

## High-performance liquid chromatography on dynamically modified silica

### IX<sup>a</sup>. Modification of silica with 3-(N,N-dimethylpalmitylammonium) propanesulphonate for reversed-phase chromatography

STEEN HONORÉ HANSEN\* and JETTE TJØRNELUND

*PharmaBiotec Research Center, Department of Organic Chemistry, The Royal Danish School of Pharmacy, 2 Universitetsparken, DK-2100 Copenhagen (Denmark)*

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#### ABSTRACT

Reversed-phase high-performance liquid chromatography on silica has been performed using 3-(N,N-dimethylpalmitylammonium) propanesulphonate as an additive to the aqueous mobile phases. The adsorption of the surfactant has been investigated as a function of its concentration in the eluent and the pH of the eluents. The separation mechanisms are reversed-phase as well as silanolophilic interactions, and are compared with those observed when using quaternary long-chain alkyltrimethylammonium ions as the additive to generate the reversed phase.

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#### INTRODUCTION

Reversed-phase high-performance liquid chromatography (RP-HPLC) on dynamically modified silica using quaternary long-chain alkyltrimethylammonium ions as the modifying agent has been thoroughly investigated during the last decade [1–3]. It has been shown that these RP-HPLC systems exhibit an excellent reproducibility of selectivity even when using silica column packing materials of various origins [4,5]. The reproducibility of selectivity as well as the peak shape of the amine solutes are superior to deactivated chemically bonded octadecylsilyl (ODS) column packing materials [6,7].

A few investigations of the use of 3-(N,N-dimethylalkylammonium) propanesulphonates as additives to the eluents in RP-HPLC on chemically bonded phases have been reported [8–10].

This paper reports the modification of bare silica with 3-(N,N-dimethyl-

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\* For Part VIII, see ref. 3.

palmitylammonium) propanesulphonate (PAPS) as a function of the concentration of PAPS in the eluent and the pH of the eluent. The retention mechanisms of the solutes are discussed and compared with the retention mechanisms observed when using quaternary long-chain alkyltrimethylammonium compounds for the modification of the silica surface. The structures of the two types of modifiers are shown in Fig 1.

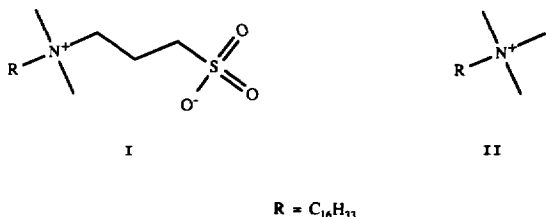


Fig. 1. (I) 3-(N,N-Dimethylpalmitylammonium) propanesulphonate (PAPS) and (II) N,N,N-trimethylhexadecylammonium (CTMA) ion.

## EXPERIMENTAL

### Chemicals

3-(N,N-Dimethyldodecylammonium) propanesulphonate (DAPS), 3-(N,N-dimethylmyristylammonium) propanesulphonate (MAPS), PAPS and 3-(N,N-dimethylstearylammmonium) propanesulphonate (SAPS) were obtained from Fluka (Buchs, Switzerland). 4,4'-Diethoxyazobenzene was prepared by coupling diazotized 4-ethoxyaniline to phenol followed by ethylation using diethyl sulphate; the crude product was recrystallized from methanol-water.

The column packing materials were as follows: Partisil (Whatman, NJ, USA), Zorbaxsil (DuPont, Cheshire, UK), Spherosil XOA 600 (Prolabo, Paris, France), LiChrosorb Si 60 (Merck, Darmstadt, Germany), Chromosorb LC6 (Johns-Manville, Denver CO, USA) and Spherisorb S5W (Phase Separations, Clwyd, UK).

All other chemicals were of analytical-reagent grade from Merck.

### Apparatus

The chromatographic investigations were performed using one of two systems. The first system consisted of a Waters (Millipore, Milford, MA, USA) liquid chromatograph with a 6000A pump, a 710B WISP autoinjector, a Shimadzu (Tokyo, Japan) CTO-6A column oven and a 490E programmable multiwavelength detector operated at 254 nm and controlled by a Waters Maxima 820 data system. The second system used a Kontron (Tegimenta, Switzerland) chromatograph consisting of an Anacomp 220 HPLC controller, a 220 pump, an MSI 660 autosampler, a 480 column oven, a 432 UV detector operated at 254 nm and a Model 800 plotter.

