Effects of pH and temperature on the chromatographic performance and stability of immobilized poly(methyloctylsiloxane) stationary phases

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ABSTRACT

The effects of mobile phase pH, temperature, buffer type and buffer concentration on the selectivity and stability of four stationary phases, with different PMOS loadings, prepared by the thermal immobilization of poly(methyloctylsiloxane) on to silica (PMOS-SiO$_2$), were evaluated with both hydrophobic and hydrophilic basic solutes. These solutes show longer retention times at near neutral pH, where both the silanols and the basic solutes are partially ionized, and shorter retention times in more alkaline pH, where the silanols are mostly ionized and the basic solutes are not ionized. Increases in temperature and buffer concentration also result in shorter retention times. These PMOS-SiO$_2$ stationary phases are quite stable at low pH and are also stable at ambient temperature (23 °C) using pH 7 phosphate. The PMOS-SiO$_2$ stationary phases are more stable at higher pH using triethylamine (pH 11) and borate (pH 10) buffers than with phosphate and carbonate buffers. Temperature increases stationary phase degradation, while buffer concentration has a minimal effect on stationary phase degradation, indicating that these PMOS-SiO$_2$ stationary phases have stabilities similar to the equivalent chemically bonded phases.

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1. Introduction

The preparation and characterization of RP-HPLC stationary phases requires considerations of both the mobile phase and the stationary phase [1–6]. The objective of our first paper [7] was to show that seventeen PMOS-SiO$_2$ stationary phases with different C% have high ion-exchange properties in near neutral mobile phases using the Tanaka test [8] but afford good peak shapes and high efficiencies for highly basic solutes and benzodiazepines at neutral pH. Chemometrics showed that these PMOS-SiO$_2$ stationary phases were very different from most commercial stationary phases, using the Euerby classification protocol [9]. In a more recent paper [10] we explored the PMOS-SiO$_2$ ion-exchange properties, previously observed with the Tanaka test, using chromatographic evaluations of several basic test solutes at different mobile phase pH values. Using different buffer types, this study showed that at different mobile phase pH, the buffer type caused large variations in the retention factors of these basic solutes with the PMOS-SiO$_2$ stationary phases studied. On the other hand mobile phase pH and

different buffer types have lesser effects on the retention of these basic solutes with commercial C8 stationary phases. This paper [10] also showed that the selectivities for basic solutes afforded by a PMOS-SiO$_2$ stationary phase are totally different from those of chemically bonded C8 stationary phases, confirming the results obtained in the first study.

In this manuscript we want to show how the mobile phase at different pH with the same buffer type and mobile phases at the same pH with different buffer types affect basic solute retentions using four PMOS-SiO$_2$ stationary phases with different carbon contents. Stationary phase stability was then evaluated using these same variables (pH, buffer type and concentration), as well as temperature.

2. Experimental

2.1. Chemicals and reagents

The mobile phases were prepared with ultrapure water from a Millipore Direct-Q^{TM} system (Billerica, USA). Methanol and isopropanol were from Tedia (Fairfield, USA). Tetrahydrofuran was from J.T. Baker (Phillipsburg, USA). Pentane was purchased from Merck (Darmstadt, Germany).

The compounds used to prepare mobile phases were: HCl (36.5–38%), KH$_2$PO$_4$ (98%), K$_2$HPO$_4$ (99%) and KHCO$_3$ (99.7–100.5%)

from Synth (Diadema, Brazil), KOH from Merck (Rio de Janeiro, Brazil), phosphoric acid (85%) from Casa da Química (Diadema, Brazil), N-(2-hydroxy-1,1-bis(hydroxymethyl)ethyl)glycine (tricine) (99%) from Sigma (St. Louis, USA), sodium borate from Fisher (Fairlawn, USA), triethylamine (TEA) (99%) from Vetec (Duque de Caxias, Brazil) and ammonium hydroxide (28–30%) from LabSynth (Diadema, Brazil).

The silica used to prepare the stationary phases was Kromasil, lot no AT 1959, from Akzo Nobel (Bohus, Sweden), with 5 μm particle size, 11.1 nm pore size and 313 m^2/g specific surface area. The polysiloxane used was poly(methyloctylsiloxane), number-average molar mass, M_n, 6200 and weight-average molar mass, M_w, 16,000, obtained from United Chemicals Technologies (Bristol, USA).

