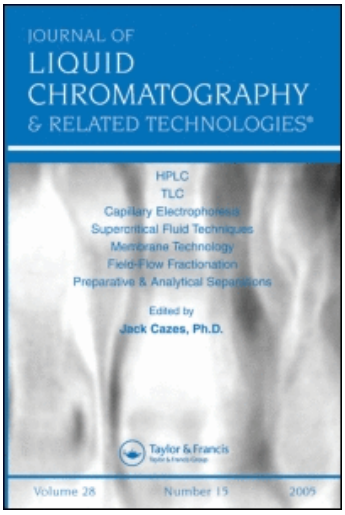

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HPLC OF ANTIPHLOGISTIC ACIDS ON SILICA DYNAMICALLY MODIFIED WITH CETYLPYRIDINIUM CHLORIDE

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ABSTRACT

The cetylpyridinium chloride (CPC) which contains an aromatic ring and a hydrophobic cetyl group differs in its structure from the generally used cationic counter ions. Thirteen of antiphlogistic acids and their derivatives were investigated. Silica as a stationary phase and an eluent containing CPC were used. The conclusion can be drawn that CPC functions as an ion pairing agent and its use in the aqueous eluent results in the formation of a "dynamically modified" silica surface. The adsorption isotherm for CPC on the bare silica also was determined. Comparative data are shown on the retention of several antiphlogistic acids with CPC and cetrimide containing aqueous eluents.

INTRODUCTION

In the last years several papers have appeared describing high-performance liquid chromatography (HPLC) on bare silica "dynamically modified" by the addition of different quaternary ammonium salts to the eluent. In our opinion, standard papers on

this topic are those published by Hansen et al. [1-6], who developed the work of Ghaemi and Wall [7]. Hansen at al. mainly used long-chain quaternary ammonium salts in aqueous eluents. They studied the circumstances of formation of the dynamically-coated stationary phase. In keeping with the report by Ghaemi and Wall, they found that, by this means, they could create stationary phase functions opposed to a polar eluent as a reversed phase. It was established, that the N-acetyl-N,N,N-trimethylammonium ion (cetrimide,CTMA) is dynamically adsorbed to silica gel, displacing a proton of the silanol group and the hydrocarbon chains are pointing away from the silica surface, thus the system behaves as an ion pair forming reversed phase. The various investigations clarified the roles of the composition of the mobile phase i.e. the CTMA concentration, the pH and the ionic strength in the retention of organic compounds (1,2). Gazdag, Szepesi and Hernyes [8] reported data on the degree of CTMA binding on silica gel as a function of the experimental conditions. They published the retention diagrams of 15 acidic compounds as functions of the pH of the mobile phase and its CTMA and methanol concentration. Budvári, Szász et al. [9] studied acidic compounds, belonging to three groups of drugs also in silica gel and CTMA containing systems, to obtain further data on structure-HPLC behaviour and possibilities of drug analytical application.

As it is expected, the adsorption of the counter-ion and the selectivity of the chromatographic process largely depend on the number of carbon atoms in the used quaternary ammonium counter ion and on its structure. It seemed of interest to study the behaviour of such a quaternary ammonium counter-ion which

contains both a long alkyl chain and an aromatic ring. It might be assumed that an aromatic nucleus in the counter-ion will result in a nucleophilic interaction and occasionally influence the selectivity of the chromatographic process. On the other hand, the aromatic nucleus can exert a similarly important effect in the binding on the silica surface. Following this logic our interest turned to the cetylpyridinium cation, which simultaneously contains an aromatic ring and a hydrophobic cetyl group and has a quaternary ammonium ion character. It should be expected, that a cetylpyridinium salt adsorbs on a silica surface and functions as a reversed phase due to the hydrophobic nature of the cetyl group.

Cetylpyridinium chloride (CPC) was used in a HPLC system by Bidlingmayer and Warren [10]. They worked with a μBondapak C_{18} column, and the mobile phase contained CPC as counter-ion. The interaction between the alkylsulphonates they studied and the counter-ion (CPC) in the mobile phase resulted in the formation of ion-pairs, the alkylsulphonates thereby becoming UV-active. It was established, that CPC bonded in a considerable degree on the C_{18} column.

We set the aim to carry out more detailed research on the usefulness of CPC in HPLC systems. In our present experiment the retention times of antiphlogistic acids were found to depend on the concentration of CPC in the eluent, while the strong adsorption of CPC on the silica surface was also established. The conclusion can be drawn that the use of CPC in a methanol-water-phosphate buffer eluent results in a "dynamically-modified" surface on the silica. The acids migrate as ion-pairs.

