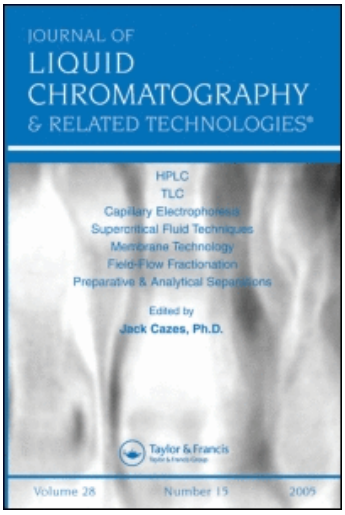

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STUDY OF THE COMPETITIVE BINDING OF TBA AND CP IONS ON C_{18} SURFACE

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ABSTRACT

The adsorption behavior of tetrabutylammonium (TBA) and cetylpyridinium (CP) cations was studied on Hypersil 5-ODS sorbent. After establishing that CP has about 5-fold stronger affinity to the C_{18} surface than the structurally different, symmetric, spherical molecule of TBA, the adsorption from their common solution was also examined. The data of "competitive binding" seem to show that TBA should have certain selective binding sites on the C_{18} surface from which it can not be displaced by the CP, even in the case where the latter is present in a 4-fold excess in the mobile phase.

The maximal coverage was found to be 52.9%(CP) and 12%(TBA), i.e., every second C_{18} group is covered by CP cations and only 1/8th of C_{18} groups may interact with TBA. The accessible, proton releasing silanol content, related to the total C_{18} content of the column, was found to be very low.

INTRODUCTION

The retention determining role of the stationary phase concentration of ion pairing agent is a generally accepted view in reversed phase ion pair (RPIP) HPLC.[1-6] Correspondingly, the binding to the C_{18} surface of the different IP'ing agents was the subject of several works.[7-15] In this respect, numerous structural types of the cationic and anionic IP'ing agents were examined during the last decades, but only a few compounds are widely used in the daily practice of RPIP HPLC routine and research.

From the cationic IP'ing agents the cetyltrimethylammonium, CTMA(Chloride=cetrimide) tetrabutylammonium, TBA (bromide, sulfate or dihydrogenphosphate) are the most often used IP'ing substances. Although the adsorption onto the C_{18} surface under different experimental conditions was examined for both of these agents (CTMA[9,10,14], TBA[13,15]) a comparative study on the binding property of the two substances has not been yet performed. Such an examination offered interesting observations by the fact that TBA and CTMA represent two different structural subtypes of the quarternary ammonium IP'ing compounds. While TBA is a symmetrically substituted "spherical" cation, the cetyl chain makes CTMA asymmetric.

Assuming that the binding on the C_{18} surface takes place through one of the butyl radicals (TBA) and the cetyl group (CTMA), it can be expected that the nitrogen, i.e., cationic head, facing towards the mobile phase, will have quite different interacting (hydrophobic,electrostatic) properties due to the different volume, carbon content, etc., of the environmental trimethyl and tributyl moieties.

In this work, instead of CTMA as asymmertric quarternary ammonium compound, cetylpyridinium, CP (chloride) was applied. This compound emerged as IP'ing agent in a work of Bidlingmeyer[16] and was studied by Budvári-Bárány et al.[17] in a dynamically modified silica-based RP system. The aromatic nucleus content, resulting in UV activity (i.e., UV detectability) and enabling the molecule to certain selective interactions, may be the source of potential advantages of CP over CTMA being applied as an IP'ing agent or functioning, in case of cationic or neutral solutes, as a displacing modifier.

Since the chromatographic selectivity of TBA or CP is largely determined by the quality and concentration of the anchoring cationic head, a study of the competitive binding of TBA and CP on C_{18} (i.e., when they are present simultaneously at the same eluent) seemed for us—also technically—an attractive task. While the breakthrough of TBA was signaled by refractometry, the pyridine moiety (i.e., UV activity) of CP allowed the application of dual (UV spectrophotometric-refractometric) detection for selectively revealing the breakthrough of CP and the selective binding sites on the C_{18} surface.