The test solutes were: uracil (mobile phase volume marker, 98%), butylbenzene (BB) (>99%) and amitriptyline hydrochloride (ami) (99%) from Aldrich (Milwakee, USA), HPLC grade toluene from Tedia and quinizarin (96%), nortriptyline hydrochloride (nor) (98%), (−)-nicotine (ni) (98–100%), (±)-chlorpheniramine maleate (Ch) and procainamide hydrochloride from Aldrich (Steinheim, Germany). Codeine sulfate (co), diphenhydramine hydrochloride (D), propranolol (pr), salbutamol sulfate (sa) and methadone (me) were kindly donated by Dr. Marcelo Ribani from TECPAR (Curitiba, Brazil) while fluoxetine (Flux) was kindly donated by Dr. Paulo César Pires Rosa of EMS (Hortolândia, Brazil).

2.2. Preparation of the stationary phases

As described in previous papers [7,10] the four stationary phases were prepared using different amounts of PMOS (g PMOS/g silica) and different times and temperatures of thermal treatment to produce stationary phases with different carbon contents. The conditions are summarized in Table 1.

The stationary phases were slurry packed (0.8 g of stationary phase in 20 mL isopropanol-tetrahydrofuran (20:80, v/v)) into previously polished 50 mm × 3.9 mm columns, made from 316 stainless steel tubing at a constant packing pressure of 40 MPa, using a Haskel Packing Pump (Burbank, USA) with methanol as

Table 1
Percentages of PMOS per gram of silica (%PMOS), time (t) and temperature (T) of immobilization used to prepare the PMOS-SiO$_2$ stationary phases.

Code	PMOS%[a]	t (h)	T (°C)	C%	PMOS%[b]
SP1	30	8	100	5	9
SP2	30	4	130	7	10
SP3	60	4	100	9	15
SP4	45	6	140	12	20

[a] Amount of PMOS used to prepare the phases.
[b] Amount of PMOS immobilized onto the Kromasil silica surface after the packing procedure.

propulsion solvent. The pressure was maintained until the passage of 200 mL of methanol to remove excess PMOS that was not imobilized [7,10]. All columns were conditioned for at least 2 h with mobile phase at 0.5 mL/min before the chromatographic evaluations. For comparison, a column was also packed with Kromasil silica having no polysiloxane phase.

2.3. Mobile phase preparation

All mobile phases were prepared volumetrically. The pH were measured in the aqueous phase with a calibrated pH meter, Qualxtron model 8010 (Jundiaí, Brazil), before the addition of organic modifier. Lower pH adjustments were made with hydrochloric acid solutions for organic buffers and phosphoric acid solutions for phosphate buffers, while potassium hydroxide solutions were used to adjust neutral and higher pH with both inorganic and organic buffers.

2.4. Chromatographic evaluations

All the chromatographic evaluations were performed using a modular HPLC system with a Shimadzu LC 10AD pump (Kyoto, Japan), a Rheodyne model 8120i injection valve (Cotati, USA) with 5 μL loop, a Shimadzu CTO-10AC column oven and a Shimadzu Model SPD-10 AV UV–vis detector. Data were processed using.

Table 2
Influence of mobile phase pH on the retention factors (k) of some basic solutes on PMOS-SiO$_2$ stationary phases and on bare silica. All the tests were carried out in 80:20 (v/v) methanol:phosphate buffer mobile phases at 23 °C. Solute identification: pr, propranolol; ami, amitriptyline; nor, nortriptyline; ni, nicotine; co, codeine; Ch, chlorpheniramine; sa, salbutamol; D, diphenhydramine; me, methadone.

pH	pr	ami	nor	Ni	co	Ch	sa	D	me
	SP1								
6	2.1	5.8	4.9	1.2	4.0	8.7	1.2	3.7	5.1
7	2.3	2.3	8.7	0.6	2.3	5.5	1.6	2.5	6.7
8	1.4	2.8	6.4	0.3	1.4	2.7	0.9	1.4	3.7
11	0.9	1.8	4.2	0.2	1.1	1.6	0.6	0.9	2.3
	SP2								
6	1.8	5.4	4.1	1.2	3.7	8.4	1.1	3.3	4.2
7	2.3	4.7	9.2	0.6	2.0	5.6	1.3	2.4	6.3
8	0.7	1.5	3.6	0.2	0.9	1.5	0.5	0.7	1.8
11	0.6	1.2	3.0	0.1	0.8	1.1	0.5	0.5	1.4
	SP3								
6	2.1	5.8	5.8	0.8	2.8	8.5	1.1	3.1	5.1
7	2.3	5.2	10.4	0.7	2.4	6.6	1.6	2.2	5.8
8	0.8	1.9	4.7	0.1	0.9	1.8	0.4	0.6	2.1
11	0.7	1.5	3.4	0.2	0.8	1.3	0.4	0.6	1.5
	SP4								
6	9.0	38.3	38.0	2.2	4.7	34.5	1.2	17.0	25.8
7	7.9	22.8	41.8	1.2	3.0	19.5	1.2	9.2	25.3
8	5.0	12.9	29.2	0.8	2.4	10.8	0.9	5.4	15.4
11	1.8	5.3	10.5	0.4	1.3	3.0	0.2	1.9	4.5
	Bare silica								
6	0.8	1.7	1.6	0.7	3.0	4.0	1.0	1.5	1.8
7	1.7	0.8	2.4	0.3	1.6	2.2	1.5	0.7	1.8
8	3.0	3.0	5.0	0.9	3.6	6.6	3.4	2.8	2.8
11	0.2	0.1	0.8	0.0	0.6	0.4	0.4	0.1	0.6

