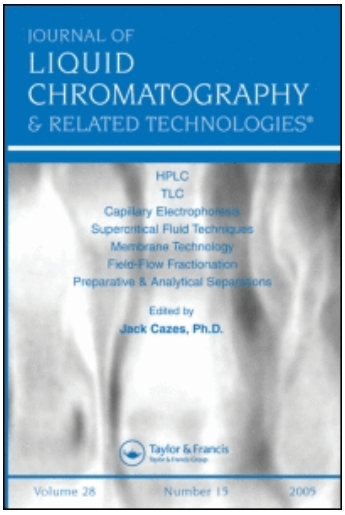

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Further Investigations in HPLC Systems Containing Silica and Polar Bonded Silica Packing Dynamically Modified by Camphorsulfonic Acid

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FURTHER INVESTIGATIONS IN HPLC SYSTEMS CONTAINING SILICA AND POLAR BONDED SILICA PACKING DYNAMICALLY MODIFIED BY CAMPHORSULFONIC ACID

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ABSTRACT

The investigation of chromatographic (HPLC) systems containing camphorsulfonic acid (CSA) has been continued. Similarly to the previous finding for silica, on polar bonded silica packings CSA forms double layer. The adsorbed amount of CSA was determined by the evaluation of break-through curves. It was found that the addition of triethylamine in a small excess to the CSA containing eluent results in the formation of a versatile chromatographic system. The adsorbed amount of CSA and TEA from such eluents was determined by the estimation of the break-through curves were gained by simultaneous refractometric and UV spectrophotometric detection. The migration of the compounds follows a normal phase mechanism evidenced by the behavior of the examined medicinal compounds.

INTRODUCTION

In a previous paper [1] the adsorption of camphorsulfonic acid (CSA) on bare silica was followed by the break-through method, using methanol-chloroform-CSA and acetonitril-chloroform-CSA mixture as mobile phase. In the same study the formation of CSA-double layers was suggested and the amount of CSA bonded in the 1. and 2. layer has been determined. The different

sorptional feature of the two CSA-layers was revealed. It seemed reasonable to extend the research to the study of CSA-bonding power of cyanopropylsilica and other polar bonded silica sorbents representing a polarity weaker than that of the bare silica and thereby the field of application of CSA-silica systems should be widened [2]. In trying to increase the versatility, i.e. selectivity of the system, as an organic modifier triethylamine was added in excess to the mobile phase. The separation power of a similar chromatographic system containing cyanopropylsilica, CSA as ion exchanger and diethylamine as a counter base, was carefully studied by Szepesi et al. [3,4]. Whether in our case, the retention bahavior of the substances tested should reflect a normal- or rather a reversed phase character of the silica/CSA-TEA, etc containing system, was a further question to be answered.

EXPERIMENTAL

Materials

All of the model stubstances, but not the fluoroquinolones, and the steroids were a quality of Ph.Hg.VII. (Pharmacopoeia Hungarica VII.).

Fluoroquinolones were synthetized at Chinoin Pharmaceutical Works (Budapest) and used without further purification.

The steroids were synthetized at Gedeon Richter Pharmaceutical Works (Budapest) and used without further purification.

Methanol HPLC grade (Chemolab, Budapest)

Chloroform Ph.Hg.VII., was used after purification by the Ph.Hg. method.

Triaethylamin, TEA (LOBA Chemie, Fishamend, Germany)

(+)10-Camphorsulfonic acid (CSA) monohydrate (Merck).

Chromatography

The HPLC apparatus was comprised in Waters (Millipore, USA) Mode 501 solvent delivery system, LABOR MIM (Budapest, Hungary) Model QE 308 variable wavelength UV photometer, Yokogava (Tokyo, Japan) Type 3051 recorder. For the determination of triethylamine adsorption the effluent was monitored with RI detection (Waters Differential Refractometer, Model R 401).

The packings were filled into stainless steel columns (250 x 4 mm I.D.). Adsorbents were purchased from BST (Bioseparation-Technique Ltd., Budapest, Hungary) in a particle size 5 μm of each: Silica Si-100-S, Diol Si-100-S, Cyanopropyl Si-100-S, Phenylsilica Si-100-S.

Break-through curves

The amount of adsorbed CSA and TEA was determined by the evaluation of the correspondent break-through curves. The equipment used for the break-through measurements is a modified version was described in a previous publication [1]. For the simultaneous measurement of CSA and TEA adsorption an apparatus working with two parallel switched pumps, columns and detectors (UV, RI) was applied [5].

