

CHROM. 12,552

## INFLUENCE OF MOBILE PHASE COMPOSITION AND NATURE OF SAMPLE ON RETENTION DATA FROM HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

J. PUNČOCHÁŘOVÁ, J. KRÍŽ, L. VODIČKA and D. PRŮŠOVÁ

*Institute of Chemical Technology, Laboratory of Synthetic Fuels, Suchbátarova 5, 166 28 Prague 6 (Czechoslovakia)*

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### SUMMARY

The dependences of retention data, expressed as capacity factors  $k'$ , linear capacity of the column  $\theta_{0,1}$ , height equivalent to a theoretical plate (HETP) and peak shape, on the sample size are described. The measurements were carried out on a silica stationary phase with twelve different mobile phases. Cyclohexanol and cyclohexanone were the injected samples. It was found that the shape of the adsorption isotherm and consequently the symmetry of the sample elution peak is determined by the composition of the mobile phase. Also, the retention times decrease or increase with size of the injected sample depending on the adsorption type, *i.e.*, depending on the sample type and the mobile phase. A suitable mobile phase composition may result in an expansion of the region of the linear capacity of the column.

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### INTRODUCTION

In chromatographic studies, it is advantageous for reproducibility of retention data if measurements are carried out in the linear region of the relevant adsorption isotherm. When working on an analytical scale and using a sensitive detector it is possible to confine the measurements within this linear region. However, with a less sensitive detector, when it is necessary to increase the size of the injected sample, the dependence of the retention data on the sample size must be determined. In preparative liquid chromatography (LC), the non-linear region of the isotherm must also be exploited. Thus, with the growing importance of preparative LC, the problem of the dependence of retention data and peak shape on the degree of coverage of the adsorbent by the sample is really significant.

In classical adsorption chromatography, the separation process is described by means of a convex isotherm. The adsorbents used are usually somewhat deactivated by water. When using solvents that are not moderated in any way or that contain only small amounts of water, elution of water from the adsorbent to the mobile phase starts on contact with the adsorbent; on becoming activated the latter causes retention volumes to increase. Thus it is very difficult to reach a constant equilibrium between the mobile phase and adsorbent in such systems.

Highly efficient columns are now used in high-performance liquid chromatography (HPLC) which here longer lives. Thus it is desirable to maintain the equilibrium between the mobile phase and the adsorbent for a prolonged time. Also, moderators other than water may be chosen for chromatography of polar compounds on polar adsorbents. Such reagents (*e.g.*, alcohols, ethers) are very soluble in the basic non-polar solvent, and if they are present in the mobile phase in high amounts (*e.g.*, 1%) it is possible to maintain equilibrium between the polar adsorbent and mobile phase for a long time. However, there still remains the question of the real nature of the chromatographic system when using these moderators.

In the present paper, we describe the dependences of retention data, expressed as capacity factors  $k'$ , linear capacity of the column  $\theta_{0,1}$ , height equivalent to a theoretical plate (HETP) and peak shape, on the size of the injected sample of cyclohexanol and cyclohexanone, with the mobile phase composition being variable.

### THEORETICAL

Three types of isotherm may be distinguished in chromatographic systems: linear (1), convex (2) and concave (3).

The connection between retention data, peak shape and type of adsorption isotherm has been described by Snyder<sup>1,2</sup>. The first part of the isotherm corresponding to small amounts of injected sample is often linear. A convex isotherm<sup>1,3</sup> is typical of classical adsorption liquid chromatography. It is expected in cases of prevailing monolayer adsorption. Concave isotherms are infrequent in adsorption LC and are connected with multilayer adsorption<sup>1,4</sup>. In such systems there is usually a strong interaction between molecules already adsorbed, and the adsorbent-sample interactions are relatively weak. Under these conditions, hydrogen bonds may be formed, which then represent an important contribution to the adsorption energy of the LC system<sup>1,5,6</sup>.

In the case of adsorption LC, the chromatographic system is characterized by two simultaneous isotherms, *i.e.*, the sample isotherm and the solvent isotherm on the given adsorbent<sup>1,6</sup>. Mutual competition between sample molecules and solvent molecules during localization on active sites of the adsorbent bed results in the final form of the separation process. An ideal situation for chromatographic measurements would result in perfect reproducibility of measured data over the complete concentration range employed. In practice, this means performing the measurements in the region of linear capacity of the column,  $\theta_{0,1}$ .

The linear capacity of a column is defined<sup>1</sup> as the covering of the adsorbent by the sample (grams of sample per grams of adsorbent) which results in changes in retention data of less than 10%.

Three contributions of the solvent to the adsorption energy of a chromatographic process have to be considered: interactions between solvent molecules and sample molecules in the mobile phase; interactions between solvent molecules and sample molecules in the stationary phase; and interactions between solvent molecules and adsorbent. Formation of solvent-sample "complexes" in the liquid and the adsorbed phases may increase or decrease sample adsorption according to the degree of adsorption of this "complex" compared to that of the free sample. As there is a competition between sample molecules and solvent molecules on the active sites of

the adsorbent surface, more strongly adsorbed solvents are considered to be stronger.