Table 3
Retention factors (k) of some basic solutes on PMOS-SiO$_2$ stationary phases and on bare silica. All the tests were carried out in 80:20 (v/v) methanol:tricine buffer (pH 8; 20 mmol/L) mobile phases at 23 °C. Solute identifications as in Table 2.

	pr	ami	nor	Ni	co	Ch	sa	D	me
SP1	5.3	10.0	14.1	1.4	4.7	14.0	3.3	6.4	12.7
SP2	2.2	5.4	5.7	0.9	3.3	8.0	1.4	3.3	5.1
SP3	2.5	6.4	7.1	0.9	3.1	9.3	1.3	3.4	5.8
SP4	9.2	30.6	39.2	1.7	4.4	30.1	1.4	13.8	26.3
Bare silica	2.8	3.0	4.2	1.1	3.7	7.1	2.7	3.0	4.4

ChromPerfect software from Justice Innovations (Mountain View, USA). All tests were conducted at a flow rate of 0.5 mL/min.

2.5. Stability evaluations

Column stability tests were performed using a modular HPLC system from Shimadzu equipped with a LC-10AD LC pump, a SPD-10A UV–vis detector, a CTO-10AS column oven, a SIL-10AD automatic injector and a SCL-10A system controller. Data were acquired and processed using the CLASSVP program (Shimadzu). The columns under test were continuously purged with fresh mobile phase, not recycled, at 0.5 mL/min. Detection was at 254 nm.

2.6. Software

All graphs were constructed using OriginPro 7.5 SRO v7.5714(B714) (Northampton, USA).

3. Results and discussion

3.1. Effects of mobile phase pH and buffer type on retention factors

The PMOS-SiO$_2$ stationary phases present mixed-mode retention mechanisms due to the combination of hydrophobic interactions that occur between the solutes and the octyl groups and ion-exchange interactions that occur between the basic solutes and the free silanols [11]. Thus retention occurs due to the contributions of both processes, consistent with the "three site model" [12]. This observation is confirmed by comparison of the retention factors obtained with the four PMOS-SiO$_2$ stationary phases and with a bare silica stationary phase (the same silica used to prepare the PMOS-SiO$_2$ stationary phases) (Table 2). If the basic solutes had been retained on PMOS-SiO$_2$ stationary phases only by ion-exchange interactions, these solutes would have also been highly retained on the bare silica stationary phase.

Different selectivities are due to different degrees of ionization of the test solutes and of the free silanols on the stationary phase surface as well as to the different interactions of different buffers with the stationary phase. The mobile phase pH and the pK_a of the solutes in aqueous mixtures with organic modifiers, defined as $_w^s$pH and $_w^s$pK_a, are functions of organic modifier type, organic modifier amount and temperature [13–16]. Thus, different buffers result in different $_w^s$pH and also in different interactions of the solutes with the stationary phase. Since the PMOS-SiO$_2$ stationary phases retain basic solutes by synergic interactions, which involve both ion-exchange and hydrophobic interactions, the degrees of ionization of both the silanols and the basic solutes have significant influences on the retention factors of basic solutes on PMOS-SiO$_2$, independent of the C%.

As shown in Table 2, the retention factors of propranolol, nortriptyline, methadone and salbutamol increase as the pH increases from 6 to 7 but decrease as the pH goes from 7 to 11. However, the retention factors of the other test solutes (nicotine, codeine, chlorpheniramine and diphenhidramine) decrease as the pH goes from 6 to 11. These effects are more evident on SP1, SP2 and SP3 than on SP4, which has a higher C% and, thus, fewer free silanols to ionize.

