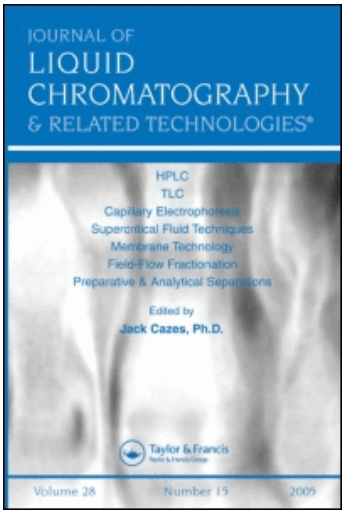

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A. Lore[a]; Zs. BudvÁri-BÁrÁny[a]; Gy. SzÁsz[a]; A. Shalaby[b]
[a] Institute of Pharmaceutical Chemistry Semmelweis University of Medicine, Budapest, Hungary [b] Department of Pharmaceutical Chemistry, University of Zagazig, Zagazig, Egypt

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INVESTIGATIONS IN HPLC SYSTEM CONTAINING SILICA DYNAMICALLY MODIFIED BY CAMPHORSULFONIC ACID

A. LŐRE[1], ZS. BUDVÁRI-BÁRÁNY[1], GY. SZÁSZ[1], AND A. SHALABY[2]
[1]*Institute of Pharmaceutical Chemistry*
Semmelweis University of Medicine
Budapest, Hungary
[2]*Department of Pharmaceutical Chemistry*
University of Zagazig
Zagazig, Egypt

ABSTRACT

The adsorption of camphorsulfonic acid (CSA) by silica was followed by the break-through method using methanol-water-CSA and acetonitril-water-CSA mixture as mobile phases. The break-through curves comprise two distinct waves, indicating the formation of CSA-double layer on the silica surface. The amount of CSA bound in the 1. and 2. layer is a function of the methanol-, acetonitril- and CSA-content of the eluent. Following the retention of a model substance along the break-through curve from methanol-water-CSA eluent, the different sorptional feature of the two CSA layers was revealed: retention increase followed by the depression of

the retention could be observed before and after the first break-through point respectively. Further study of these appearances is in progress.

INTRODUCTION

In a previous paper (1) HPLC behaviour of imidazo-quinazolone derivatives has been reported. Using silica as stationary phase an increase, followed by a depression of retention was observed when camphorsulphonic acid (CSA) concentration of the methanol-water-CSA eluent was gradually increased (1). At first impression, considering the highly polar character of the stationary phase, this experience should indicate molecular complex formation between CSA and the model compounds. However, assuming that equilibration of the system is accompanied by the adsorption of CSA on the silica surface, the organization of a dynamically modified reversed phase, ion pair formation, resulting in a retention increase could be also assumed. The lack of literature data initiated us to begin a detailed study related to the CSA--adsorption on silica surface.

CSA as HPLC-ion pairing agent was used on cyanopropyl stationary phase by Szepesi et al (2) on diol stationary phase by Petterson and Schill (3) for enantioseparation of eburnane-alkaloids and β -blocking agent respectively. Ladányi et al. (4) separated 8-azagonane-12-one enantiomers by HPLC on silica gel stationary phase, while Brugman et al. (5) have studied the retention behaviour of nucleobases and nucleosides on silica gel using CSA as ion pairing agent. A

relatively great number of authors used CSA in RPHPLC systems, unless the mechanism of CSA-action would have been investigated.

The binding of potential pairing species in normal- as well as in reversed phase systems is generally examined by the break-through method (6,7,8). Basing on this method the binding of CSA by silica gel surface could have been followed.

EXPERIMENTAL

Materials

The model substance, 3-methyl-2-(4'-methylphenyl)-imidazo(5,1-b)-quinazol-9-one (structure see in Fig. 4) was synthetized in our laboratory (9). Its quality was checked by chromatography.

(+)-10-camphorsulfonic acid monohydrate (Merck).

Chloroform was purchased from Interkémia (Budapest, Hungary) and was used after purification by the method of the Hungarian Pharmacopoeia (10).

Methanol (analytical grade) was obtained from Reanal (Budapest).

Acetonitril (for HPLC) was a product of Chemolab (Budapest).

Chromatography

The HPLC equipment consisted of the following parts: Waters (Millipore, USA), Model 501 solvent delivery system, LABOR MIM (Budapest, Hungary), Model OE 308 variable wave-

length UV photometer as detector, Yokogawa (Tokyo, Japan), Type 3051 recorder was utilized. A stainless-steel column (250x4.6 mm I.D.) packed with 5 μm LiChrosorb Si_{60} (BST, Budapest) was applied. As mobile phase chloroform-methanol (99:1, 97:3, 95:5) mixtures containing different concentrations (0.005-0.05 M) of camphorsulfonic acid were used.