All analytical columns from Knauer (Bad Homburg, Germany), 120 × 4.6 mm I.D., were slurry-packed in methanol with the respective column packing material. The column systems were operated at 40°C, and the analytical columns were protected against dissolution by the use of saturation columns, 150 × 4.6 mm I.D., packed with LiChrorep Si 60 (15–25 μm) and situated between the pump and the autoinjector.

The hold-up volumes of the chromatographic systems were determined using deuterium oxide.

A Shimadzu UV-265 UV-VIS spectrophotometer was used for the determination of the critical micelle concentration (CMC) by solubilization of 4,4'-diethoxyazobenzene. A Waters 431 conductivity detector was used for the determination of the CMC by conductance measurements.

#### *Determination of adsorption isotherm*

Eluents consisting of methanol-water-potassium phosphate buffer pH 7.5 (50:45:5, v/v/v) with 0–50 mM of PAPS were used for the experiments. The amount of PAPS adsorbed was determined as described in the following sections. The contents of the column packing materials were determined as described previously [4]. The surface area of LiChrosorb Si 60 was determined as described by Hansen *et al.* [11].

#### *Determination of CMC*

The CMC was determined by two methods. The first method, the solubilization of 4,4'-diethoxyazobenzene, was performed as described previously [12]. The determination of the CMC was also performed by measuring the conductance of solutions consisting of methanol-water-0.2 M potassium phosphate buffer pH 7.5 (50:45:5 v/v/v) with 0–20 mM of PAPS. The conductance data were normalized by division by the conductance of the solution without added PAPS. These results were plotted as a function of the concentration of PAPS. The CMC was determined as the concentration of PAPS corresponding to a minimum in the relative resistance [10].

#### *HPLC of 3-(N,N-dimethylalkylammonium) propanesulphonates*

The determination of the amounts of PAPS absorbed on to the silica surface was performed by an elution method. After equilibration the analytical column was disconnected from the system and eluted with methanol-water (90:10, v/v) into a 50-ml calibrated flask. The concentration of PAPS in the 50-ml eluate was determined by the following HPLC method.

The purity of PAPS was investigated using a 120 × 4.6 mm I.D. Knauer column packed with Spherisorb octyl 5 μm (Phase Separations) and operated at ambient temperature. The mobile phase consisted of methanol-water-0.2 M potassium phosphate buffer pH 7.5 (80:17:3, v/v/v), and the flow-rate was 1.0 ml/min. A Waters 410 differential refractometer was used for the quantitative determination of the compounds. A linear response was obtained in the range 0.012–50 mM for all four 3-(N,N-dimethylalkylammonium) propanesulphonates. The  $k'$  values for DAPS, MAPS, PAPS and SAPS were 1.32, 2.08, 3.35 and 5.49, respectively.

## RESULTS AND DISCUSSION

#### *Adsorption isotherm*

A standard chromatographic system similar to those used for the modification of silica with quaternary long-chain alkyltrimethylammonium compounds was chosen [12]. The columns packed with bare silica were equilibrated with methanol-0.2 M potassium phosphate buffer pH 7.5-water (50:5:45, v/v/v) containing different amounts of PAPS. The adsorption isotherm (Fig. 2) does not show a Langmuir

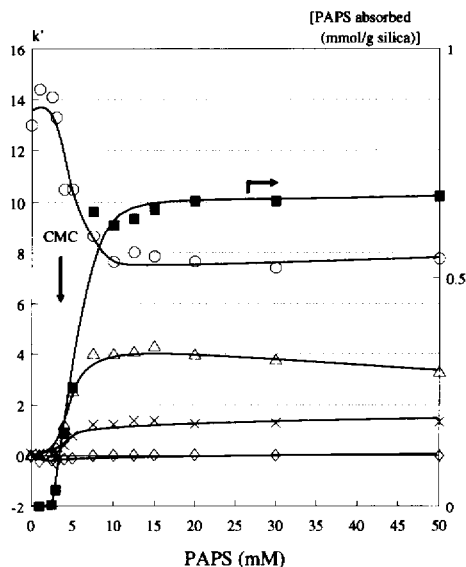


Fig. 2. Adsorption isotherm for PAPS (■). Retention ( $k'$  values) versus the concentration of PAPS in the eluent for:  $\times$  = phenol;  $\triangle$  = toluene;  $\diamond$  = benzoic acid;  $\circ$  = N,N,N-trimethylanilinium ion. Chromatographic conditions: column, LiChrosorb Si 60,  $5 \mu\text{m}$ ,  $120 \times 4.6 \text{ mm}$  I.D.; eluent, methanol–water– $0.2 \text{ M}$  potassium phosphate pH 7.5 (50:45:5, v/v/v) with different amounts of PAPS added; flow-rate  $1.5 \text{ ml/min}$  at  $40^\circ\text{C}$ .