According to Subirats et al. [15] a phosphate buffer solution prepared at pH 7.0 has a $_w^s$pH of 9.6 after the addition of methanol to prepare a 80:20 methanol:buffer mobile phase, while, according to Buckenmaier et al. [16] the basic solutes nortriptyline and diphenhydramine have pK_a values of 10.1 and 9.2, in water, but $_w^s$pK_a values in 80:20 methanol:water of 9.2 and 8.2, respectively. Thus, according to the Henderson–Hasselbalch equation for $_w^s$pK_a and $_w^s$pH, these solutes would be 37.2% and 3.5% protonated, respectively, at a nominal pH of 7.0. Thus the retention factor of diphenhydramine decreases, while that of nortriptyline increases when the pH of the phosphate buffer solution used to prepare the mobile phases increases from 6 to 7, as the mobile phase prepared with a phosphate buffer at pH 7.0 has a $_w^s$pH almost 2 units higher than the $_w^s$pK_a of diphenhydramine. This solute is almost 100% unprotonated at this pH. Its deprotonation results in decreased retention since the unprotonated solute has reduced ion-exchange interactions with the free silanols. Nortriptyline still nearly 50% protonated at a nominal pH 7.0 in phosphate buffer, while the increase in pH results in deprotonation of the free silanols on the stationary phase surface. The increased deprotonation of the free silanols results in increased retention of nortryptyline, since the partially protonated test solute has ion-exchange interactions with the free silanols. Similar explanations can be given for the other solutes.

The $_w^s$pH obtained with tricine is lower than the $_w^s$pH obtained with phosphate buffer. Thus the $_w^s$pH obtained with tricine buffer does not deprotonate the basic solutes, but is high enough to deprotonate the free silanols, resulting in increased ion-exchange

Table 4
Effects of phosphate buffer concentration (mmol/L) on retention factors of some basic solutes on PMOS-SiO$_2$ stationary phase SP3 and on bare silica. The tests were carried out in 65:35 (v/v) methanol:phosphate buffer (pH 7) at 23 °C. Solute identification as in Table 2.

mmol/L	pr	ami	ni	Co	Ch	sa	D	Me
	Retention factors on PMOS-SiO$_2$							
10	8.8	35.6	2.0	4.9	30.1	3.3	13.8	34.7
50	6.4	27.8	1.6	4.1	22.4	2.1	10.3	24.9
100	2.5	14.2	1.0	2.6	10.8	0.7	5.3	9.4
	Retention factors on bare silica							
10	6.8	2.9	1.1	3.1	6.4	3.2	2.5	6.8
50	1.6	1.3	0.7	2.0	3.2	1.6	1.1	1.6
100	0.7	0.6	0.6	1.3	1.4	0.6	0.5	0.7

interactions and retention factors higher than those obtained with phosphate buffer (Table 3).

3.2. Effects of buffer concentration on retention factors

The mobile phase buffer concentration also has a large impact on solute retention in ion-exchange chromatography but only a minor effect in RP-HPLC [17]. Thus it is important to examine how changes in buffer concentration can affect retention on PMOS-SiO$_2$ stationary phases. 10, 50 and 100 mmol/L phosphate buffers at pH 7 were chosen to ensure that the effect would be large enough to be detected. The mobile phases were 65:35 (v/v) methanol:phosphate buffers. Due to the high aqueous content, nortriptyline was removed from the test solute mixture.

The effects of buffer concentration on the retention factors of the test solutes are shown in Table 4. For all the solutes, the retention factors decrease as the buffer concentration is increased. However, the decreases in the retention factors with the increases in buffer concentration are different for the various solutes. This means that when the concentration of the mobile phase additive changes, the retentions of the different solutes do not change proportionally. Thus, selectivity (band spacing) will vary as the buffer concentration is changed. The elution order of salbutamol and nicotine was inverted as the buffer concentration increased, confirming the influence of the ion exchange mechanism for the PMOS-SiO$_2$ stationary phases.

There are strong synergistic sites on the PMOS-SiO$_2$ stationary phases with combined reversed-phase and ion-exchange interactions in accordance with the Neue et al. [12] "three site model". The overall retention for bases is described by the relationship: $k = k_{RP} + k_{IEX} + k_{RP}k_{IEX}$. These synergic interactions between the reversed-phase and ion-exchange sites are confirmed once more when some basic solutes are evaluated under the same conditions using a bare silica stationary phase, as also shown in Table 4. The retention factors obtained using a bare silica stationary phase are lower than those obtained with the PMOS-SiO$_2$ stationary phases and the reduction observed in retention factors with the buffer concentration increase is greater in the PMOS-SiO$_2$ stationary phase than with the bare silica stationary phase. If basic solutes were retained on the PMOS-SiO$_2$ stationary phase just by ion-exchange interactions, they should be more retained on the bare silica stationary phase. However, basic solutes are more retained on the PMOS-SiO$_2$ stationary phases, because these stationary phases retain basic solutes by isolated hydrophobic (k_{RP}) and ion-exchange (k_{IEX}) interactions and also by synergic ion-exchange and hydrophobic interactions ($k_{RP}k_{IEX}$), while the bare silica stationary phase retains basic solutes just by ion-exchange interactions.

