate salt (ME, ≥98%). Stock solution (1.0 M) for preparation of phosphate buffers was made from 85% *ortho*-Phosphoric acid solution (puriss. p.a. for HPLC). Acetic acid buffers were prepared from acetic acid (99.8%) and anhydrous sodium acetate (>99%). Ammonium bicarbonate buffers were prepared from ammonium bicarbonate (>99.5%). Buffer pH was adjusted with 1.0 M sodium hydroxide (NaOH, FIXANAL) where needed. For preparation of the protolytic forms of metoprolol, dichloromethane (LiChrosolv quality), sulphuric acid (95%) and dry diethyl ether (distilled and provided by a department of organic chemistry) was used. All bought chemicals were from Sigma–Aldrich.

3.4. Procedures

3.4.1. General procedures

All buffers were prepared to 20 mM total concentration of the buffering component before mixing with the organic modifier. Phosphate buffers were used at $^{w}_{w}$pH 3, 6, 7, and 8. Acetate buffers were used at $^{w}_{w}$pH 4 and 5. Ammonium bicarbonate buffers were used at $^{w}_{w}$pH 9, 10 and 11. All buffers were filtered through a 0.22-μm type GV DURAPORE membrane filter from Millipore (Cork, Ireland).

All experiments were performed at a temperature of 25.0 °C and flow rate of 0.70 mL/min.

3.4.2. Analytical study

The analytical experimental series on the effect of pH on the retention time were performed in 1 pH-unit steps from $^{w}_{w}$pH 3.0 to $^{w}_{w}$pH 11.0. 5.0 μL of a 0.25 mM solution of each solute was injected at each pH step. Fixed modifier percentages were used; for the hybrid Silica C18 Kromasil Eternity column 50.0% MeOH was used at all pH steps, and for the polymeric PLRP-S column 30.0% ACN was used at all pH steps. For buffers systems used at the respective $^{w}_{w}$pH level, see Section 3.4.1.

3.4.3. Overloaded study

The overloaded test series was performed in 1 pH-unit steps from $^{w}_{w}$pH 5.0 to $^{w}_{w}$pH 10.0, by injecting 100.0 μL of 5.0, 10.0 and 20.0 mM of each solute at each pH step. The modifier percentage was determined so that the analytical retention time was approximately 16.0 min for the peak apex, see Table 2. For buffers used at the respective $^{w}_{w}$pH, see Section 3.4.1.

3.4.4. Preparation of different protolytic species of metoprolol

ME base was produced by dissolving racemic ME tartrate in a 0.1 M NaOH water phase (100 mL NaOH water phase per gram of ME tartrate), and then shaking this alkaline water phase with an equivalent (1:1) volume of dichloromethane. This extracts uncharged ME base from the water phase to the organic phase. The dichloromethane phase was then evaporated with the rotary evaporator and the metoprolol base crystals were dried and transferred to a dedicated container.

Table 2
Determined modifier percentages for each $^{w}_{w}$pH and solute.

$^{w}_{w}$pH	Eternity, MeOH-percentages			PLRP-S, ACN-percentages		
	PP	ME	BT	PP	ME	BT
5	41	27	10	29	18	11
6	41	27	10	29	23	12
7	41	29	10	29	33	14
8	41	40	11	29	50	16
9	41	40	13	29	47	19
10	41	50	17	29	45	26

ME HCl was produced by dissolving the above produced ME base in dry diethyl ether (≈5 g in 100 mL), placing the solution on ice and then bubbling HCl gas through the diethyl ether solution. The HCl gas was produced by slowly dripping concentrated H_2SO_4 on NaCl and gently carrying the generated gas over to the reaction vessel with N_2 as a carrier gas. When the ME HCl was precipitated the crystals were subsequently poured onto a filter paper, washed by vacuum filtration with dry diethyl ether and transferred to a dedicated container.