Break-through curves

The schematic diagram of the apparatus used for break--through curve determinations is shown in Fig. 1.

In contrast with the equipments described in previous papers (6,7) the apparatus used works with only one pump. To balance the pressure (i.e. to eliminate pressure surges) an auxiliary column (the same size and packing as the first column) fitted to a second injector, was applied. On the beginning of the experiment the chromatographic system was flushed up with CSA-free eluent and thereafter the break--through curves of the eluents containing different amount of CSA have been recorded. Spectrophotometric detection at 290 nm was applied. Flow rate: 1 ml/min., chart speed: 20 cm/h.

Retention behaviour of the model substance along the break-through curve.

Following the method of break-through determination described above, chloroform-methanol-CSA 97:3:0.03 M mixture as eluent was used. From the moment when CSA containing eluent was allowed to enter the system and thereafter in every two

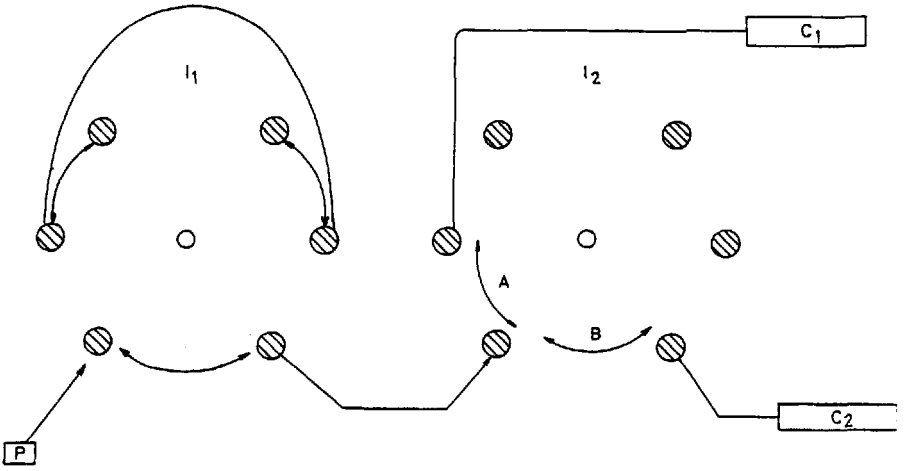

Figure 1

The chromatographic system for break-through determination (schematic diagram).

- I_1 Injector for sampling
- I_2 Injector for two ways valve function
- P Pump
- C_1 Column for analytical purposes
- C_2 Column for balancing of pressure surges
- A Switching position: open to C_1
- B Switching position: open to C_2

minutes, equal amounts of the model substance have been injected. The latter was solved in a chloroform-methanol 97:3 mixture (concn. 0.016%) and 20 µl aliquots were injected.

RESULTS, DISCUSSION

A series of break-through curves are plotted in Figure 2. On the curves, parallel with the increase of CSA-concen-

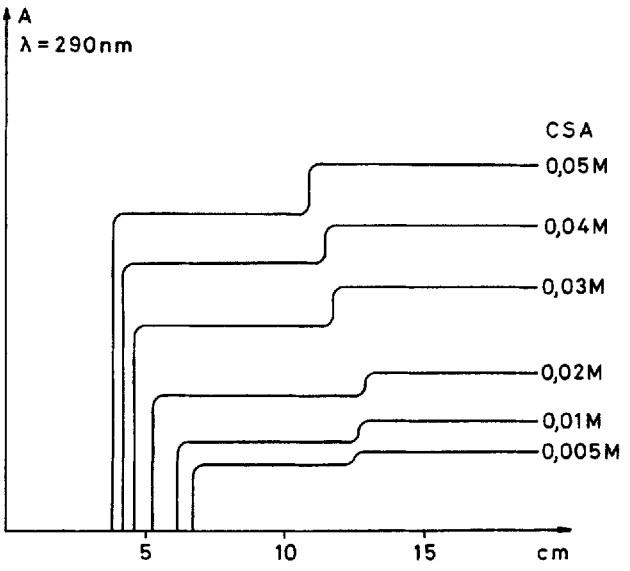

Figure 2

Break-through curves for CSA

Chromatographic system: LiChrosorb Si_{60}/Chloroform-Methanol--CSA 97:3 : 0.005-0.05 M.

trations, a second rise may be more and more definitely observed. This phenomenon is to be interpreted with the formation of CSA double layer on the silica-surface. The total bound amount of CSA is shown in Table 1. It is to be noted that surface area parameter for LiChrosorb Si_{60} (in bulk) was taken as 550 m^2/g, a data is given by the manufacturer (E. Merck; 11). Considering the fact that this is a data only for approximate information and the adsorbed amounts of CSA were calculated by using this value, consequently, the