3.3. Effects of temperature

The effects of temperature on retention factors and retention apparent enthalpies ($\Delta H°$, kJ K^{-1} mol^{-1}) in the temperature range from 16 to 60 °C are shown in Table 5. The large variations in the retention factors with small variations in temperature are due to the high apparent enthalpies of retention. For example, nicotine at 16 °C has the same retention factor as of butylbenzene but the apparent enthalpy of retention of nicotine is two times higher than the apparent enthalpy of retention of the neutral solute. Since the apparent enthalpies of retention are high and uncorrelated (the plots of the equation $\ln(k) = -\Delta H°/RT + \Delta S°/R + \ln(\phi)$ are not parallel), small variations in temperature result in large variations in selectivity. At 60° poorer selectivity is observed, since the retention factors are lower. However, at 16 °C some of the critical pairs seen at 40 °C are easily resolved, and vice versa. For example, the pairs chlorpheniramine-nortriptyline and methane-fluoxetine are easily separated at 16 °C but not at 40 °C, while the separation of
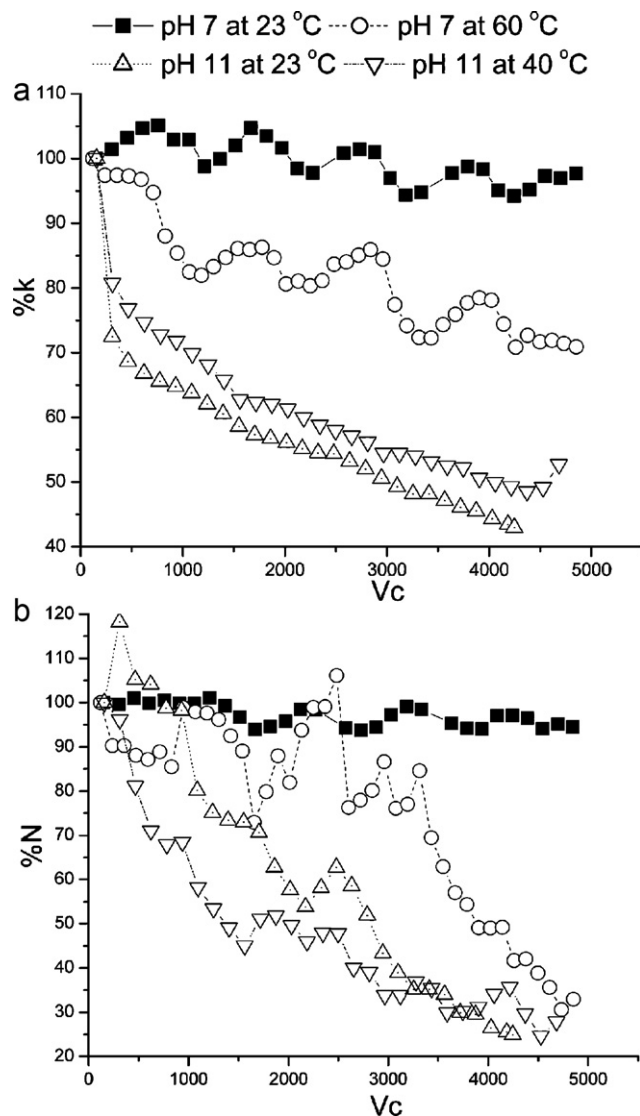

Fig. 1. The effects of pH at higher temperatures using PMOS-SiO$_2$ stationary phase SP4 (20% PMOS). Percent variation of (a) retention factor and (b) efficiency as functions of the passage of 65:35 (v/v) methanol:phosphate buffered mobile phases. The test solute was amitriptyline.

the pair amitriptyline-nortriptyline is difficult at 16 °C and easy at 40 °C.