3.4.5. pH matching of the protolyte samples (metoprolol) in the overloaded study

To assure the absence of pH mismatch in the protolytic samples (ME) the following procedure was used. First, the modifier percentage for the desired analytical retention time was determined for each $^{w}_{w}$pH step (see Section 3.4.3). Then two strong solutions (50.0 mM) of metoprolol base and metoprolol HCl respectively were made separately in the eluent containing the determined modifier percentage. The $^{S}_{w}$pH in the eluent was measured and the two strong solutions of different ME forms were then mixed with known volumes of each solution until the measured eluent $^{S}_{w}$pH was reached. The sample concentrations of 20, 10 and 5 mM in each injection series were diluted with the eluent from the high concentration pH-matched solution. pH was measured in all sample solutions and eluents to assure the absence of a pH difference.

BT and PP did not change the pH of any solution at any concentration used in this study, thus no pH adjustment was necessary with these probes.

4. Results and discussion

Overloaded elution zones for amino alcohols (metoprolol and propranolol) using eluents with pH close to the pK_a of the solute have been previously studied [3,10]. To further investigate this, 100 μL of 5.0, 10.0 and 20.0 mM of metoprolol at $^{w}_{w}$pH 7 were injected on eight different reversed phase columns available on the market, see Fig. 1. It is intriguing to see that all columns with C18 surface chemistries show obvious elution bands originating from a type II adsorption isotherm regardless of the base matrix under these conditions, while the purely polymeric poly(hydroxymethacrylate) column (Shodex ODP2 HP-4D) lacks this appearance and show bands that look more like type I peaks.

To even further investigate this phenomenon, two high pH resistant columns were chosen for extended studies: Eternity C18, a recently released organic hybrid silica C18 column (ethyl groups inserted as an organic/inorganic interfacial gradient from the surface and down), and PLRP-S, a polymeric column with polystyrene/divinylbenzene particles. Three different probes were used: a "neutral" compound, 3-phenyl-1-propanol (PP), a quaternary amine benzyltriethylammonium chloride (BT) and finally a base, metoprolol (ME). First, retention factors at different pH with the same amount of modifier were determined, followed by the analysis of overloaded elution zones at different pH around the pK_a of metoprolol.

4.1. Analytical study

The three chosen probes were studied with analytical injections, to see how the retention time varies across the pH range. This was done by injecting 5 μL of a 0.25 mM solution of each probe in 1 pH-unit steps from $^{w}_{w}$pH 3 to $^{w}_{w}$pH 11.

PP is neutral under these conditions and is used as a control substance in this study, to assure the well-being and consequent behavior of the columns throughout the study. This is important since it has been reported that polymeric columns suffer from swelling and shrinking. As can be seen in Fig. 2, PP (dashed line with open triangular symbols) performance is unaffected by pH in the whole pH range studied in the analytical study, on both columns. The uniform behavior of the neutral probe indicates that the column materials from a neutral probes perspective are unaffected by pH. The efficiency for PP on Eternity was approximately 11,000 plates over the whole pH range studied, and for PLRP-S it was approximately 4200 plates. The asymmetry factor (5%) was approximately 0.97 for Eternity and 1.16 for PLRP-S.

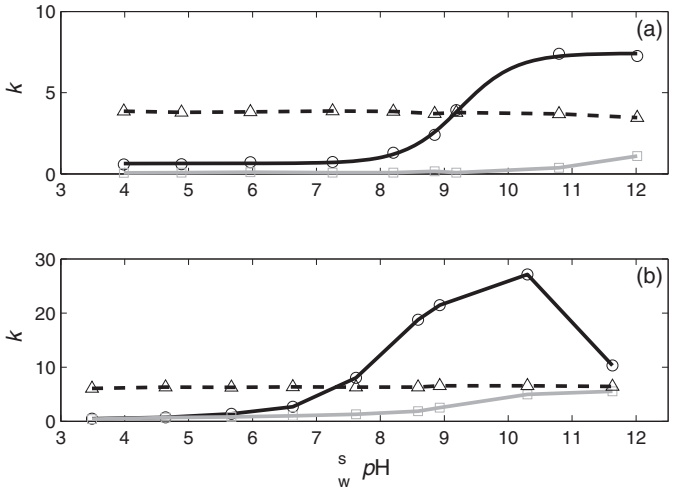

Fig. 2. Retention factors versus $^{s}_{w}$pH using the (a) Kromasil Eternity column and (b) the PLRP-S column. 5 μL of a 0.25 mM solution of each probe was injected, at different pH. ME is the solid black line and open circle symbols, BT is shown with the gray solid line with open rectangular symbols, and PP is the black dashed line with open triangular symbols.