An increased loading of the column (*i.e.*, a higher amount of injected sample) results in a decrease in the column efficiency, a shift in the retention values and also, in some cases, in a change of the sample peak shape.

The effect of loading of the adsorbent with the sample on the HETP value can now be subjected to a more detailed study owing to the increased efficiency of liquid chromatography columns. Such columns were not available when Snyder<sup>7</sup> found HETP to be affected by sample sizes higher than  $10^{-4}$  g per g of adsorbent. Done<sup>8</sup> used much more efficient columns, which enabled him to observe a dependence of HETP on the sample size even for loadings of  $10^{-6}$  g of sample per gram of adsorbent. He also found that capacity factors decrease with increasing sample size, which is considered to be the classical dependence, the region of linear capacity corresponding to up to  $10^{-4}$  g of sample per gram of adsorbent (adsorbent, silica gel; mobile phase, *n*-hexane-dichloromethane). Endele and Halász<sup>9</sup> found a similar increase of *h* and decrease in capacity factors *k'* with increasing sample size for aromatic compounds (benzene, diphenyl, terphenyl, nitrobenzene) in the *n*-heptane-silica gel system. The maximum sample size for the region of linear capacity was  $2 \cdot 10^{-4}$  g of sample per gram of silica gel. Generally, the maximum loading for commercial types of silica gels is  $10^{-4}$  g of sample per gram of silica gel (with respect to the surface).

Majors<sup>10</sup> has also reported the traditional type of dependences of *h* and *k'* on the sample size: *k'* decreases and *h'* increases with increasing sample size, which corresponds to a non-linear isotherm. Water content has a significant effect on retention times. Boehme and Engelhardt<sup>11</sup> found that the retention times were decreased to one fifth when the water content was increased from 20 to 40 ppm. Kirkland<sup>6</sup> used water and alcohols for measuring the adsorbent activity. An increase in the moderator content results in higher homogeneity of the adsorbent surface. The nature of the dependences of *h* and *k'* on the sample size is a function of the mobile phase used. Classical dependences were found for both hexane moderated by water and methylene chloride moderated by water. In case of a diethyl ether-water mobile phase, both *k'* and *h* are strongly affected at low water concentrations (up to 10%). However, a further increase in water content has only a negligible effect on both parameters. Use of 5% water-saturated diethyl ether yields a linear dependence of *k'* on the degree of coverage in the complete range examined. When methylene chloride is moderated by alcohols, a marked asymmetry of the polar sample peak is found, which shifts with changing alcohol content: with increasing alcohol content in the mobile phase, the tailing moves from the side of the growing signal to that of the decreasing signal. In a system moderated by 2-propanol (in contrast to water-moderated systems), *k'* increases with increasing sample size. Kirkland has suggested that the nature of the adsorption isotherm may change upon increasing the moderator (alcohol) content.

## EXPERIMENTAL

### *Apparatus*

A Varian 8500 liquid chromatograph equipped with a syringe pump connected to an RI detector and an A 25 dual-channel strip chart recorder (Varian, Palo Alto, Calif., U.S.A.) was used. Sample injection was performed by the stop flow technique

with 5- and 10- $\mu$ l syringes (Hamilton, Bonaduz, Switzerland). Two Micropak Si-10 columns (Varian, 50 cm  $\times$  2 mm I.D. and 25 cm  $\times$  2 mm I.D.) packed with LiChrosorb Si 60 (10  $\mu$ m) were used in these measurements.

Retention data were calculated on a HP 9830A calculator connected with a HP 9866A thermal printer (Hewlett-Packard, Avondale, Pa., U.S.A.). Graphical treatment of data was carried out on the same calculator equipped with a HP 9862A plotter.

### Reagents

2-Propanol (analytical grade; Lachema, Brno, Czechoslovakia) was used without further treatment. *n*-Heptane (Reakhim, Moscow, U.S.S.R.) and diethyl ether (Lachema) were dried over sodium before use, rectified and stored over Nalsit A4 molecular sieves (CHZID, Bratislava, Czechoslovakia). Chloroform (analytical grade, Lachema) was shaken with 20% NaOH, then distilled water, dried over phosphorus pentoxide and distilled on a glass sieve column with no access to moisture.

### Mobile phase

Mobile phases were prepared from degassed components by weighing. The following mobile phases were used: *n*-heptane-2-propanol; *n*-heptane-diethyl ether; *n*-heptane-diethyl ether-2-propanol and *n*-heptane-chloroform-2-propanol. The compositions of the twelve eluents used is given in Table I.