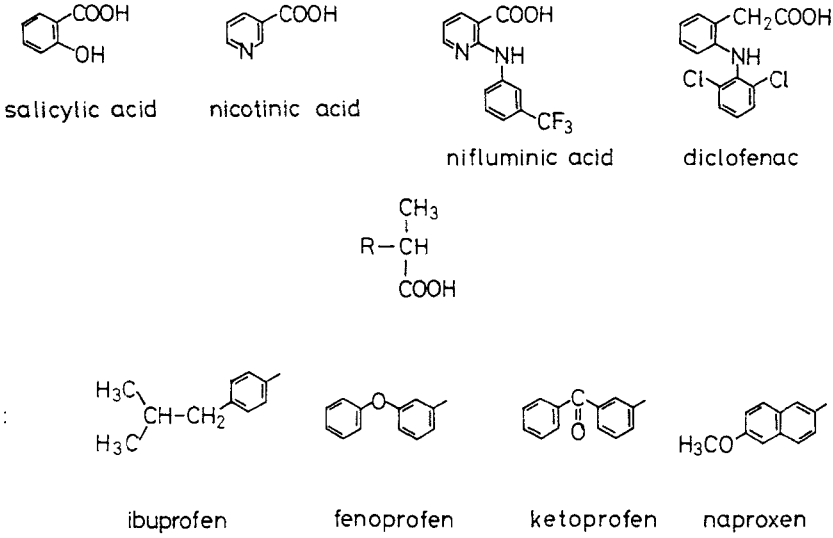


Fig. 1 Structures of the investigated antiphlogistics

EXPERIMENTAL

Model substances

Salicylic acid (Pharmacopeia Hungarica VII.), Diclofenacum natrium (Biogal, Debrecen, Hungary), Phenoprofen (Chinoin, Budapest), 2-(3-Phenoxyphenyl)-2-cyanopropionic amide (Chinoin), 3-(Phenoxyphenyl)-cyanoacetic acid methyl ester (Chinoin), Ketoprofen (Chinoin), Ibuprofen (Chinoin), 4-Aminohydratropic acid ethyl ester (Chinoin), 4-Acetaminohydratropic acid methyl ester (Chinoin), Naproxen (Syntex), Nifluminic acid (Gedeon Richter, Budapest), Nicotinic acid N-oxide (Gedeon Richter), 2-Chloronicotinic acid (Gedeon Richter).

Chemicals

Methanol (Reanal, Budapest), Potassium dihydrogen phosphate, Sodium hydrogen phosphate (Reanal, Budapest, Hungary), Cetylpyridinium chloride (Fluka), Cetrimide (Reanal), LiChrosorb Si 60, 10 μm (Merck).

Chromatography

Chromatographic testing was performed on a Liquochrom, Model 2010, high-pressure liquid chromatograph (LABOR MIM, Budapest). A variable-wavelength UV detector (LABOR MIM) was used.
All experiments were performed on a 250x4.6 mm I.D. column, packed with LiChrosorb Si 60 (10 μm) (Chrompack, Middelburg, the Nederlands). The eluent was methanol-water-phosphate buffer (pH = 5) (60:30:10) with the addition of various concentrations (0.002 - 0.05 M) of CPC and (0.002 - 0.04 M) cetrimide.

Buffer solution: 0.178 g of disodium hydrogen phosphate was dissolved in 100.0 ml of water, and 0.680 g of potassium dihydrogen phosphate was dissolved in 500.0 ml of water. 495.25 ml of the latter solution and 4.75 ml of the former solution were mixed. This gave a buffer with pH = 5. The pH of the eluent, if necessary, was adjusted to 5, using 0.1 M solution of sodium hydroxyde or phosphoric acid.

The column was equilibrated with the mobile phase overnight. The flow rate was 1 ml/min. Following each experiment the column was brought to the initial state by elution with water-phosphoric acid (1:1) mixture then methanol, and prior to the next experiments the column was equilibratet with the eluent.