Negative surface charges (silanols on silica matrixes) will contribute to the retention of positively charged solutes. The quaternary amine has a positive charge at all pHs. This feature could give information about changes of the stationary phase surface charges due to pH. As can be seen in Fig. 2a and b, the analytical retention factor for BT (gray solid line with gray open rectangular symbols) increases at high pH on both columns; just slightly on the C18 column and more pronounced on the polymeric column. The increase on the polymeric column also starts at a lower pH compared to the hybrid phase. Why the retention of BT increases at high pH on the polymeric column cannot be explained in the same way as for the silica column [14], since the polymeric surface lack residual silanols. There are however other negatively charged synthesis residues capable of cationic interactions on the polymeric phase, as described by others [15,16]. The efficiency for BT on Eternity was around 9000 plates at low $^{S}_{w}$pH, but drops down to about 6000 plates when $^{S}_{w}$pH increases above 9. The asymmetry factor increases correspondingly above $^{S}_{w}$pH 9, from approximately 1.16 at low $^{S}_{w}$pH to approximately 2.1 at high $^{S}_{w}$pH. On PLRP-S, the efficiency drops dramatically from about 8000 plates at low $^{S}_{w}$pH to less than 400 plates at high $^{S}_{w}$pH. The asymmetry factor increases in an opposite pattern, from 1.01 at low $^{S}_{w}$pH to approximately 3.5 at high $^{S}_{w}$pH.

The pH effect on the retention time for ME, which has an estimated p*K*a of about 9.60–9.70 [17,18] is shown for the hybrid silica column in Fig. 2a and for the polymeric column in Fig. 2b (black solid line with open circle symbols). The line in Fig. 2a shows the fitting of the retention factors of ME in 50% methanol (from the analytical test series on the Eternity column) as a function of $^{S}_{w}$pH, to Eq. (1). The apparent pK_a was estimated to 9.24 using Eq. (1) in 50% methanol (pK_a values for metoprolol were also estimated in the sample making for the overloaded study, see Table 3), which is lower than the value measured in aqueous solution. At low to moderate $^{S}_{w}$pH values the retention factor for metoprolol is low, as can

Table 3
Experimental details and calculated results from the overloaded ME series. α is the ratio of uncharged solute to the total concentration.

${}^{w}_{w}$pH bufffer	ME on Eternity (MeOH)					ME on PLRP-S (ACN)				
	% Modif.	Approx. buffer conc. [mM]	${}^{s}_{w}$pH eluent	App. pK_a ME	α	% Modif.	Approx. buffer conc. [mM]	${}^{s}_{w}$pH eluent	App. pK_a ME	α
5	27	14.6	5.3	6.76	0.036	18	16.4	5.2	6.66	0.033
6	27	14.6	6.5	7.87	0.041	23	15.4	6.4	7.77	0.042
7	29	14.2	7.6	8.74	0.064	33	13.4	7.7	8.79	0.076
8	40	12.0	8.7	9.31	0.196	50	10.0	8.9	9.19	0.315
9	40	12.0	8.9	9.53	0.194	47	10.6	8.9	9.42	0.220
10	50	10.0	10.9	9.54	0.962	45	11.0	10.4	9.49	0.890

be expected, since the molecule in this range is positively charged. As the ${}^{S}_{w}$pH increases, there is also an increase in the retention factor for metoprolol on both columns. This increase in retention factor starts at somewhat lower ${}^{S}_{w}$pH values for the polymeric column than for the silica column; this is likely due to ion exchange interactions, which affect the retention at a lower pH on the polymeric column than on the silica column (see BT discussion above). The drastic decrease in retention factor at high pH on the polymeric column was observed and explained in Ref. [16], as a cease in the strong cation exchange mechanism when the solute becomes uncharged. This effect was especially prominent with acetonitrile eluents, which are also used in this study. The efficiency for ME on Eternity is somewhat inconsistent in the intermediate ${}^{S}_{w}$pH interval, but increases from approximately 7500 plates at low ${}^{S}_{w}$pH to 8400 plates at high ${}^{S}_{w}$pH. The asymmetry factor follows an equally scattered behavior at intermediate ${}^{S}_{w}$pH, but increases from approximately 1.06 at low ${}^{S}_{w}$pH to 1.17 at high ${}^{S}_{w}$pH. On the PLRP-S column, the efficiency decreases dramatically from about 7000 plates at low ${}^{S}_{w}$pH to about 140 plates already from around ${}^{S}_{w}$pH 6.6. The asymmetry factor increases from 1.07 at low ${}^{S}_{w}$pH to around 3 at high ${}^{S}_{w}$pH.