3.4. Stability evaluations

3.4.1. Stability evaluations with phosphate buffers

Temperature and pH are both variables that affect selectivities for basic solutes, and both high pH mobile phases and higher temperatures rapidly degrade stationary phases, as shown in Fig. 1. A test carried out at 23 °C using 65:35 (v/v) methanol:phosphate buffer at pH 7 did not cause any loss in retention or efficiency, while a test test carried out using the same pH 7 mobile phase at 60 °C resulted in a 25% retention loss and an 80% efficiency loss in the same mobile phase volume. A test carried at 23 °C using phosphate buffer at pH 11 resulted in a rapid retention loss of 50% and an efficiency loss of 80%. Slightly faster degradation occurs at 40 °C than at 23 °C. These results reinforce other results that phosphate buffered mobile phases should not be used at temperatures of 60 °C or at pH higher than 7. On the other hand, a highly alkaline mobile phase did not provide better separations with basic solutes on these

Table 5

Effect of temperature on retention factors for basic and neutral solutes on PMOS-SiO$_2$ stationary phase SP3. The test were carried out in 80:20 (v/v) methanol:phosphate buffer (pH 7; 20 mmol/L). Solute identification as in Table 2 also Flux, fluoxetine; proc, procainamide; Q, quinizarin; BB, butylbenzene. $\Delta H°$ is calculated using the formula ln(k) = $\Delta H°/RT + \Delta S°/R + \ln(\phi)$, where T is the absolute temperature, R is the gas constant (8.31441 J K^{-1} mol^{-1}) and ϕ is the volume ratio of mobile phase to stationary phase (Vm/Vs).

T (°C)	pr	ami	nor	ni	co	Ch	D	me	Flux	proc	Q	BB
	k											
16	4.7	9.6	9.7	2.6	6.1	14.7	6.2	10.1	6.7	6.5	1.9	2.6
40	3.4	5.4	7.4	2.0	3.6	7.2	3.7	6.7	6.4	4.0	1.7	2.2
60	2.2	2.9	4.3	1.6	2.2	3.3	2.2	3.6	3.5	2.3	1.4	1.9
	$\Delta H°$ (kJ K^{-1} mol^{-1})											
	−13.9	−21.7	−14.4	−9.3	−18.6	−26.9	−18.8	−18.7	−11.0	−18.6	−5.2	−5.4

PMOS-SiO$_2$ stationary phases than did analyses carried out with phosphate at pH 7.

3.4.2. Stability evaluation with ammonia and triethylamine buffers

The effect of mobile phase pH and buffer type were also evaluated using pH 10.5 ammonia and pH 11.5 triethylamine mobile phases (Fig. 2). The effect of buffer concentration was evaluted by using 5 mmol/L and 180 mmol/L triethylamine mobile phases, while the effect of temperature was studied with 20 mmol/L triethylamine mobile phases at 23 °C and 60 °C.

The test carried out at 23 °C using 65:36 (v/v) methanol:triethylamine buffer at pH 11.5 caused only a 20% loss in retention factor, while efficiency was unchanged up to the end of the test. When the same mobile phase was used at 60 °C, a 25% retention factor loss and an 80% efficiency loss was seen. The test carried out using a triethylamine concentration
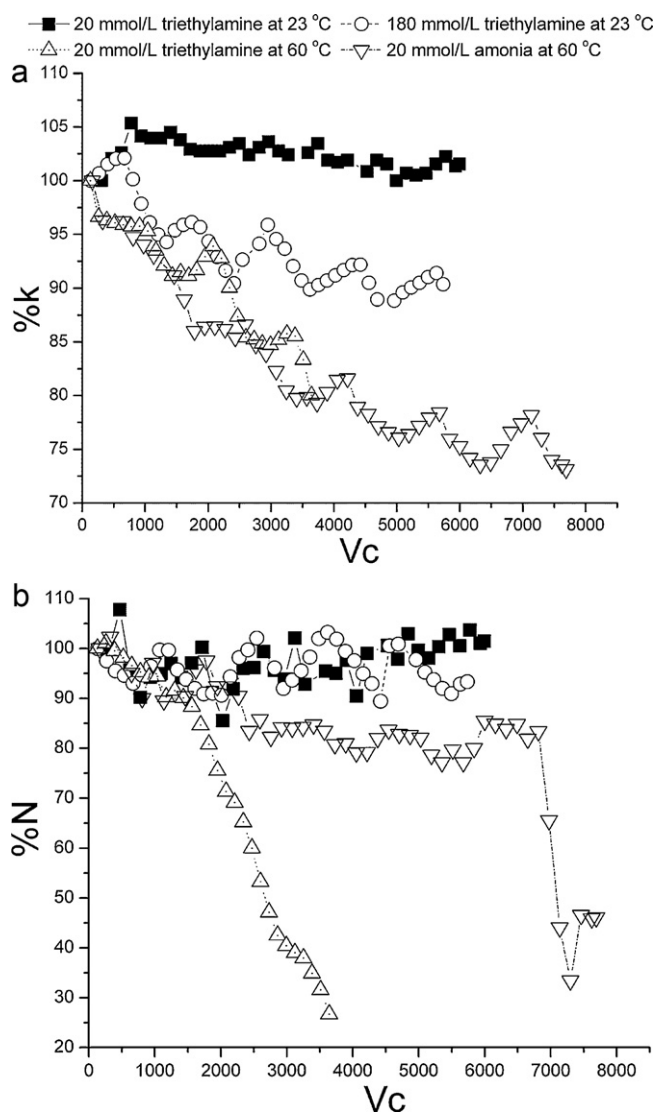