RESULTS AND DISCUSSION

The relationships between the concentration of CPC in the eluent and the retention times of the antiphlogistic acids are

Table 1

Retention data of the compounds

Compound	Retention time sec						
	eluent						
	1	2	3	4	5	6	7
1. Salicylic acid	126	158	167	174	237	382	623
2. Diclofenacum natricum	140	195	217	227	403	1078	1612
3. Phenoprofen	148	168	182	185	253	425	580
4. 2-(3-Phenoxyphenyl)-2-cyanopropionic amide	176	179	186	185	187	189	188
5. 3-(Phenoxyphenyl)-cyanoacetic acid methyl ester	177	179	184	186	188	209	219
6. Ketoprofen	146	174	186	186	344	373	490
7. Ibuprofen	152	173	186	186	281	466	530
8. 4-Aminohydratropic acid ethyl ester	148	168	183	185	248	466	590
9. 4-Acetaminohydratropic acid methyl ester	187	189	190	189	190	196	191
10. Naproxen	180	181	195	199	277	431	567
11. Niflumic acid	128	195	206	217	266	281	351
12. Nicotinic acid N-oxide	130	165	176	194	241	315	375
13. 2-Chloronicotinic acid	134	159	170	194	231	282	374

1: Methanol-water-buffer (pH = 5) 60:30:10
2: 0.002 M CPC
3: 0.003 M CPC
4: 0.004 M CPC
5: 0.01 M CPC
6: 0.02 M CPC
7: 0.04 M CPC

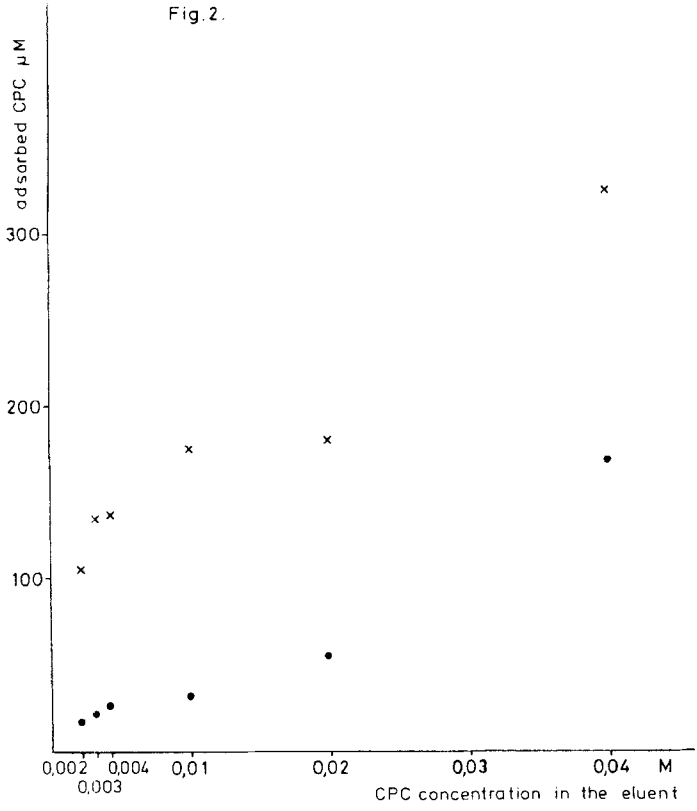
given in Table 1. On the increase of the CPC concentration in the eluent, the retention times of the acids are lengthened. We presume that ion-pair formation occurs between the acids (anions) and CPC ions, the resulting ion-pairs are bound more strongly than the free acids (anions) on the dynamically-coated stationary phase. Table 1 also reveals that elevation of the concentration of CPC in the eluent increases the difference between the retention times of the acids.

The synthesis intermediates No. 5 and 9 show that they can not interact strongly with the stationary phase, and increase of the concentration of CPC on the silica surface does not influence the retention times of these esters. The acid amide (compound No. 4) shows a light retention increase.

The behaviour of 4-amino-hydratropic acid ethyl ester (compound No. 8) must be emphasized. On increase of the concentration of CPC in the eluent, the retention value for this compound increases considerably. This is presumably attributable to the larger alkyl group of the ester.

Table 1 reveals that the application of CPC in the mobile phase increases the selectivity of the chromatographic system. For some acid-pairs the sequence of chromatographic migration is reversed.