### Procedure

Retention data (Table II) for cyclohexanol and cyclohexanone were measured at room temperature, using silica gel as the stationary phase. The flow-rate of mobile

TABLE I  
MOBILE PHASE COMPOSITION (% w/w)

No.	<i>n</i> -Heptane	2-Propanol
1a	99	1
1b	98	2
1c	97	3
	<i>n</i> -Heptane	Diethyl ether
2a	65	35
2b	50	50
2c	35	65
	<i>n</i> -Heptane-diethyl ether (65:35)	2-Propanol
3a	99.9	0.1
3b	99.5	0.5
3c	99.0	1.0
	<i>n</i> -Heptane-chloroform	2-Propanol
4a	98 (50:50)	2
4b	99.5 (95:5)	0.5
4c	99.5 (50:50)	0.5

phase was 30 ml/h. Prior to these measurements, the column was stabilized by washing with new mobile phase for 12 h (flow-rate 30 ml/h). The dead volume of the column was determined by measuring the retention time of an unretained sample—*isooctane*.

TABLE II

## RETENTION TIMES (sec) OBTAINED ON VARIOUS MOBILE PHASES

OL = Cyclohexanol; ON = cyclohexanone. Mobile phases numbered as in Table I.

Sample size (g)	Mobile phase															
	1a	1b	1c	1a	2a	2b	2c	3b	3c	3a	3b	3c	4a	4b	4c	
	OL	OL	OL	ON	OL	OL	OL	OL	OL	ON	ON	ON	OL	OL	OL	
2.0 · 10 <sup>-6</sup>			380					745								
2.5 · 10 <sup>-6</sup>			380			821	668	789		370	299		325			
5.0 · 10 <sup>-6</sup>			378			805	670	758	593	370	299	265	322			
1.0 · 10 <sup>-5</sup>		520	376	408	559	828	630	750	598	363	298	268	322			
2.0 · 10 <sup>-5</sup>		519	375	409	523	802			592	368	295	261	323	556		
2.5 · 10 <sup>-5</sup>	375	520	371	410	518	798	635	751	592	362	293	263	321	562		
5.0 · 10 <sup>-5</sup>	375	520	372	411	500	790	615	768	598	360	290	259	321	550	1380	
1.0 · 10 <sup>-4</sup>	379	520	370	428	470	777	648	759	602	354	288	259	320	551	1382	
1.5 · 10 <sup>-4</sup>	380	520	371	457	518	748	612		630	360	288	259	322	555	1400	
2.0 · 10 <sup>-4</sup>	375	520	375	438	512	761	618	768	613	350	282	254	328	560	1390	
2.5 · 10 <sup>-4</sup>	380	522	376	440	510	750	620	768	637	348	285	252	328	561	1390	
3.0 · 10 <sup>-4</sup>		520		468	508	742	580	763	610		282	255	325	570	1454	
4.0 · 10 <sup>-4</sup>	381	528	380	456	491	743	600	770	600	346	282	253	328	561	1411	
4.7 · 10 <sup>-4</sup>	380	527		487		682	530	779	609	329	275	242			1393	
5.0 · 10 <sup>-4</sup>	388	531	382	487	490		610	782	610	338	281	254	328	562	1370	
6.6 · 10 <sup>-4</sup>	385	530	388	500	461	679		782	611	320	270	237	339		1432	
8.5 · 10 <sup>-4</sup>	383	535	387	518	449	678	512	790	615	319	266	225	342	582	1458	
9.5 · 10 <sup>-4</sup>		530	390													
1.13 · 10 <sup>-3</sup>	381			510	432	649	520	793	616	312	260	230	342	593	1433	
1.9 · 10 <sup>-3</sup>	390	533	390		411	620	530	808	624	291	258	226	346	600	1492	
3.78 · 10 <sup>-3</sup>	390	541	398	611	378		428	808	635	280	244	220	353	612	1482	
5.67 · 10 <sup>-3</sup>	389	550	389		360	720	402				231	211	350	609	1536	

*Working procedure and conditions*

The sample size was varied in the range 10<sup>-6</sup>–10<sup>-2</sup> g per gram of silica gel. Low sample concentrations (up to 5 · 10<sup>-4</sup> g) were injected as solutions of the sample in *isooctane*; higher concentrations of sample were injected into the column *in situ*. Retention data obtained were expressed as capacity factors using the relation

$$k' = \frac{t_R - t_m}{t_m}$$

where  $k'$  = capacity factor,  $t_R$  = retention time of sample and  $t_m$  = retention time of unretained component. Plots of the dependence of  $k'$  on the sample size were constructed, relative to 1 g of silica gel packing.