Mobile phase, substance application, recording.

As eluent the mixture of chloroform and methanol 99:1 containing given concentration of CSA and/or TEA was used. The eluents were filtered and degassed prior to chromatography. The flow rate was 1.0 ml/min. A Model 7125 sampling valve (Rheodyne, Berkley, USA) was applied.

The column-temperature was controlled by recirculating water through an isolated stainless jacket from termostat (Ultrathermostate MLW Type U2C, Freital, Germany).

The model substances were solved in the correspondent eluents. The basic compounds, due to solubility problems, were applied in their base form.

The chromatograms were obtained and recorded, the retention data were collected by a Hewlett-Packard integrator Model 3396 Ser 2.

Each retention data was calculated as an average of three parallel runs. The mobile phase hold-up time was signalled by the solvent peak of methanol

RESULTS AND DISCUSSION

Table 1 involves the data of adsorption for CSA on silica and three polar bonded silica packings. The results were gained by the evaluation of the break-through curves. The main message of the table is, that in accordance with the earlier (1) and present finding for silica, CSA forms two distinct layers (i.e.

Table 1

The adsorption of CSA on silica and polar bonded silica columns (Mobile phase: chloroform - methanol 99:1).

Column	CSA in the eluent M	CSA adsorbed uM/g	
		Layer 1.	Layer 2.
SILICA	0.005	242.2	-
	0.010	240.0	115.0
	0.020	249.2	249.2
SILICA-DIOL	0.005	40.6	28.32
	0.010	65.6	37.32
	0,020	97.2	99.2
CYANOPROPYL-SILICA	0.005	49.2	21.0
	0.010	76.0	39.12
	0.020	110.8	119.52
PHENYL-SILICA	0.005	56.0	13.12
	0.010	85.2	52.8
	0.020	124.0	111.2

double layer) on the surface of each of the three polar bonded silica adsorbents. Another characteristic of the table is the increase of the adsorbance by increasing the CSA-concentration of the eluent. This is true for both of the CSA-layers. At a CSA concentration 0.02 M the amount of adsorbed CSA by the second layer equals with the one adsorbed by the first layer. This unexpectedly high value in the 2nd layer may come from the simultaneous adsorption of CSA by the empty 2nd layer and the continous occupation of the free sites of the first layer. The amount of CSA adsorbed by the polar bonded silica layers is about 1/3rd of that bonded by the bare silica. The gradual increase of CSA in the 1st. layer parallels with a steep rise of CSA-adsorption into the 2nd. layer.

Table 2 shows the amount of adsorbed CSA and TEA in the same chromatographic system, when the TEA was present in a minute excess. In the latter case double layer formation does not take place. The comparison of the

Table 2

Simultaneous adsorption of TEA and CSA by different silica packings

Column	Eluent $CHCl_3$ - MeOH 99:1			Ratio of adsorbed TEA:CSA
	+0.012 M TEA	+0.012M TEA + 0.010 M CSA		
	Adsorbed amount uM/g			
	TEA	TEA	CSA	
SILICA	352.4	329.2	261.2	1.26
SILICA-DIOL	74.88	74.4	46.4	1.62
CYANOPROPYL-SILICA	152.64	149.2	114.8	1.31
PHENYL-SILICA	160.0	144.8	108.8	1.33

corresponding data of the Tables 1 and 2 shows, that TEA, excepted cyanopropyl silica, strongly reduces the adsorbed amount of CSA by bare silica as well as polar bonded silica surfaces. On the other hand, CSA has small influence on the adsorption of TEA by the silicas. The TEA and CSA are adsorbed in an amount corresponding to a mol ratio approximately 1.3, excepted diol silica, where the ratio index is higher, 1.6. This fact allows to conclude, that TEA and CSA occupies the major part of the silica surface in the form of a (1:1) salt-like compound and in addition, a part of the superfluous TEA adheres to the free silanol groups of the surface. Considering the moderately polar character of the weakly alkaline (non adsorbed TEA) mobile phase the stationary phase in this chromatographic system must definitely exert a polar sorbent activity, since all the solutes migrate according to the rules of a normal phase mechanism.