The amount of the adsorbed CPC was determined by the breakthrough method of Bartha and Vigh [11] with an adjustment we applied earlier for the determination of camphorsulfonic acid's adsorption [12]. The results are shown in Fig. 2. It can be seen, that the amount of CPC adsorbed is dependent on the CPC concentration of the eluent. On the other hand, the pH of the mobile phase has also a strong influence on the CPC-binding by the



Column : 250mm x 4,6mm I.D. (Lichrosorb Si 60 10µm)
pH = 7,5 — ×
pH = 5,0 — •

Fig. 2 The adsorption isotherm of CPC

silica. The amounts of the bonded CPC are similar to those have been reported for CTMA at pH = 5 by Gazdag et al [8] as well as for palmityl ammonium propanesulfonate (PAPS) at pH = 7.5 by Hansen and Tjørnelund [13]. The fact, that the amount of the adsorbed CPC increases parallel with the CPC concentration of the

Table 2

Comparison of the retentions (t_R,sec) in CPC and CTMA containing HPLC systems

Compound	CPC concentration mM			CTMA concentration mM		
	10	20	40	10	20	40
Salicylic acid	237	382	623	208	227	264
Nicotinic acid				224	248	278
2-Chloro nicotinic acid	231	282	374	217	234	259
Nicotinic acid N-oxide	241	315	375	229	248	277
Nifluminic acid	266	281	351	239	278	386
Diclofenac	403	1078	1612	238	284	310
Fenoprofen	253	425	580	221	246	337
Ibuprofen	281	466	530	205	220	
Naproxen	277	431	567	221	248	308

System: Silicagel/Methanol-Water-Phosphate buffer (pH = 5) 60:30:10 CPC or CTMA.

eluent, and no maximum or saturation is observable may be a consequence of the fact, that the critical micellar concentration (CMC) for CPC is at about 0.04 M (determined by conductance measurement). Due to solubility problems eluents with CPC concentration higher than 0.04 M have not been used in these experiments. Hansen and Tjørnelund [13] found that the adsorption of PAPS showed a steep increase after reaching the CMC value.

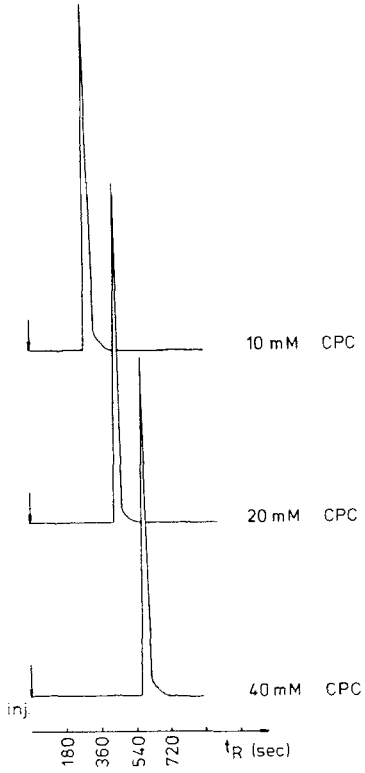


Fig. 3 Variation in retention times of naproxen as a function of cetylpyridinium chloride concentration

To estimate the retaining property and selectivity of the HPLC systems containing silica stationary phase modified by CPC and CTMA respectively the retention data for a series of acidic compounds are collected in Table 2.

Fig. 3 shows a series of naproxen's chromatograms as a function of cetylpyridinium chloride concentration.

REFERENCES

1. Hansen, S.H., J. Chromatogr. 209, 203 (1981)

2. Hansen, S.H., Helboe, P., Thomsen, M. and Lund, U., J. Chromatogr. 210, 453 (1981)
3. Hansen, S.H., Helboe, P., Lund, U., J. Chromatogr. 240, 319 (1982)
4. Hansen, S.H., Helboe, P., Lund, U., J. Chromatogr. 260, 156 (1983)
5. Hansen, S.H., Helboe, P., Lund, U., J. Chromatogr. 270, 77 (1983)
6. Hansen, S.H., Helboe, P., J. Chromatogr. 285, 53 (1984)
7. Ghaemi, Y. and Wall, R.A., J. Chromatogr. 174, 51 (1979)
8. Gazdag, M., Szepesi, G., Hernyes, M., J. Chromatogr. 316, 267 (1984)
9. Budvári-Bárány, Zs., Radecyky, G., Shalaby, A. and Szász, Gy., Acta Pharm. Hung. 59, 49 (1989).
10. Bidlingmeyer, B.A., Warren, F.V., Jr., Anal. Chem. 54, 2351 (1982)
11. Bartha, A., Vigh, Gy., J. Chromatogr. 260, 337 (1983)
12. Lőre, A., Budvári-Bárány, Zs., Szász, Gy., Shalaby,A., J. Chromatogr. 14, 2065 (1991)
13. Hansen, H., Tjørnelund, Jette, J. chromatogr. 556, 353 (1991)

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