behaviour. At low concentrations of PAPS only a very small amount of the surfactant is adsorbed on to the silica surface. At a certain concentration, corresponding to the CMC, a steep increase in the amount of PAPS adsorbed is observed until a plateau is reached, corresponding to an adsorption of about  $0.7 \text{ mmol/g}$  of silica. The surface area of LiChrosorb Si 60 is about  $550 \text{ m}^2/\text{g}$ , and the adsorbed amount therefore corresponds to a coverage of about  $1.2 \mu\text{mol/m}^2$  of silica, which should be compared to the about  $8 \mu\text{mol/m}^2$  of silanol groups normally considered to be available on a fully hydroxylated silica surface.

The CMC of PAPS in the eluent was determined to be  $3.2 \text{ mM}$  by the solubilization method and  $4 \text{ mM}$  by measuring the conductance of the mobile phases. By HPLC analysis PAPS was found to contain 1.1% DAPS, 1.4% MAPS and 2.0% SAPS.

### *Influence of pH*

The effect of the pH of the eluents on the retention of solutes was investigated in the pH range 2–10 (Fig. 3). The change in the retention of the solutes when changing the pH is very similar to that observed in RP-HPLC on chemically bonded ODS phases.

The retention of non-ionic solutes is virtually constant throughout the pH interval investigated, which indicates the constant amount of PAPS absorbed on to the surface of the silica. Anionic solutes are only retained in the system at a pH where they are non-ionized, *i.e.* at low pH values. The anionic solutes have negative capacity

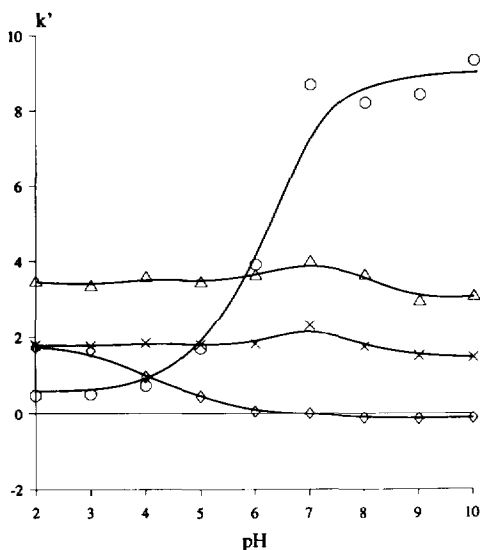


Fig. 3. Retention ( $k'$  values) versus pH of buffer solutions in eluent. Symbols as in Fig. 2. Chromatographic conditions as in Fig. 2, with various pH values in buffer solutions and with 7.5 mM PAPS added.

factors  $k'$  at higher pH values, indicating ionic exclusion from the partly ionized silica column packing material. The retention of the quaternary ammonium compound increases with the ionization of the silanols corresponding to the  $pK_a$  value (about 7) of the silica. The retention of amines also increases with the increase in ionization of the silanols (Fig. 4). At high pH values (about 8–10), where the retention is due to a mixture of reversed-phase and ion-exchange interactions (Fig. 4C), the retention decreases as a result of the increasing deprotonization of the amines.

The retention of cationic solutes was studied in detail to compare the modification of silica with N,N,N-trimethylhexadecylammonium (CTMA) ions (Fig. 4A) and PAPS (Fig. 4C). The relative retention between imipramine and some related cations at pH values above 8 (Fig. 4C) are similar to that found when chromatographing these substances on bare silica with the same mobile phases but without adding any surfactant (Fig. 4B). However, larger  $k'$  values are obtained due to the additional reversed-phase effect, and due to the additional separation mechanism some changes in selectivity are observed. Comparing the PAPS system (Fig. 4C) with the CTMA system (Fig. 4A) reveals fundamental differences in the separation mechanisms as the quaternary ammonium ion only exhibits a small retention in the latter system, whereas it has the largest retention time of the solutes using no modification (Fig. 4B) or modification with PAPS.

#### Retention mechanisms

When no or only a small amount of PAPS is adsorbed on the silica surface, no RP effect is observed, but above the CMC reversed-phase separations are performed (Fig. 5), similar to those seen in RP-HPLC on chemically bonded phases.