*Linear capacity*

*Moderator concentration.* Low concentrations of strongly polar substances have a favourable effect on the chromatographic properties of the mobile phase. Fig. 1 demonstrates the dependence of the capacity factor of cyclohexanone on the

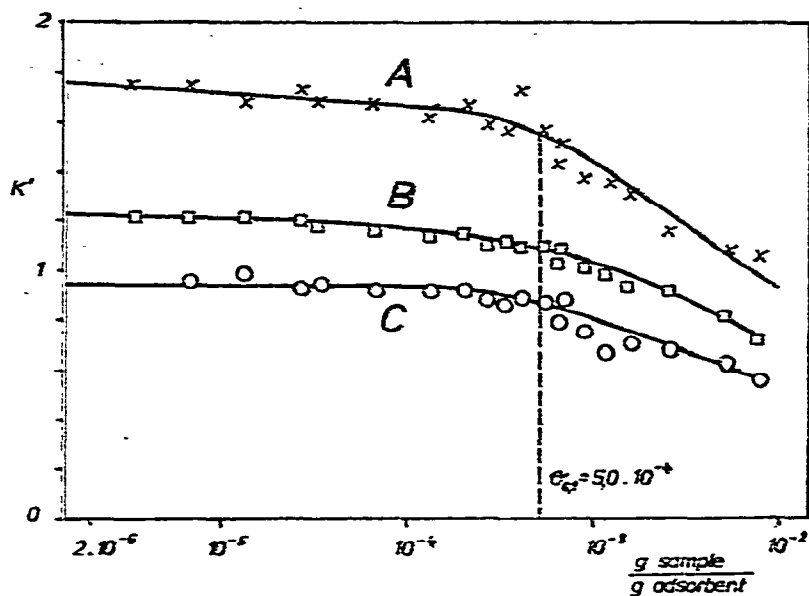


Fig. 1. Dependence of capacity factor on the degree of coverage of stationary phase by the cyclohexanone sample. Column: Micropak Si-10 (500 × 2 mm I.D.). Mobile phases: heptane-diethyl ether (65:35)-2-propanol (99.9:0.1) (A); (99.5:0.5) (B); (99:1) (C).

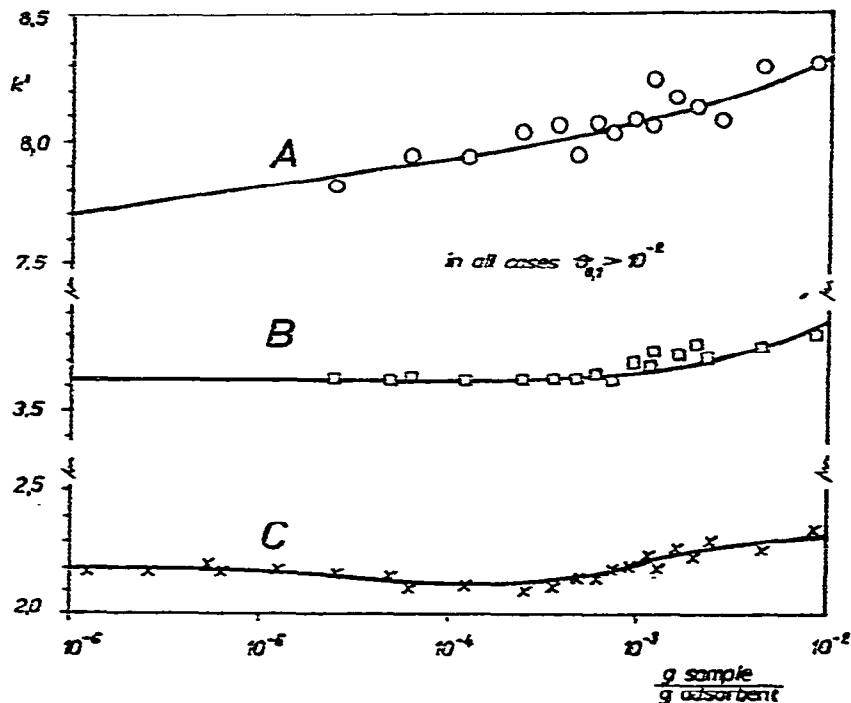


Fig. 2. Dependence of capacity factor on the degree of coverage of stationary phase by the cyclohexanol sample. Column: Micropak Si-10 (250 × 2 mm I.D.). Mobile phases: heptane-2-propanol (99:1) (A); (98:2) (B); (97:3) (C).

sample size. The mobile phase was based upon a mixture of heptane and diethyl ether (65:35, w/w), the elution strength of which was increased by addition of a small amount of 2-propanol (0.1, 0.5 and 1%). The value of the capacity factor decreased with increasing sample size, the classical type of dependence. For higher contents of 2-propanol, the capacity factor of cyclohexanone decreased, the dependence of  $k'$  on sample coverage became more linear, but the value of the linear capacity remained unchanged.

Fig. 2 shows the dependence of  $k'$  on sample size for cyclohexanol. The mobile phase is heptane with a increasing content of 2-propanol (1,2 and 3%, respectively). The capacity factor increases with increasing sample size, in contrast to the classical adsorption. The existence of an adsorbed multilayer is suggested. The capacity factor decreased with increasing content of 2-propanol. The region of linear column capacity includes the whole concentration interval studied, even at the lowest amount of moderator.