Fig. 2. The effect of ammonia and triethylamine buffer concentrations, pH and temperature using PMOS-SiO$_2$ stationary phase SP4. Percent variation of (a) retention factor and (b) efficiency as functions of the passage of 65:35 (v/v) methanol:buffered mobile phases. The test solute was amitriptyline.
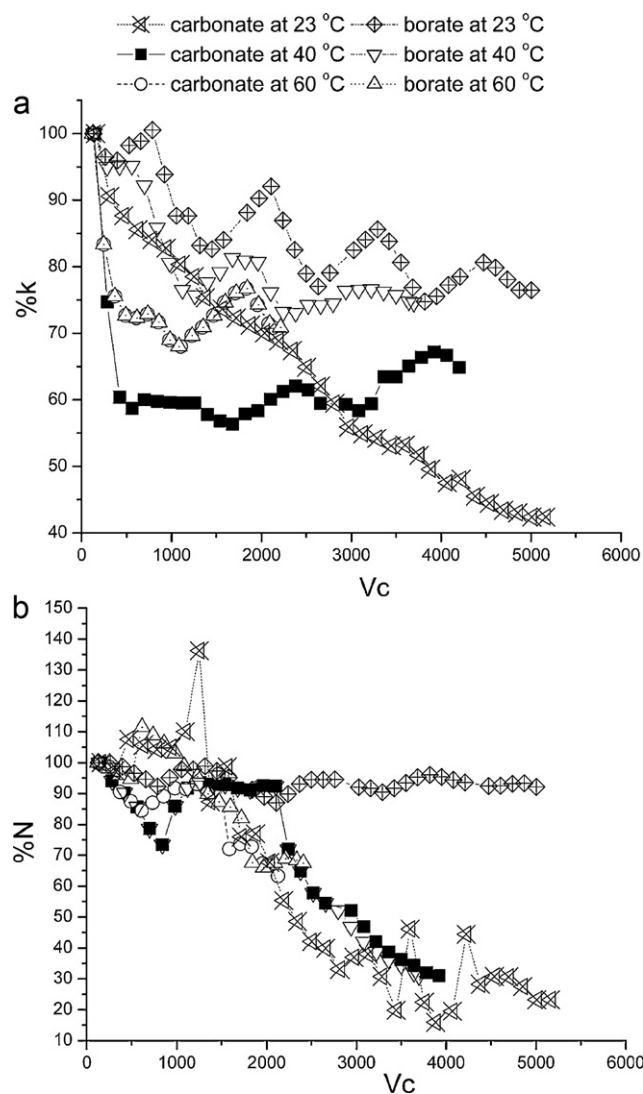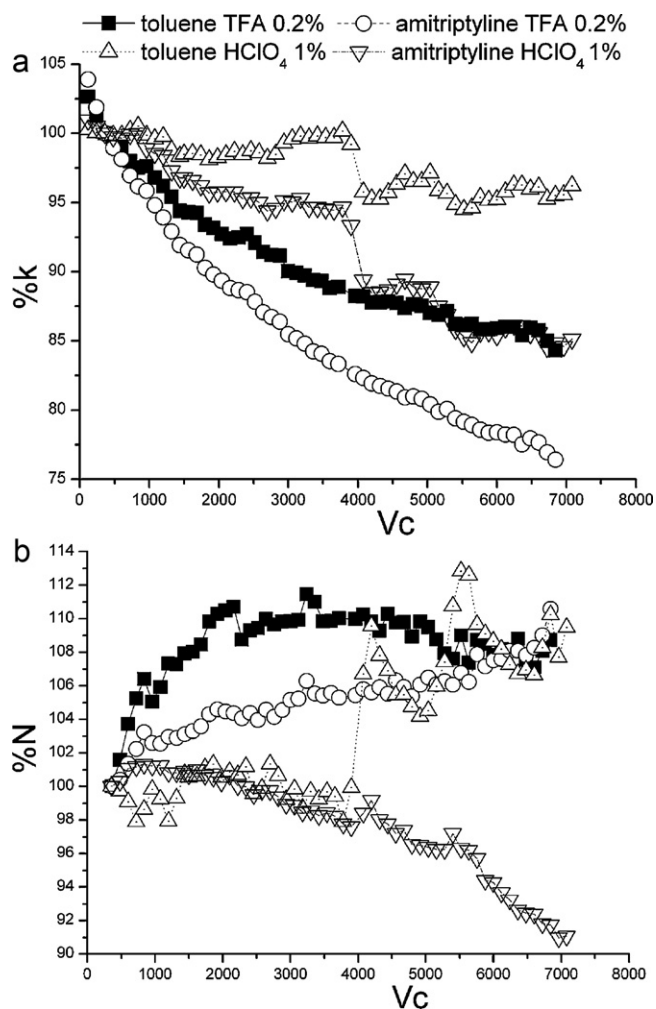