Only a small reduction of the cationic capacity is seen when PAPS is absorbed

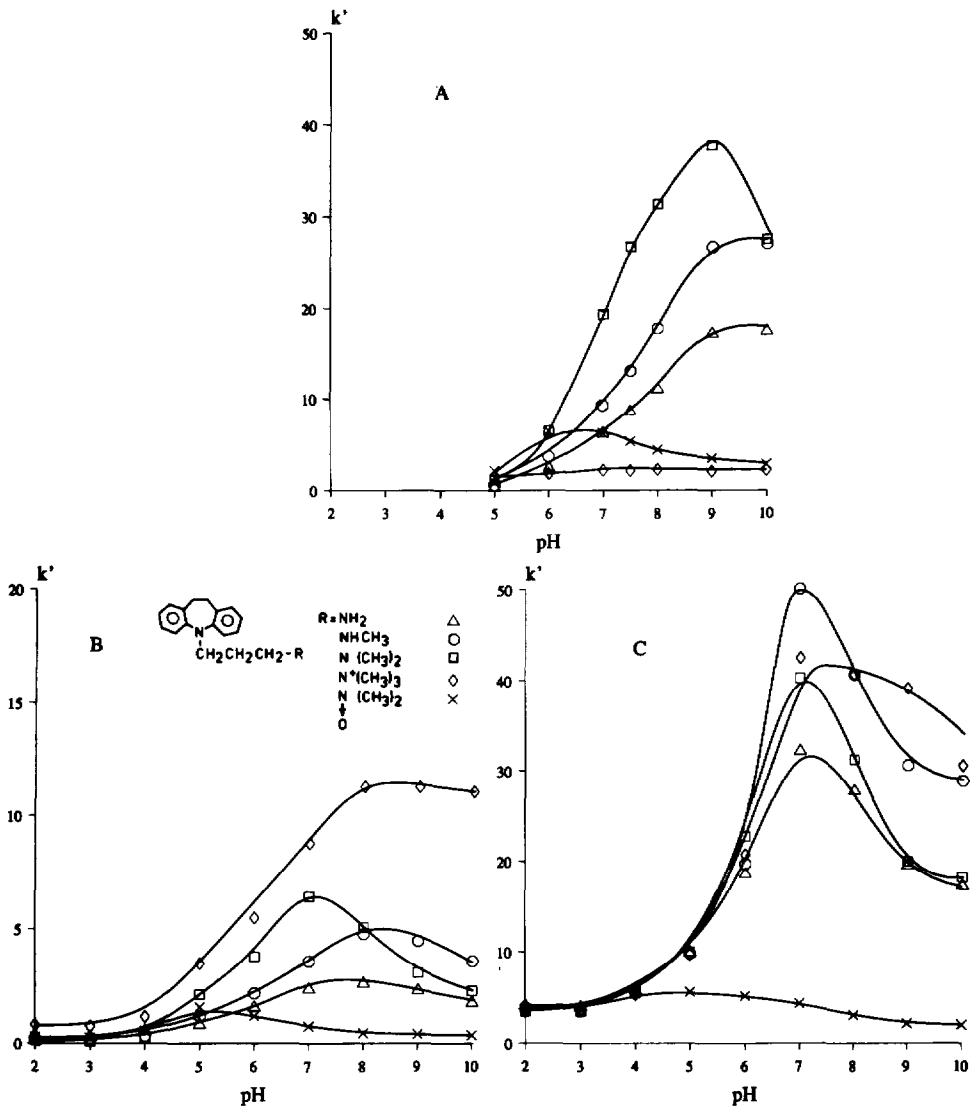


Fig. 4. Retention ( $k'$  values) of imipramine and some closely related compounds *versus* pH of buffer solutions in eluent. Chromatographic conditions: column as in Fig. 2; eluents, methanol-water-0.2 M potassium phosphate pH 7.5 (50:45:5, v/v/v). (A) With 2.5 mM of N,N,N-trimethylhexadecylammonium bromide added; (B) with no surfactant added; (C) with 7.5 mM of PAPS added. Flow-rate, 1.5 ml/min at 40°C.

on to the surface at pH values above 5 (Fig. 4C), as indicated by the retention of the quaternary ammonium compound. When using quaternary long-chain alkyltrimethylammonium ions, the cation-exchange properties of the silica are effectively eliminated (Fig. 4A).