4.2. Overloaded study

4.2.1. Metoprolol (basic ionizable probe)

In Fig. 1 the peak shapes of ME is shown at ${}^{w}_{w}$pH 7 for eight different RP columns. All columns with C18 surface chemistries show the same kind of peak deformation regardless of the base matrix, whereas the bands on the purely polymeric phase have more or less right-angled triangular shape (type I).

To further investigate this, the same kind of overload study was conducted, but using different eluent pH. Fig. 3 shows the same types of injections for the Kromasil Eternity column (Hybrid silica C18) and the PLRP-S column (polystyrene/divinylbenzene) respectively. The difference in behavior of this ionizable compound on these two different RP phases is remarkable. While the purely polymeric column shows type I isotherm behavior throughout the whole pH range used, the C18 column displays a dramatic change in peak shape. It starts with type I behavior at low pH (${}^{w}_{w}$pH 5 (not shown) and 6) with a sharp front and a very diffuse rear. At ${}^{w}_{w}$pH 7 the front is sharper and the band is more compressed regarding both front and rear (the type II peak shape from Fig. 1 is not observed at 20 mM here, but appears at higher concentrations).

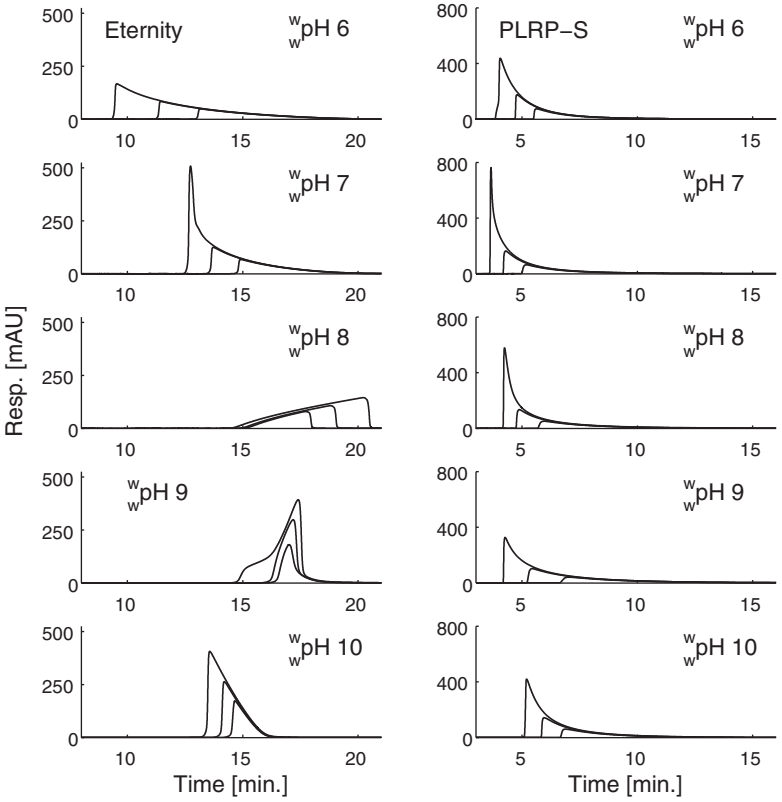

Fig. 3. Overloaded injections of pH matched samples of 5, 10 and 20 mM ME on (left column) Eternity and (right column) PLRP-S at different eluent pH (see legend). The organic modifier was methanol on the Eternity column and acetonitrile on the PLRP-S column. For more experimental conditions, see Section 3.