Fig. 3. The effect of carbonate and borate buffers at several temperatures on PMOS-SiO$_2$ stationary phase SP4. Percent variation of (a) retention factor and (b) efficiency as functions of the passage of 65:35 (v/v) methanol:buffered mobile phases. The test solute was amitriptyline.

Fig. 4. Stability at low pH using PMOS-SiO₂ stationary phase SP4. Percent variation of (a) retention factor and (b) efficiency as functions of the passage 50:50 (v/v) methanol:0.2% trifluoroacetic acid mobile phase at 80 °C and 50:50 (v/v) methanol:1% perchloric acid mobile phase at 60 °C. The test solutes were toluene and amitriptyline.

of 180 mmol/L at 23 °C caused a 10% retention loss although the efficiency was unchanged up to end of the test. The test carried out at 23 °C using 65:36 (v/v) methanol:ammonia buffer at pH 10.5 resulted in a 20% retention factor loss while the efficiency was unchanged up to the passage of 6500 Vc. After this volume the retention factor continued its slow decrease, indicating that the polymer was still present. However, the test done in 50:50 (v/v) methanol:triethylamine buffer (pH 12; 20 mmol/L) at 23 °C is less aggressive than tests done with phosphate buffer, causing only a ∼10% decrease in the retention factor, while the efficiency for toluene was actually slightly better at the end of the test than at the beginning.

3.4.3. Stability evaluations with carbonate and borate buffers

Claessens and van Straten [18] have shown that carbonate is more aggressive than phosphate and that both are much more aggressive than borate for chemically bonded phases (Fig. 3). These results should be compared with those obtained with phosphate buffer (Fig. 1), which causes similar efficiency losses (85%) and higher retention factor losses (65%) after the passage of only 400 Vc at 60 °C.

3.4.4. Stability at low pH

The PMOS-SiO₂ stationary phases present good peak shapes in acidic mobile phases, as do other stationary phases prepared by the immobilization of polysiloxanes onto silica surfaces [19]. The results of the low pH stability tests with 0.2% TFA and 1% perchloric acid at 80 °C and 60 °C, respectively, shown in Fig. 4 indicate slow efficiency and retention factor losses for toluene and amitriptyline.

The reasonable stabilities presented for the PMOS-SiO₂ stationary phases at low pH even at high temperatures (60 °C and 80 °C) are probably due to the fact that the cleavage of Si–C bond in PMOS is more difficult than in chemically bonded phases due to steric hindrance, while silica is highly stable in most acidic media.

4. Conclusions

The retention mechanisms for basic solutes with these PMOS-SiO₂ stationary phases involve both ion-exchange, due to exposed silanols, and hydrophobic interactions with the C8 chain of the polysiloxanes immobilized onto the silica. An increase in the pH of the mobile phase reduces the ion-exchange interactions between the basic solutes and the stationary phase, resulting in lower retention factors. The influence of the ion-exchange retention mechanism for basic solutes with these PMOS-SiO₂ stationary phases confers unique selectivities for basic solutes as well as good peak shapes, observations similar to those made for other stationary phases that have significant ion-exchange contributions to retention.

The PMOS-SiO₂ stationary phases are relatively stable in acidic mobile phases even at 60–80 °C. These stationary phases also present reasonable stabilities with alkaline mobile phases using ammonia, triethylamine and borate buffers and in neutral mobile phase using phosphate buffer, all at ambient temperature, but are unstable in alkaline mobile phases, even at ambient temperature, using phosphate and carbonate buffers. Higher temperatures (60–80 °C) accelerate stationary phase degradation much more than does increased buffer concentration.

Even though PMOS-SiO₂ stationary phases have poor stabilities in alkaline mobile phases using phosphate or carbonate buffers, the use of soft buffers such as tricine, tris, ammonia and triethylamine gives these phases unique selectivities and better stabilities, making them promising for basic pharmaceutical analyses.

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