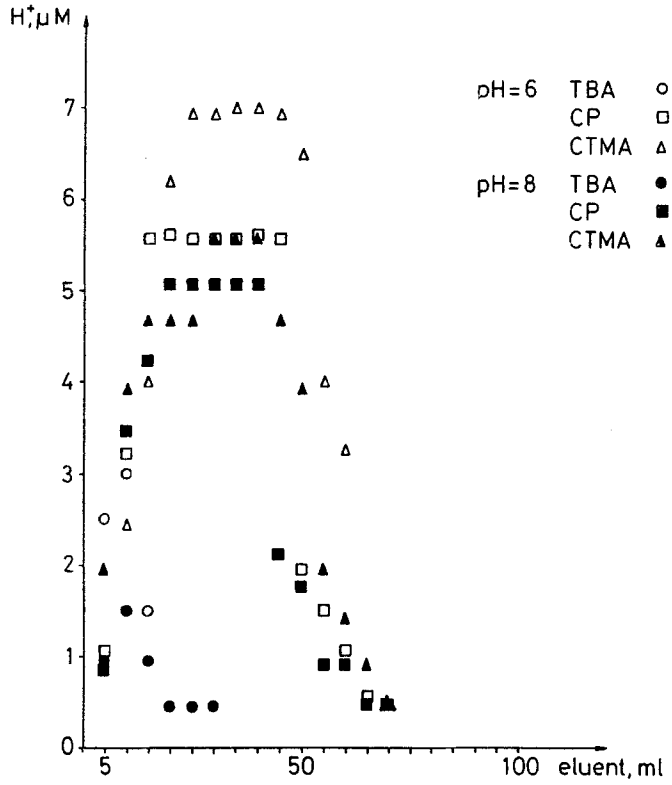

Figure 1. The amount (μM) of proton released by the IP'ing reagents on Hypersil ODS column (sorbent content: 2.5 g = 425 m^2; see, also, Table 4).

EXPERIMENTAL

Chromatography

The HPLC apparatus comprised an ISCO pump Model 2350 (USA), combined with a Valco injector unit (10 μL loop). An ISCO variable wavelengtth absorbance detector (230-800 nm) was used.

For plotting the breakthrough curves of TBA and CP (CTMA), a Waters differential refractometer, Model R401, was employed. For the breakthrough determination, in case of CP, dual (refractometric-UV spectrophotometric) detection was used. The description of the equipment is available *in a previous* work.[18] The equipment units, subsequent to the pump, were thermostatted at

$25^0C \pm 1^0C$ (Ultrathermostat MLW type U2C, Freital, Germany). The breakthrough curves and the chromatograms were recorded, the data handling was effected by a Hewlett Packard integrator Model 3396 Ser II.

The C_{18} sorbent, Hypersil 5-ODS (Shandon), particle size 5 μm, was packed in a stainless steel column (250 x 4.0 mm I.D., BST, Budapest, Hungary).

As mobile phase, sonically degassed and filtered mixtures of acetonitrile and aqueous phosphate buffer solutions (pH = 3.0; 6.0; 8.0) containing different amounts of TBA and/or CP was applied. All ready-made eluents contained sodium chloride in an amount to adjust the ionic strength to 0.1. Each data of binding or retention was calculated as an average of two or three parallel runs. The column void time was signalled by the solvent peak of acetonitrile. Following testing, the columns were brought to the initial state by washing with a 90:10 mixture of water-acetonitrile (200 mL) and then with acetonitrile (100 mL). In case the column was loaded by an eluent with higher pH (6.0 and 8.0) a prewash with a 10:90 mixture of acetonitrile-phosphoric acid (pH = 2.5) was performed.

The eluent flow rate was 1.0 mL/min. The effluent was monitored at the wavelength of maximum absorption for each compounds.