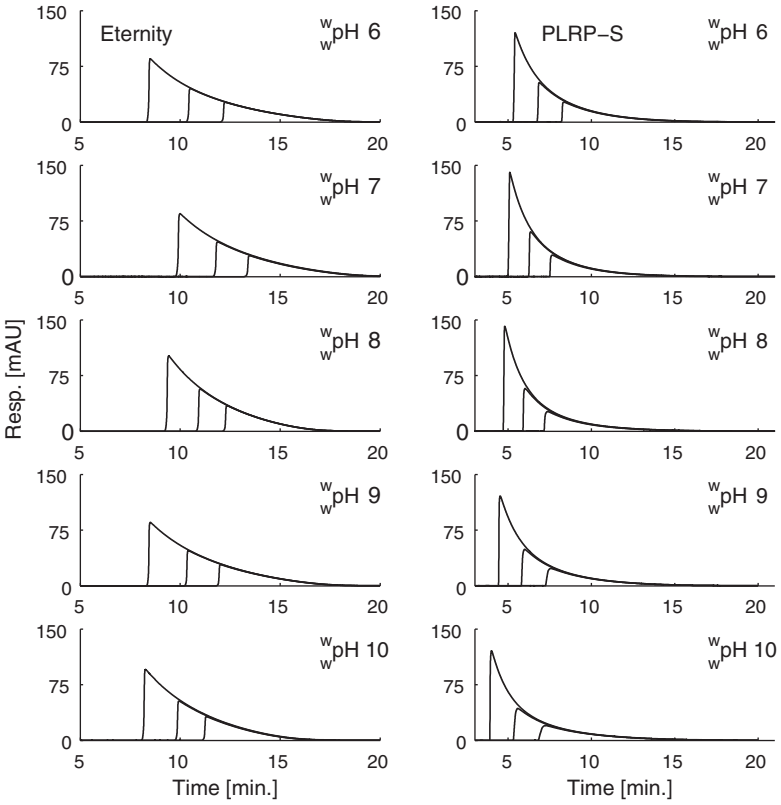

Fig. 4. Overloaded injections of 5, 10 and 20 mM BT on (left column) Eternity and (right column) PLRP-S at different eluent pH (see legend). The organic modifier was methanol on the Eternity column and acetonitrile on the PLRP-S column. For more experimental conditions, see Section 3.

When going higher in pH ($^{w}_{w}$pH 8) the bands show a dramatic switch to type III appearance, with a very diffuse front and a sharp rear. At $^{w}_{w}$pH 9 the band is once again more compressed and raises up to what looks like an intermediate between a compressed type III and type II shape. Finally, at $^{w}_{w}$pH 10 the band once again shows type I behavior, although much more compressed than at low $^{w}_{w}$pH. These transitions have been observed before, but have been assigned to pH-differences between the sample and the eluent [9], a condition that does not prevail in this study.

4.2.2. Benzyltriethylammonium chloride (positively charged quaternary ammonium probe)

BT is an aprotic quaternary ammonium ion that is constantly positively charged independently of the sample pH, due to the fully substituted nitrogen atom. As can be seen in Fig. 4, BT peak shape (type I) is basically unaffected by pH on both columns across the whole pH interval used, except for minor changes in the retention time (generally a decrease with higher pH) and a somewhat less sharp front for the lower concentrations on the PLRP-S column at higher pH.

4.2.3. Phenyl-1-propanol (neutral probe)

PP is a "neutral" probe (pK_a value 15.03 in water solution [19]) that is uncharged throughout the whole pH range. As can be seen in Fig. 5, PP peak behavior is identical regardless of pH on both columns across the whole pH range used. The only visible difference is the lower efficiency and/or higher loadability on the PLRP-S column as compared to the Eternity column. In the analytical test series of PP, the efficiency on the Eternity column is 11,000 plates and on the PLRP-S it is merely 4200 plates.