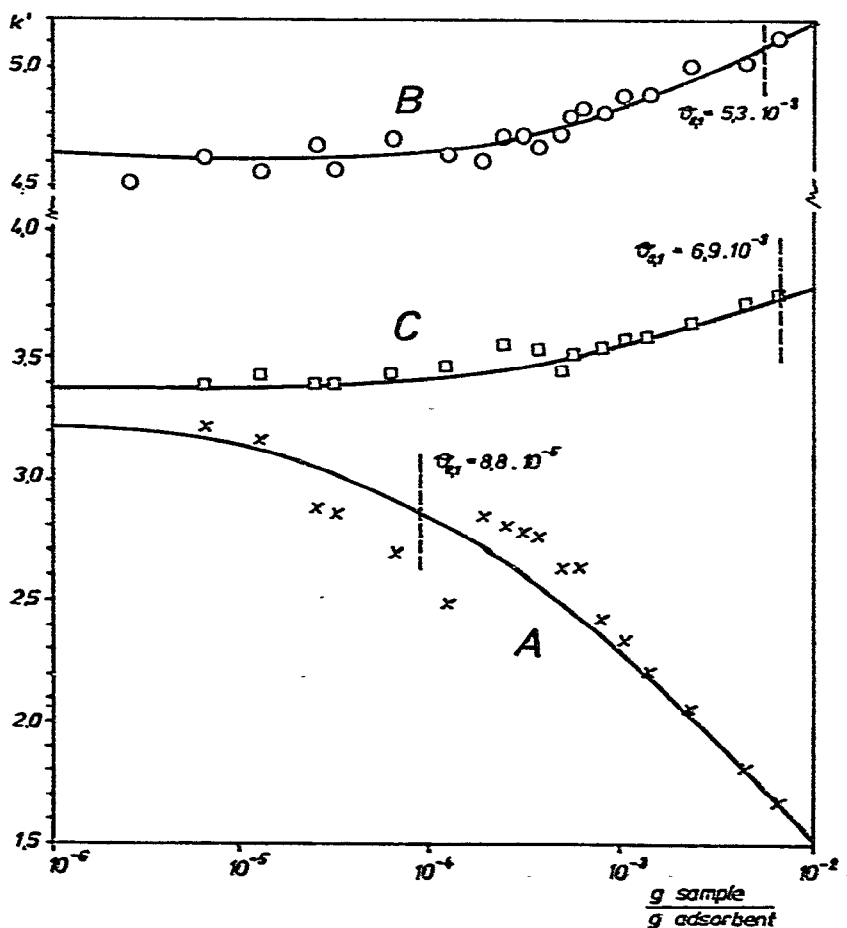


Fig. 3. Dependence of capacity factor on the degree of coverage of stationary phase by the cyclohexanol sample. Column: Micropak Si-10 (500 × 2 mm I.D.). Mobile phases: A = heptane-diethyl ether (65:35); B = heptane-diethyl ether (65:35)-2-propanol (99.5:0.5); C = heptane-diethyl ether (65:35)-2-propanol (99:1).

**Effect of mobile phase composition.** Suitable mobile phases may be also comprise mixtures of two or three individual solvents.

Curve A in Fig. 3 represents a heptane–diethyl ether mobile phase (65:35). Under these conditions, the cyclohexanol capacity factor decreases with increasing coverage of stationary phase by the sample. The region of linear capacity is relatively narrow ( $9.8 \cdot 10^{-5}$  g/g). The mobile phase was then moderated by addition of 0.5 and 1.0% of 2-propanol, respectively (curves B and C). After the addition of 2-propanol, the above dependence was reversed, the capacity factor increasing with increasing sample size. The region of linear column capacity is substantially wider for systems which contain 2-propanol ( $5.3 \cdot 10^{-3}$  g sample per g adsorbent for 0.5% 2-propanol;  $6.9 \cdot 10^{-3}$  g sample per g adsorbent for 1% 2-propanol). Absolute values of the capacity factor are higher for the heptane–diethyl ether–2-propanol system than for the heptane–diethyl ether system.

A mixture of heptane and chloroform (50:50) was also used as the mobile phase, with 0.5 and 2% of 2-propanol. When cyclohexanol was the sample, the