Materials

Cetylpyridinium chloride monohydrate, 98%, Aldrich, Tetrabutyl-ammonium hydrogen sulfate, 97%, Aldrich; Buffer solutions at the pHs 3.0 6.0 8.0 were prepared by mixing the proper volumes of 0.067 M aqueous solutions of potassium dihydrogenphosphate and sodium hydrogenphosphate (KH_2PO_4,Na_2HPO_4,$2H_2O$, analytical grade, Reanal, Budapest); the pH of the solutions were tested by potentiometry with an accuracy ±0.02 unit. Acetonitrile for HPLC, Chemolab, Budapest. Water, deionized,double distilled. Sodium chloride 99.99%, Aldrich. Potassium iodomercurate(II), K_2HgI_4, Merck. Model substances (Table 5) met the requirements of the Hungarian Pharmacopeia. From their 0.01 % methanolic solution 10 μL was injected.

RESULTS & DISCUSSION

Table 1 contains the data of binding of the TBA and CP by two Hypersil ODS columns of different batches. The binding data were obtained by the usual evaluation of the breakthrough curves. Prior to each breakthrough experiment, the column was purged with the method which was described in the experimental section.

Table 1

The Amount of Adsorbed Ion Pairing Reagent, μM

	Column I			Column II		
	5*	**10***	**20***	**5***	**10***	**20***
TBA	93	125	144	102	120	144
CP	600	624	635	581	597	624
I**	6.5	5.0	4.4	5.7	5.0	4.3

Stationary phases: I-II: Hypersil 5-ODS (Shandon); Mobile phase: Phosphate buffer 0.067 M (pH = 3) - acetonitrile 90: 10 + TBA or CP, I = 0.1 (NaCl); * IP'ing reagent conc'n in the mobile phase mM; ** CP/TBA.
NOTE: The adsorbed amount of ion pairing reagents at pH 6 and 8 varied within ± 10% compared to the values at pH 3.

Table 2

Ratio of the Adsorbed Amount from CP-TBA Common Solution

		Ratio of CP/TBA Concentrations in Eluent, mM							
		5/5	**10/10**	**20/20**	**10/5**	**20/5**	**20/10**	**5/10**	**5/20**
pH 3	Cp[b]	572	615	609	592	600	594	600	558
	TBA[b]	93	96	114	74	59	81	105	102
	CP/TBA	6.2	6.4	5.3	8.0	10.2	7.3	5.7	5.4
pH 6	Cp[b]	538	552	528	567	618	576	546	541
	TBA[b]	84	90	96	78	69	78	106	93
	CP/TBA	6.4	6.1	5.5	7.3	9.0	7.4	5.2	5.8
pH 8	Cp[b]	570	554	567	540	555	516	545	540
	TBA[b]	83	90	114	70	62	75	104	120
	CP/TBA	6.9	6.1	5.0	7.7	9.0	6.9	5.2	4.5

Stationary phase: Hypersil II 5-ODS (Shandon); Mobile phase: Phosphate buffer 0.067 M - acetonitrile 90:10 + CP or TBA; I = 0.1 (NaCl); [b]Bonded amount, μM.

The breakthrough for TBA, signalled by the refractometric detector, strictly coincided with the point where continous monitoring of the effluent by Mayers reagent (aqueous solution of K_2HgI_4) began to show a positive response. In this way, with a chemical indication of the breakthrough, we could establish, with a great accuracy, that the effluent practically did not contain TBA at all before the breakthrough: the limit of visual detectability (appearance of white turbidity) 0.8 μg/mL.

The breakthrough of CP was signalled by both detectors. The values in Table 1 show that CP has an affinity about 5-fold stronger than that of TBA towards the ODS surface. It may also be seen that the adsorbed amount of TBA fairly depends upon the TBA concentration in the mobile phase, while CP binding seems quasi-independent of the mobile phase concentration. As a consequence, I^x (CP/TBA binding) values decrease parallel to the increasing of the mobile phase concentration of TBA and CP.

Table 2 shows the binding ratio CP/TBA (I^x) when the two reagents are present in a common eluent in different concentration ratios, and also, the pH influence on the binding is shown. What is to be seen, at 1:1 ratios with increasing concentrations is that I^x decreases down to 4.9 from 6.4. This experience allows the assumption that TBA should have some selective binding sites on the C_{18} surface and these sites cannot be occupied by the CP; this reasoning seems to be confirmed by the fact that CP, although the bound amount of TBA somewhat decreases, cannot substitute for it. The coverage under the experimental conditions, i.e., the number of CP and TBA groups/nm^2, is rather low (see Table 3). Only half (52.9%) of the C_{18} chains may be considered as covered (interacting) with CP cations, and only 1/8th (12%) of the C_{18} groups are occupied by TBA cations.