4.3. Observations of pH stability

Similar behaviors around the pK_a of a base as displayed by ME on the Eternity column has been observed previously [7,10]. An isotherm model explaining similar peak deformations of bases as seen in the present study has been suggested [8]. In this previously described model it is assumed that α varies with the concentration of the solute and that the deformation of the peaks arises from a pH difference between the mobile phase and the injected sample, causing a titration effect during the elution of the band. When this is assumed, the model in Eq. (2) can account for the apparent adsorption isotherm switch causing the distorted peak shapes that occur close to the pK_a of the solute. In the present study however, the pH of the sample solution and the mobile phase is carefully matched, thus no pH-mismatch prevails and α is constant throughout the column. To investigate if the suggested model is valid also in the case of pH-matched solutions of the solute, elution profiles were numerically calculated using the equilibrium dispersive model solved using the Rouchon algorithm [20] for this adsorption isotherm model (Eq. (2)) [8]. The following parameters were used: $q_{S,N} = 46.1$ g/L, $q_{S,I} = 0.369$ g/L, $b_I = 1.15$ L/g, $b_N = 0.079$ L/g, $\varepsilon = 0.65$, $V_{inj} = 10$ μL, flow = 1 mL/min. The ratio α was varied between 0 and 1. Two distinct injection concentrations were simulated (0.05 and 0.5 M), results are shown in Fig. 6.

As can be seen in Fig. 3a, the adsorption isotherm type switch of the ME bands in the regions close to the pK_a most certainly still occurs, even though no pH-mismatch is present. From the simulation in Fig. 6, it can be seen that the previously described adsorption isotherm model does not account for these peak shapes under pH-matched conditions, where this model instead suggests that all bands regardless of concentration and α shows type I behavior. The reason for band distortions in this case must hence have another explanation. In studies of the effect of weakly buffered solutions on

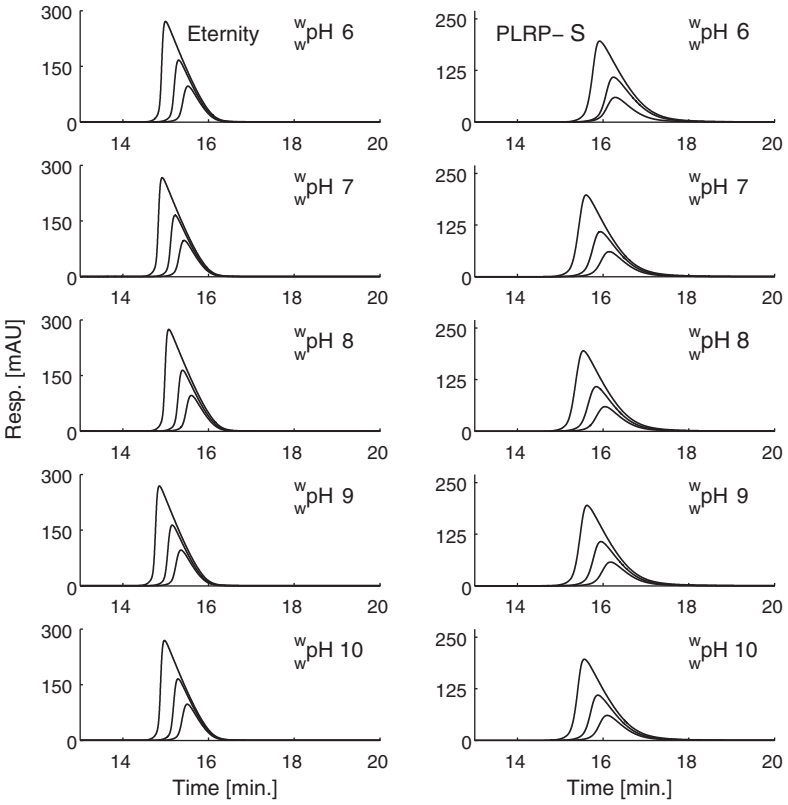

Fig. 5. Overloaded injections of 5, 10 and 20 mM PP on (left column) Eternity and (right column) PLRP-S at different eluent pH (see legend). The organic modifier was methanol on the Eternity column and acetonitrile on the PLRP-S column. For more experimental conditions, see Section 3.

the response of certain electrodes, a deviation from the expected response was assigned to a pH gradient in the near vicinity of the electrode surface [21]. The surface would then have a lower pH than the bulk solution. In this study, where the basic solute itself in many cases is the component that contributes most to the buffering capacity of the solution, an opposite gradient could be formed. If it is assumed that the uncharged form of the solute has a higher affinity towards the stationary phase, a gradient with higher pH at the surface than in the bulk eluent could arise. In this way, a pH difference effect could occur despite the absence of an initial pH difference.