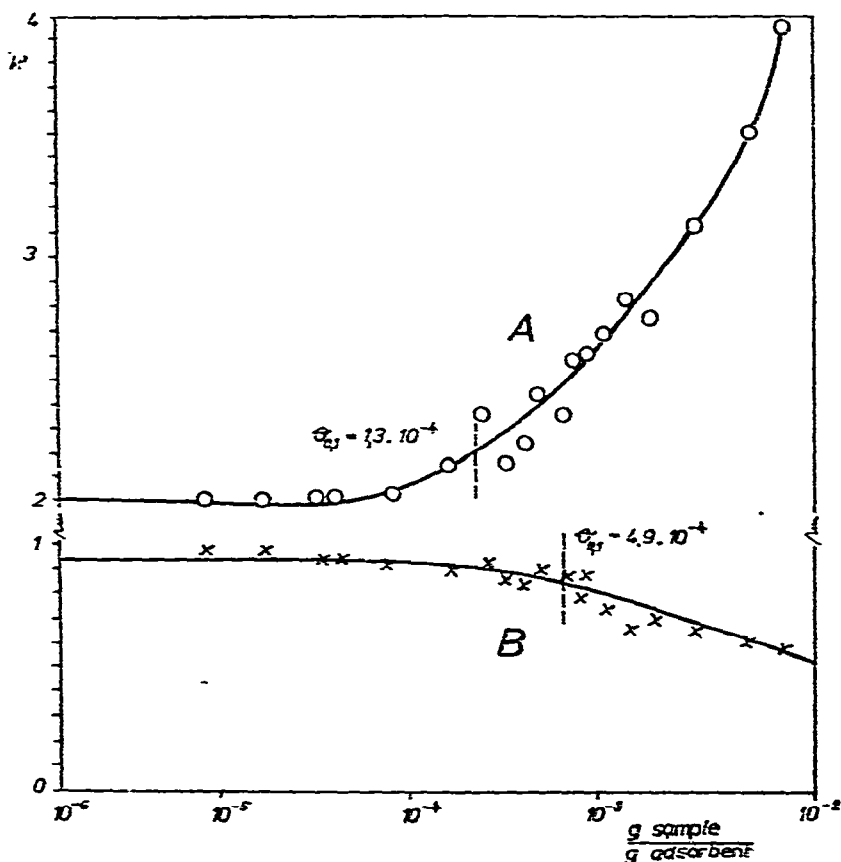


Fig. 4. Dependence of capacity factor on the degree of coverage of stationary phase by the cyclohexanone sample. Column: Micropak Si-10 (500 × 2 mm I.D.). Mobile phases: A = heptane–2-propanol (99:1); B = heptane–diethyl ether (65:35)–2-propanol (99:1).



capacity factor increased with increasing sample size. Use of 2% 2-propanol resulted in a substantial decrease in the capacity factor and a linear dependence of  $k'$  on the size of the injected sample.

Fig. 4 shows the dependence of  $k'$  on sample concentration for cyclohexanone. In the case of curve A, heptane with 1% 2-propanol served as the mobile phase. The higher the cyclohexanone sample size, the higher was its capacity factor value. Cyclohexanone is almost unretained when 2-propanol is present in higher concentration in the mobile phase. If this curve is compared with curve B, which corresponds to the same concentration (1%) of 2-propanol in another mobile phase (heptane-diethyl ether, 65:35), the striking difference in dependences can be seen (lower capacity factors for higher cyclohexanone sample sizes in B).

### HETP

Table III gives the dependence of HETP values on the sample size. This dependence should be evaluated in terms of both the sample character and the mobile phase composition.

TABLE III

HETP VALUES ( $\times 10^{-3}$  mm) OBTAINED ON VARIOUS MOBILE PHASES

For details see Table II.

<i>g Sample per g adsorbent</i>	<i>Mobile phase</i>														
	<i>1a</i>	<i>1b</i>	<i>1c</i>	<i>1a</i>	<i>2a</i>	<i>2b</i>	<i>2c</i>	<i>3b</i>	<i>3c</i>	<i>3a</i>	<i>3b</i>	<i>3c</i>	<i>4a</i>	<i>4b</i>	<i>4c</i>
	OL	OL	OL	ON	OL	OL	OL	OL	OL	ON	ON	ON	OL	OL	OL
$10^{-5}$	10	10	10	11	8	12	15	12	11	15	14	11	15	10	12
$10^{-4}$	12	10	12	16	35	28	30	13	11	22	18	15	19	10	18
$10^{-3}$	10	13	15	134	88	79	92	28	22	54	45	43	21	12	32
$10^{-2}$	42	34	32	—	—	—	—	—	193	210	—	—	63	50	225

If the sample size is increased from  $10^{-5}$  to  $10^{-4}$  g sample per g adsorbent, the HETP value is almost unchanged for most systems examined. The dependence of HETP on the sample sizes of cyclohexanone and cyclohexanol in the heptane-diethyl ether-2-propanol mobile phase (see Fig. 5) serves as an example here. A substantial decrease in efficiency is found, however, when using a heptane-diethyl ether mobile phase. Thus, when using heptane-diethyl ether (65:35) and increasing cyclohexanol sample size from  $10^{-5}$  to  $10^{-4}$  g sample per g adsorbent, the efficiency decreases to  $\frac{1}{4}$  of the original value. Addition of 2-propanol to this mobile phase results in an increase in efficiency (Fig. 6). If the sample size is further increased to  $10^{-3}$  g sample per g adsorbent, a tenfold decrease in efficiency is found in some cases (heptane-diethyl ether, 65:35; cyclohexanol as sample), while in other cases the efficiency remains unchanged (heptane-2-propanol; cyclohexanol as sample).

The effect of the nature of the sample may be illustrated with the samples cyclohexanol and cyclohexanone in the heptane-2-propanol mobile phase. Thus cyclohexanone yields a tenfold increase in HETP (Fig. 7), while for cyclohexanol the efficiency remains unchanged.