Table I

The adsorption of CSA from chloroform-methanol eluent

CSA conc. mol (mobile phase)	CSA adsorbed $\mu g/m^2$								
	until the 1st b. th. p.*			until the 2nd b. th. p.			total amount		
	A	B	C	A	B	C	A	B	C
0.005	48.50	19.00	8.32	32.91	2.24	0.43	81.41	21.24	8.75
0.01	74.41	37.95	18.80	41.32	6.20	1.79	135.73	44.15	20.59
0.02	95.46	54.83	32.43	62.60	10.37	4.58	158.06	65.20	37.01
0.03	121.24	76.10	47.92	80.61	15.20	7.07	201.86	91.20	54.99
0.04	146.36	86.57	55.92	80.40	22.66	10.67	226.76	109.17	66.59
0.05	180.21	106.49	67.71	74.04	26.63	9.87	254.25	133.12	77.58

Methanol in mobile phase vol.%

A:1 B:3 C:5

*break-through point

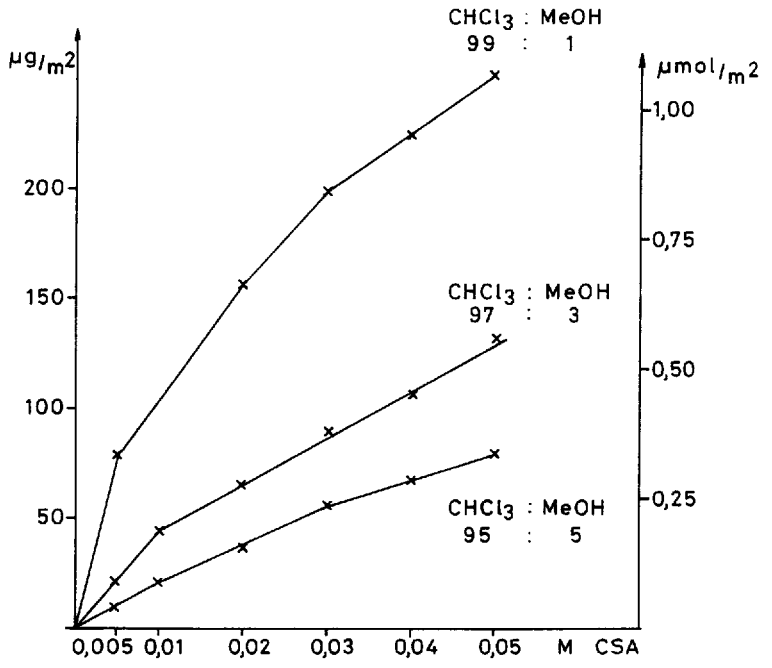

Figure 3

CSA-adsorption on silica surface as a function of the methanol and CSA content of the mobile phase.

data in Table 1 showing the extent of CSA-adsorption as function of the CSA and methanol concentration of the eluent, also must be regarded as rough estimations. The influence of methanol on CSA binding is based on the interaction between methanol and the silanol groups as well as methanol and CSA. It can be seen in Table 1, that the amount of CSA was bound by the second layer, is much more smaller than that is by the first one but it is still significant, especially in the case