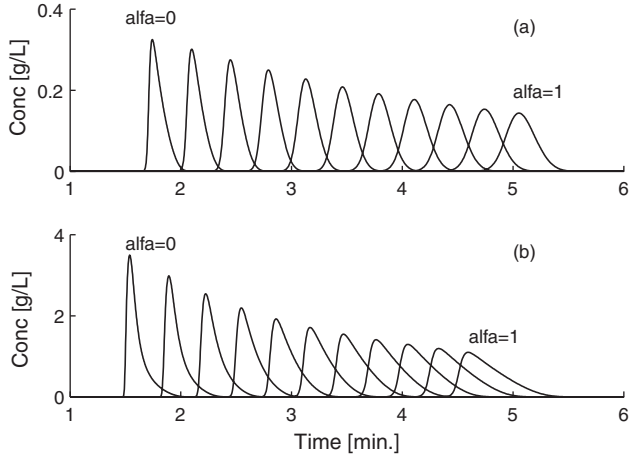

Fig. 6. Simulation of chromatograms with different fractions of charged to total concentration. Parameters used were $q_{S,N}$ = 46.1 g/L, $q_{S,I}$ = 0.369 g/L, b_I = 1.15 L/g, b_N = 0.079 L/g, ε = 0.65, V_{inj} = 10 μL, flow = 1.00 mL/min. α was varied between 0 and 1. The injected concentration solute was (a) 0.05 M and (b) 0.5 M.

5. Conclusions

The present study comprised an analytical section and an overloaded section; in the first one, it was investigated how the retention factor of three solutes changes with $^{s}_{w}$pH. In the overloaded section the focus was on the band shapes of the basic solute, where the injected sample was carefully $^{s}_{w}$pH-matched with the eluent. It was shown that distorted bands appear on C18 modified materials (polymeric, hybrid silica and silica) even though sample and eluent is pH-matched. This occurs in spite of the prevalent theory, which suggests that such distortions arise from a concentration dependent separation factor and a pH-mismatch between sample and eluent. The purely polymeric phase (PLRP-S) used in the present work shows an increase in retention time at intermediate to high pH for a positively charged quaternary amine. This suggests that there might be negative groups on the polymeric phase, but the phenomenon of the distorted peaks is not observed on the polymeric phase lacking C18 chains. No distorted peaks were observed for the neutral and the positively charged probe at any pH on any column.

In the case of the 5.0 and 10.0 mM injections of Metoprolol the sample concentration never exceeds the buffer concentration, a condition that otherwise has been suggested as an explanation to why peak distortions occur with low concentration buffers [9,10]. The appearance of distorted peaks in spite of a lower sample concentration than buffer concentration in the present study is especially obvious at $^{w}_{w}$pH 8 and 9 (Fig. 3a) where all injected concentrations result in behavior deviating from type I.

The reason for the distorted peaks in the present work does not seem to be an initial pH-difference effect, considering the pH-matching of the sample with the eluent. Hence there is no pH-mismatch, with reservation for any effects that might emerge when the sample reaches the adsorbent after the injection moment.

Nevertheless, even if the observed peaks are not the result of a pH-effect, it still seems to be related to the pK_a, i.e. the ionization state of the solute, judging from where in the pH interval the distorted peaks occur. The pH gradient at surfaces described by Gratzl et al. [21] could be a hint in the right direction, but this remains to be elucidated.

Acknowledgements

This work was supported by a grant from the Swedish Research Council (VR) for the project "Fundamental Studies on Molecular Interactions Aimed at Preparative Separations and Biospecific Measurements". Thanks to Johan Ekeroth at EKA Chemicals for kindly providing the Kromasil Eternity column. We are also grateful to Henrik Johansson at the Department of Biochemistry and Organic Chemistry, for feedback and providing the equipment for preparing metoprolol HCl.

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