An increase in the sample size up to  $10^{-2}$  g sample per g adsorbent usually

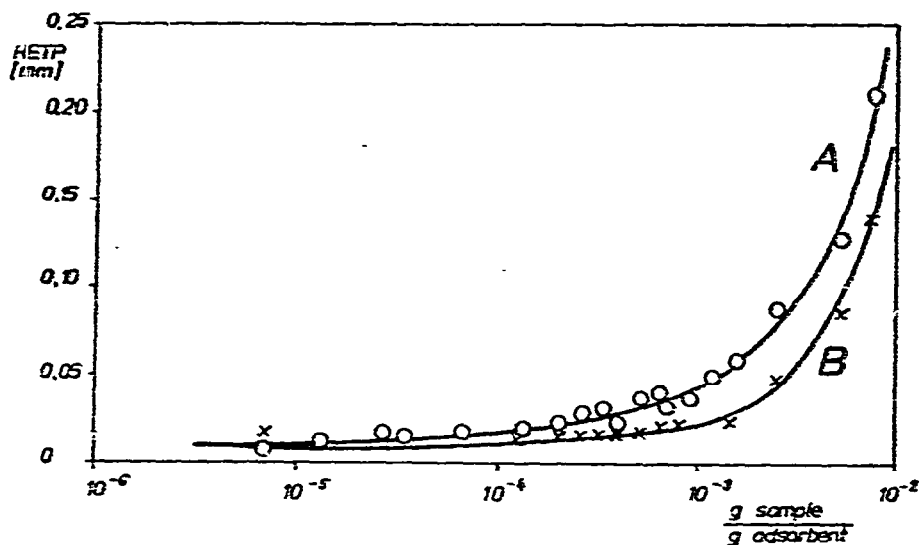


Fig. 5. Dependence of HETP on the degree of coverage of stationary phase by the sample. Column: Micropak Si-10 (500 × 2 mm I.D.). Mobile phase: heptane-diethyl ether (65:35)-2-propanol (99:1). Samples: A = cyclohexanone; B = cyclohexanol.

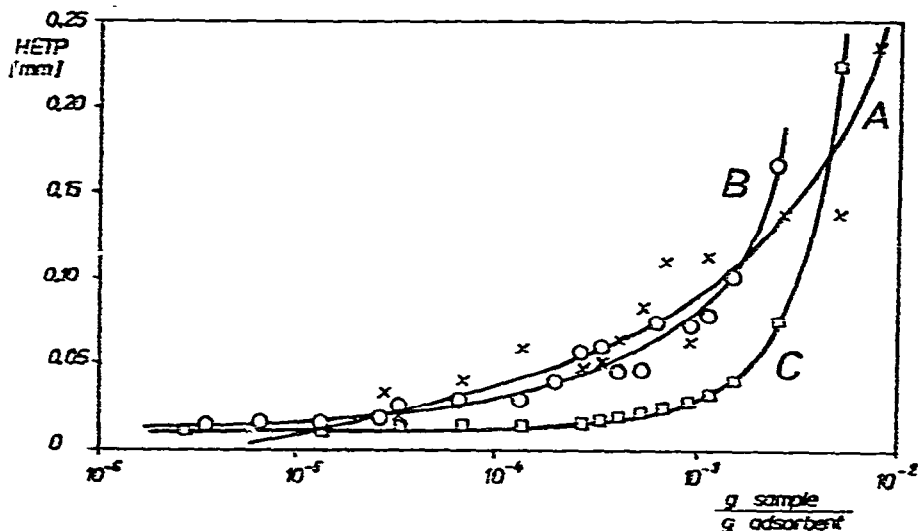


Fig. 6. Dependence of HETP on the degree of coverage of stationary phase by the cyclohexanol sample. Column: Micropak Si-10 (500 × 2 mm I.D.). Mobile phases: A = heptane-diethyl ether (65:35); B = heptane-diethyl ether (50:50); C = heptane-diethyl ether (65:35)-2-propanol (99.5:0.5).

results in a rapid decrease in efficiency, except for cyclohexanol in a heptane-2-propanol system, where the efficiency is still reasonably high.

Generally, it can be concluded that a suitable dependence of HETP on the sample size is reached when using mobile phases containing more than 1% of 2-