Table 3 show the retention for 25 structurally different medicinal compounds, having neutral, acidic or basic character. It can be seen, that the mobile phase-C, containing CSA and in a small excess TEA, is suitable for the elution of the organic bases too. We met only one application of the latter eluent type, when certain steroids were separated [6]. It is worthy of note, that with

Table 3

The retention (t_R min) of drug compounds in different CSA (TEA) containing chromatographic systems
(SI: silica; Diol:silicadiol; Phe: Phenylsilica; CNP:cyanopropylsilica; NH_2:amonosilica; NE:no elution within 90 minutes.

COMPOUND	$CHCl_3$: MeOH 99:1 A)					$CHCl_3$:MeOH 99:1 +0.010 M CSA B)					$CHCl_3$:MeOH 99:1 +0.010 M CSA +0.012 M TEA C)				
	SORBENT					SORBENT					SORBENT				
	Si	Diol	Phe	CNP	NH_2	Si	Diol	Phe	CNP	NH_2	Si	Diol	Phe	CNP	NH_2
Norgestrel	10.5	3.5	4.5	4.5	1.2	3.5	2.9	3.3	3.4	1.5	2.9	3.0	2.9	3.3	1.3
Ethisterone	12.9	3.8	5.0	4.7	1.2	3.8	3.0	3.5	3.6	1.5	3.2	3.1	3.0	3.3	1.3
Norethisterone	15.0	3.9	5.5	4.5	1.2	4.1	3.1	3.8	3.7	1.5	3.3	3.2	3.1	3.5	1.3
Benzoic acid	11.1	7.7	6.3	NE	NE	6.4	6.7	5.6	5.2	2.3	NE	NE	55.6	NE	NE
Salicylic acid	NE	8.2	5.4	NE	NE	6.9	7.8	5.2	6.0	5.6	NE	NE	NE	NE	NE
Acetylsalicylic. acid	NE	8.3	7.5	NE	NE	7.3	6.1	6.4	6.9	1.9	NE	7.5	NE	NE	NE
Hexobarbital	7.6	3.7	4.2	3.9	1.3	3.4	3.0	3.0	3.2	1.2	3.0	3.2	3.0	3.4	1.4
Phenobarbital	22.4	10.2	8.0	7.8	3.4	8.7	7.6	5.4	6.4	2.4	8.2	8.3	5.9	11.8	4.8
Caffeine	42.0	5.2	14.0	7.4	1.2	NE	9.6	NE	23.3	1.2	4.1	3.3	5.2	3.7	1.3
Theobromine	NE	15.5	36.8	26.4	1.5	NE	38.6	NE	NE	1.6	9.1	6.6	9.3	7.0	1.7
Theophylline	NE	26.3	60.5	19.7	2.8	NE	56.7	NE	NE	4.7	24.8	NE	28.6	15.1	3.2
Benzocaine	45.3	14.1	14.1	4.4	1.3	40.6	6.2	3.4	5.5	2.3	3.9	3.5	3.6	3.1	NE
Lidocaine	NE	NE	NE	NE	1.1	NE	NE	NE	NE	1.5	2.7	2.6	2.8	3.1	1.2
Tetracaine	NE	NE	NE	NE	1.3	NE	NE	NE	NE	2.4	10.0	5.5	9.1	9.5	1.2
Procaine	NE	NE	NE	NE	1.6	NE	NE	NE	NE	4.6	22.6	7.4	13.5	15.4	1.5
Ethylmorphine	NE	NE	NE	NE	1.3	NE	12.9	NE	NE	1.3	23.0	8.3	18.1	13.9	1.3
Codeine	NE	NE	NE	NE	1.3	NE	15.1	NE	NE	1.3	25.7	9.2	19.3	15.0	1.4
Chlorpromazine	NE	NE	NE	NE	1.3	NE	12.1	NE	34.2	1.2	2.8	5.5	10.7	7.6	1.2
Levomepromazine	NE	NE	NE	NE	1.3	NE	7.7 8.0	NE	28.9	1.2	6.8	3.8	6.0	5.2	1.2
Promethazine	NE	NE	NE	NE	1.3	NE	8.5	NE	29.2	1.2	9.8	4.7	9.5	6.8	1.3
Trifluperazine	NE	NE	NE	NE	1.3	NE	44.3	NE	NE	1.2	11.2	6.0	14.4	9.1	1.2
Quinine	NE	NE	NE	NE	1.2	NE	30.6	NE	NE	1.3	58.3	14.8	30.8	18.0	1.5
Quinidine	NE	NE	NE	NE	1.2	NE	24.7	NE	NE	1.3	34.8	10.4	18.4	14.2	1.4
Cinchonine	NE	NE	NE	NE	1.2	NE	57.4	NE	NE	1.3	49.7	13.8	24.3	18.8	1.4
Cinchonidine	NE	NE	NE	NE	1.2	NE	60.3	NE	NE	1.3	72.4	19.7	35.9	25.6	1.6