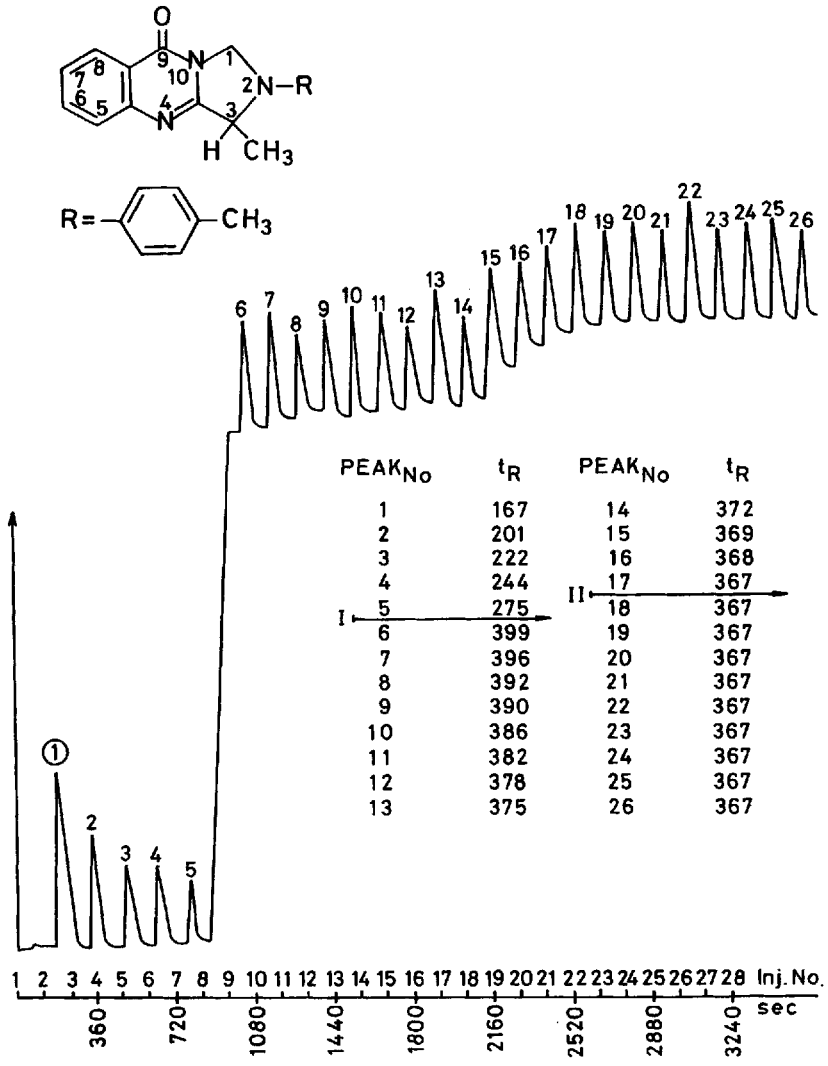

Figure 4

Retention behaviour of a model substance along the break-through curve of CSA.

Chromatographic system: LiChrosorb Si_{60}/Chloroform-Methanol--CSA 97:3:0.03 M.

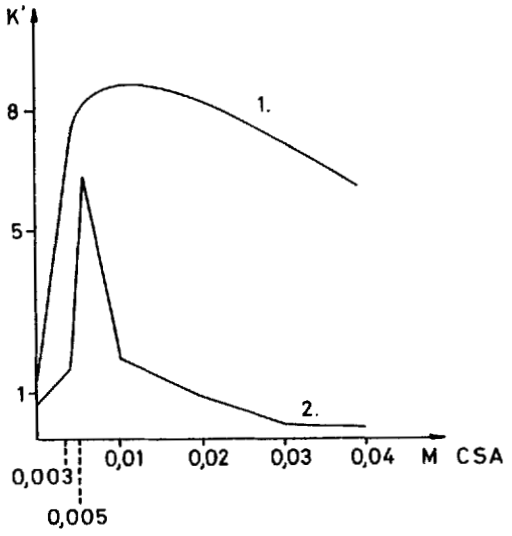

Figure 5

Retention of the model substances as a function of the methanol and CSA content of the mobile phase.

1. R ▫ propyl (see structure in Fig. 3) (LiChrosorb Si_{60}/$CHCl_3$-MeOH 95:5)
2. R = phenyl (see structure in Fig. 3) (LiChrosorb Si_{60}/$CHCl_3$-MeOH 99:1)

of methanol-poor eluents. Fig. 3 shows the total amount of CSA was bound from the eluents with various CSA content.

Our preliminary experiments reveal different sorbent properties on behalf of the two CSA-layers. Figure 4 shows the retention behaviour of an imidazo-quinazolone model substance along the break-through curve of CSA. It is certainly interesting that the retention gradually increases until the "break--through 1", then it begins to decrease until a second rise of the curve ("break-through 2").

Table II

The adsorption of CSA from chloroform-acetonitril eluent

CSA conc. mol (mobile phase)	CSA adsorbed $\mu g/m^2$		
	until the 1st b. th. p.*	until the 2nd b. th. p.	total amount
0.005	37.55	37.55	75.10
0.01	65.08	70.09	135.17
0.02	110.17	125.16	235.30
0.03	137.68	170.22	307.90
0.04	182.3	292.90	475.63

Mobile phase: chloroform-acetonitril 95:5 + CSA

*break-through point

In a previous work (10) we reported on the retention behaviour of several imidazo-quinazolone derivatives in the same chromatographic system, when methanol or CSA concentration was varied. Fig. 5 shows that in eluents with relatively higher methanol content the retention, after a peaking, gently decreases, while in eluents with low methanol content a dramatic fall and dropping under the original value may be observed. These results, in accordance with the present observations, clearly show the influence of the second layer of CSA on the retentions. When double-layer formation is enhanced by the experimental conditions (i.e. relatively low methanol and high CSA content of the eluent) its retention depressing property is manifested (see the curve No 2). In case of the chloroform-methanol 95:5 eluent the chance of double-layer formation is reduced (see data in Table 1) whereby the chromatographic process will be governed by the retention increasing effect of the first layer. For the interpretation of the above phenomenons further experiments are necessary on the mechanism of CSA-binding by silica surface.

The adsorption of CSA by the silica gel surface was examined also from acetonitril-water eluent; basically the same results, i.e. the formation of CSA double-layer could be observed, but the amount of the bound CSA was found significantly higher (Table II) than in the case of the methanol-water eluent.

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