Table 4 and Fig. 1 show the data for silanol content, which was found accessible and proton releasing by CP and TBA under the experimental conditions used. In this experiment, for the sake of comparison, CTMA was also involved. The calculations (see the scheme under Table 4) were based upon our assumption and other authors' findings[19] that CP, CTMA & TBA displace K^+ ions at the sites the latter occupied during the preconditioning of the column. In these experiments, the pH of the effluent was monitored in 5 mL portions. Summing up the pH changes caused by the different eluent portions, the total amount of protons released by CP(CTMA) or TBA was calculated. The released proton amount was taken equivalent to the accessible and proton releasing silanol content. The data indicate a silanol content which is much lower than might be expected from the findings, i.e., that residual silanol content on the silica C_{18} surface may reach 50% of that of the parent silica. Although total silanol content was not measured in this work, the silanol content found to be accessible and proton-releasing by CP or TBA was very low, compared to the

Table 3

Bonding Density

	CP	TBA
Coverage μM/Column (2.5 g)	635	144
/g (170 m^2)	254	57.6
/m^2	1.49	0.34
Coverage, group/nm^2	0.90	0.2

Coverage by ODS (Hypersil) 2.8 μM/m^2 (=1.7 $C_{18}H_{37}$/nm^2).
Coverage CP/ODS: 0.53; TBA/ODS: 0.12.

Table 4

Accessible, Proton Releasing Silanol Content

Ion	SiOH/nm^2 pH = 6	pH = 8
K	0.004	0.007
CP	0.066	0.060
TBA	0.010	0.007
CTMA	0.087	0.070

$$\sum_n^0 \left[H^+\right] M / 1.5 \bullet 10^{-3} = M$$

$$\frac{M}{10^{-6}} = \mu M / \text{column}$$

$$\frac{\mu M / \text{column}}{2.5} = \mu M / g$$

$$\frac{\mu M / g}{170} = \mu M / m^2$$

$$\frac{\mu M / m^2 \bullet 6.02 \bullet 10^{-17}}{10^{18}} = H^+ / nm^2 = SiOH / nm^2$$

literature data for residual silanols (~1.2 silanol group/nm^2). The relative coverage by accessible and proton releasing silanols at pH = 8 comes from the binding density data for CP and TBA (Table 3) divided by the number of accessible and proton-releasing silanols: CP/SiOH = 15; TBA/SiOH = 28.

Table 5

Cationic Ion Pairing Reagents' Influence on the Retention Time (min) of Cationic Compounds

Compound	0.1 M Phosphate Buffer (pH=3) - Acetonitrile				
	------ 95:5 ------			90:10	80:20
		TBA 2.5 mM	CP 5 mM		
Norephedrine	10.2	3.2	1.9	5.2	2.7
Ephedrine	15.2	3.6	2.0	6.8	3.0
Homatropine	33.9	5.5	2.4	10.6	3.3
Atropine	104.8	11.2	2.3	23.1	4.6
Codeine	27.4	4.4	1.7	7.7	2.8
Ethylmorphine	88.4	10.2	1.8	18.9	4.0
Procaine	19.1	3.0	1.6	6.8	3.0
Tetracaine	∞	∞	5.3	∞	39.3

These results provide numerical data for estimation of the contribution of salt formation in the silanophil effect. The latter was found to be almost negligible under the experimental conditions employed, confirming the decisive role of other polar interactions (H-bond, complex formation, etc.) in the silanophil effect of basic (amine) compounds.

Table 5 illustrates some potential practical aspects of applying CP or TBA as a displacing organic modifier in RP-HPLC. The model compounds in Table 5 represent four pairs of pharmaceutical amine compounds being homologs or structurally related derivatives. The reasonable applicability of such systems most clearly manifests itself in case of tetracaine which cannot be eluted (t_r>100 min) in a mobile phase with 10% acetonitrile content, while the addition of 0.005M CP results in a rapid elution with excellent peak quality in an eluent with only 5% of acetonitrile.

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