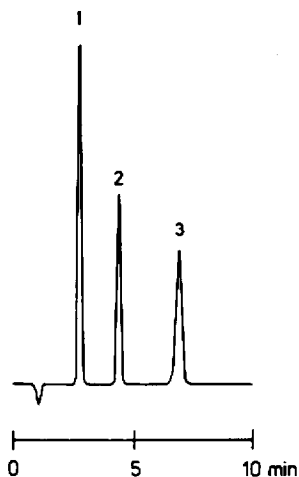


Fig. 5. Separation of (1) phenol, (2) toluene and (3) *m*-xylene on Spherisorb S5W 5  $\mu\text{m}$ , 120  $\times$  4.6 mm I.D., using methanol-water-0.2 *M* potassium phosphate pH 7.5 (50:45:5, v/v/v) with 7.5 mM of PAPS added as eluent. Flow-rate, 1.5 ml/min at 40°C.

If the adsorption of PAPS on to the silica surface is accomplished by interactions between the quaternary ammonium groups and the silanol groups in the same way as with quaternary long-chain alkyltrimethylammonium ions, but leaving free sulphonate groups for cation-exchange, differences in the silanol activity are masked. Thus, the reversed-phase system generated may be expected to show a high reproducibility of selectivity between solutes using different brands of silica.

The data in Table I show a poor reproducibility of selectivity when using different brands of silica. It has been shown previously [4-7, 13-14] that very reproducible selectivities (relative standard deviations typically 5% or below) between solutes may be obtained on different brands of silica if the silica is dynamically modified with CTMA. For comparison, data from Hansen *et al.* [6] are reprinted in Table II.

There is straightforward correlation between the surface area of the silica, the amount of CTMA adsorbed and the  $k'$  value for benzene when silica is modified with CTMA [11,13]. If the interaction between PAPS and the silica surface is due to interactions between the quaternary ammonium group and the silanols, a correlation between the retention of benzene or toluene and the surface area is expected as the retention of neutral solutes in these systems is due only to reversed-phase interactions with the long carbon chains of the absorbed surfactant. No such correlation was found, and, in addition, the adsorption of PAPS on to the silica surface is independent of the pH value of the buffer in the eluent, as indicated by the constant retention of toluene (Fig. 3) throughout the pH interval investigated.

If the adsorption of PAPS on to the silica is due to ionic interactions between the quaternary ammonium group and the silanols, an increase in the retention of neutral solutes with an increase in the ionization of silica is expected, but this was not observed. At pH values below 5, PAPS is probably adsorbed as a result of dipolar-dipolar interactions as the ammonium propanesulphonate is a strong dipole. Above

TABLE I  
SEPARATION FACTORS ( $\alpha$ ) BETWEEN PHENOL AND EIGHT DIFFERENT SOLUTES ON SEVEN DIFFERENT BRANDS OF SILICA COLUMN PACKING MATERIALS

Eluent: methanol-0.2 M potassium phosphate buffer pH 3.0 or 7.5-water (50:5:45, v/v/v) with 7.5 mM PAPS added. Temperature 40°C. - = No retention; R.S.D. = relative standard deviation.

Solute	$\alpha$	Zorbaxsil		Hypersil		Spherosil XOA 600		LiChrosorb Si 60		Spherisorb S5W		Chromosorb LC6		R.S.D. (%)	
		pH 3.0	pH 7.5	pH 3.0	pH 7.5	pH 3.0	pH 7.5	pH 3.0	pH 7.5	pH 3.0	pH 7.5	pH 3.0	pH 7.5	pH 3.0	pH 7.5
Benzoic acid	0.99	-	0.93	-	0.92	-	0.95	-	0.93	-	0.93	-	0.78	-	1.8
Phenylethylamine	0.18	1.43	0.33	2.83	0.26	1.04	0.22	0.68	0.14	1.12	0.29	1.37	0.22	1.02	27.6
Benzene	1.09	1.09	1.12	1.72	1.11	1.07	1.11	1.60	1.07	1.01	1.09	1.21	1.11	1.06	1.6
Toluene	1.92	1.90	1.95	2.85	1.93	1.76	1.86	2.69	1.94	1.73	1.90	1.74	1.89	1.88	1.6
Phthalic acid	1.15	-	0.63	-	0.73	-	0.77	-	0.79	-	0.61	-	0.85	-	22.8
Diethylamine	0.29	3.37	0.60	4.97	0.36	3.00	0.38	4.67	0.15	3.03	0.38	3.51	0.31	3.32	38.3
Morphine	0.23	1.89	0.66	3.55	0.35	1.27	0.28	2.76	0.13	1.83	0.47	1.36	0.27	1.29	51.3
Salicylic acid	0.57	0.16	1.31	0.33	1.34	0.35	0.57	0.42	0.58	0.21	1.17	0.14	1.41	0.28	40.2