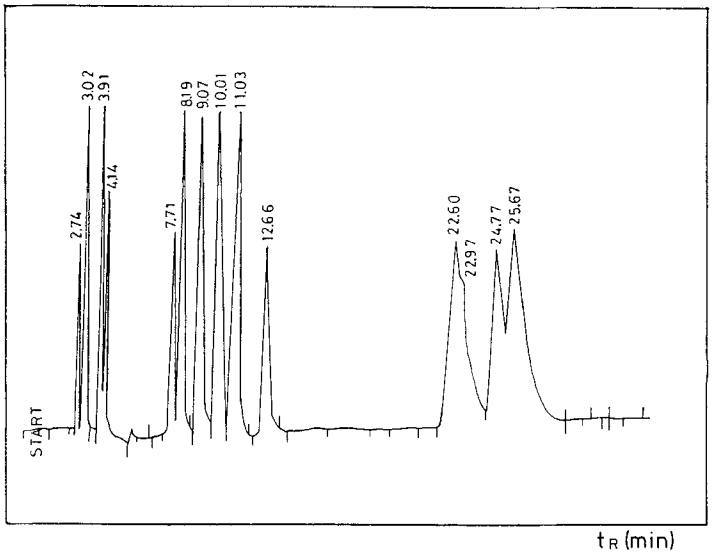

Figure 1: Representative chromatogram of 15 model compounds.

Sorbent: silica, eluent: C (see Table 3).
2.74 lidocaine, 3.02 hexobarbital, 3.91 benzocaine
4.14 caffeine , 7.71 levomepromazine,
8.19 phenobarbital, 9.07 theobromine, 10.01 tetracaine,
11.03 promathazine, 12.66 chlorpromazine, trifluperazine,
22.60 procaine, 22.97 ethylmorphine, 24.77 theophylline,
25.67 codeine.

this alkaline eluent the best separations were achieved just on silica gel surface, which, due to its too polar feature, could not be used at all when the mobile phase contained CSA in excess [2]. Another benefit using the TEA-excess containing (basic) eluent is, that it dissolves well also the salt forms of the amine pharmaceutics, which will migrate in their base form. These basic compounds could not be eluted at all with chloroform-methanol mixture with or without CSA content (Table 3, eluent A and B, compds. 12-25). In the system-C homologs (as codeine-ethylmorphine) or structurally closely related substances (see compds. 9→11, 18→21, 22→25) can be separated. The quality of separation is illustrated by Fig. 1. The chromatogram of five "*floxacins*", *is*

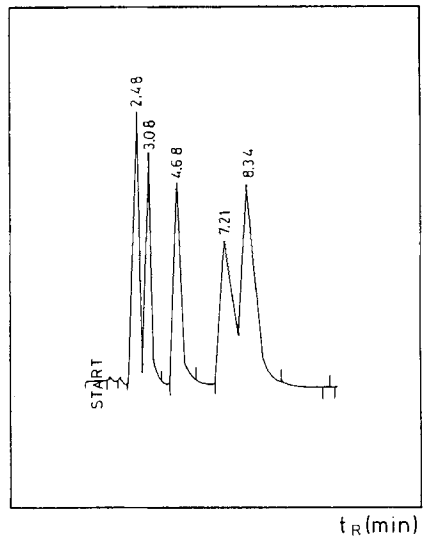

Figure 2: Chromatogram of five gyrase inhibitor fluoroquinolone derivatives.

2.48 pefloxacin, 3.08 lomefloxacin,
4.68 amifloxacin, 7.21 ciprofloxacin,
8.34 norfloxacin.

shown by Fig. 2. These gyrase inhibitor fluoroquinolone derivatives could be eluted from the aminosilica surface and separate well with the alkaline eluent (C). The strong retention of acids (benzoic-, salicylic acid, etc.) against the alkaline eluent may be explained by a substitution effect they exert on the bonded CSA.

The comparing of certain retentions evidences the normal phase character of the chromatographic system-C.: cf. the retentions caffeine-theobromine-theophylline, procaine-tetracaine, codeine-ethylmorphine, etc.

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