TABLE II  
 SELECTIVITY ( $\alpha$ ) BETWEEN IMIPRAMINE AND TEN BASIC DRUGS ON ELEVEN DIFFERENT SILICA COLUMN PACKING MATERIALS (NO. 11-21) DYNAMICALLY MODIFIED WITH 2.5 mM CTMA IN THE ELUENT.

For further details see ref. 6.

	Column packing material											R.S.D. (%)	
	11	12	13	14	15	16	17	18	19	20	21		
Imipramine	$k'$	12.7	6.7	9.4	15.9	9.1	6.5	19.0	13.1	9.3	6.5	17.5	
	$A_s$	0.9	0.8	0.9	1.1	0.9	0.9	1.9	1.0	1.7	1.2	0.9	
	$\alpha$	0.55	0.55	0.54	0.56	0.54	0.58	0.57	0.55	0.56	0.58	0.54	2.7
Desipramine	$\alpha$	0.27	0.28	0.26	0.27	0.27	0.26	0.23	0.26	0.26	0.27	0.25	5.1
Imipramine N-oxide	$\alpha$	0.91	0.88	0.85	0.89	0.87	0.86	0.82	0.91	0.88	0.89	0.92	3.3
<i>cis</i> -Clopenthixol	$A_s$	1.0	0.8	1.0	1.1	0.8	0.9	1.1	1.0	1.1	0.8	1.0	
<i>trans</i> -Clopenthixol	$\alpha$	1.04	0.99	0.97	1.01	0.98	0.96	0.92	1.03	0.98	0.99	1.06	4.0
Prochlorperazine	$\alpha$	2.43	2.23	2.13	2.50	2.25	2.07	1.84	2.17	2.04	2.11	2.49	9.2
	$A_s$	1.3	1.2	1.2	1.5	1.3	1.4	1.4	1.2	1.7	1.7	1.5	
	$\alpha$	1.92	1.79	1.63	1.96	1.85	1.76	1.74	1.70	1.74	1.80	1.92	5.7
Chlorpromazine	$A_s$	1.0	0.9	0.8	0.8	0.7	1.0	1.0	0.9	1.0	0.9	0.9	
	$\alpha$	0.93	0.88	0.78	0.93	0.88	0.85	0.82	0.85	0.92	0.89	0.93	5.6
Perphenazine	$\alpha$	0.37	0.37	0.38	0.38	0.38	0.35	0.29	0.35	0.32	0.34	0.38	8.2
Propranolol	$A_s$	2.4	1.7	1.9	2.9	2.2	2.0	2.9	2.0	2.5	1.6	2.1	
	$\alpha$	0.54	0.56	0.52	0.57	0.54	0.57	0.52	0.54	0.54	0.57	0.53	3.5
Nortriptylin	$\alpha$	0.95	0.97	0.94	0.98	0.97	0.95	0.90	0.94	0.94	0.97	0.97	2.4
Amitriptylin	$A_s$	1.0	1.0	0.8	0.9	0.7	1.0	1.3	1.0	1.3	1.0	2.1	

pH 5 the ionization of the silanols increases with increasing pH values. Whether the adsorption process at high pH values is due to dipolar-dipolar interactions, to ionic interactions, or to a mixture thereof between the quaternary ammonium group and the ionized silanols, leaving a free sulphonate group, cannot be distinguished from the results obtained in these experiments. However, the system exhibits only a small change in the cation-exchange capacity at pH values above 5.

When using quaternary long-chain alkyltrimethylammonium ions for the modification of silica, a similar reversed-phase effect is obtained, but the silanol activity and thus the cation-exchange capacity is effectively masked as a result of the ionic interaction between the quaternary ammonium group and the silanols. This results in a highly reproducible selectivity from various brands of silica [15].

## CONCLUSIONS

An RP-HPLC system has been generated using bare silica as the column packing material and aqueous eluents with PAPS added. However, the system does not provide the high reproducibility of selectivity using different brands of silica which is obtained when using silica dynamically modified with quaternary long-chain alkyltrimethylammonium ions.

## ACKNOWLEDGEMENT

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