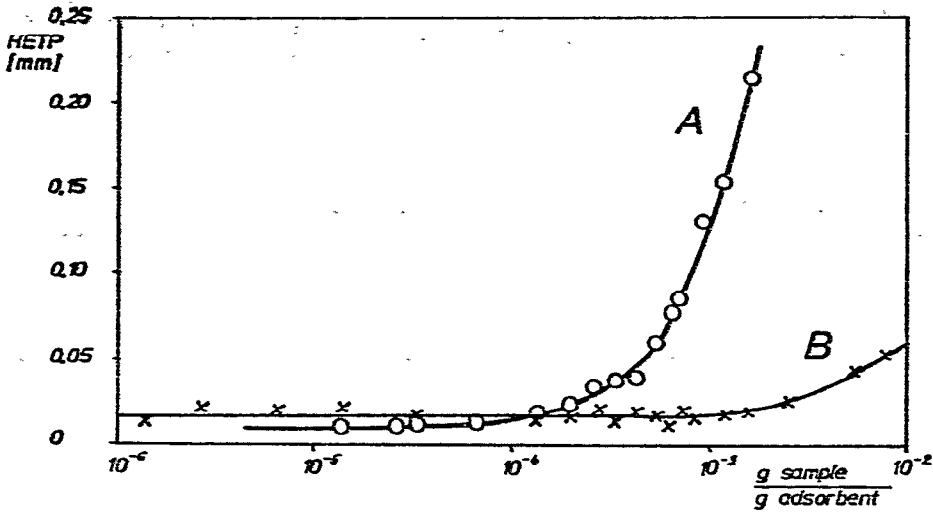


Fig. 7. Dependence of HETP on the degree of coverage of stationary phase by the sample. Column: Micropak Si-10 (500 × 2 mm I.D.). Mobile phases: A = heptane-2-propanol (99:1); B = heptane-2-propanol (97:3). Sample: A = cyclohexanone; B = cyclohexanol.

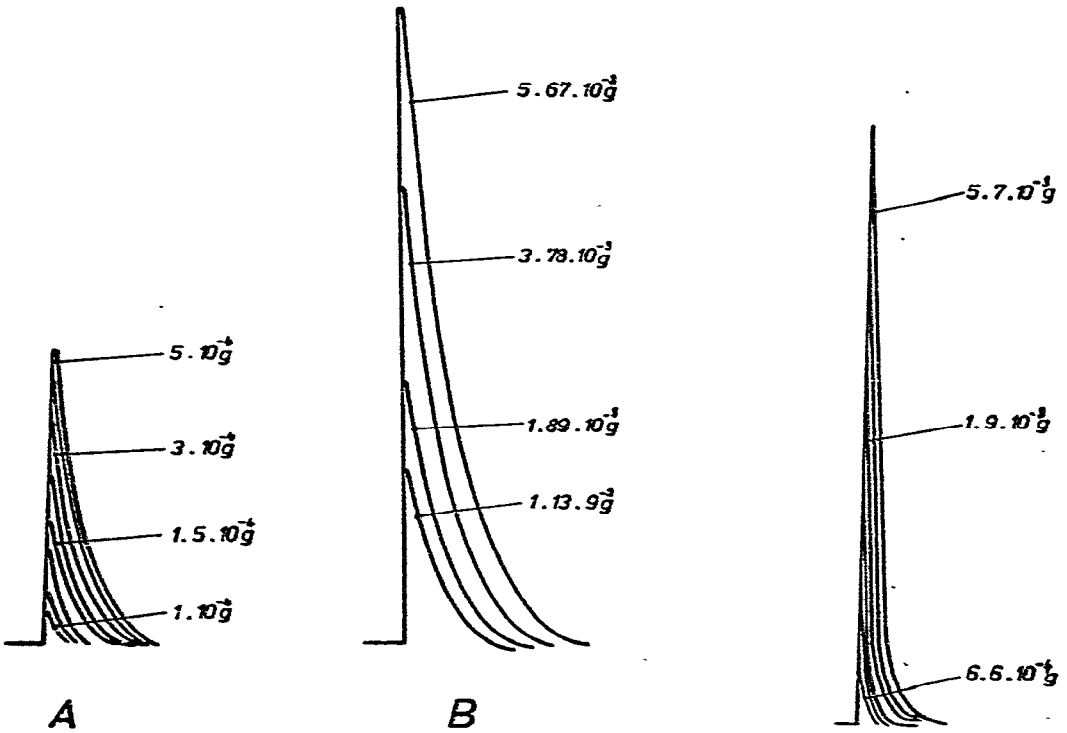


Fig. 8. Peak shapes of cyclohexanol (A and B). Mobile phase: heptane-diethyl ether (35:65).  
 Fig. 9. Peak shapes of cyclohexanone. Mobile phase: heptane-diethyl ether (65:35)-2-propanol (99:1).

propanol, *e.g.*, the system containing only heptane and 2-propanol. With respect to the nature of the injected sample, a substantially higher efficiency was found for cyclohexanol than cyclohexanone.

### Asymmetry

The peak shape in the examined systems (silica gel–mobile phase–sample) varies in accord with the type of dependence of capacity factor on the sample size<sup>1</sup>. Thus, in classical adsorption systems, which may be described by using the Langmuir isotherm, where the capacity factor decreases with increasing sample size, a normal tailing peak is obtained, which is well-known in adsorption chromatography. The peak shape of cyclohexanol in a heptane–diethyl ether mobile phase (Fig. 8) serves as an example. Cyclohexanone behaves similarly in heptane–diethyl ether–2-propanol systems (Fig. 9).

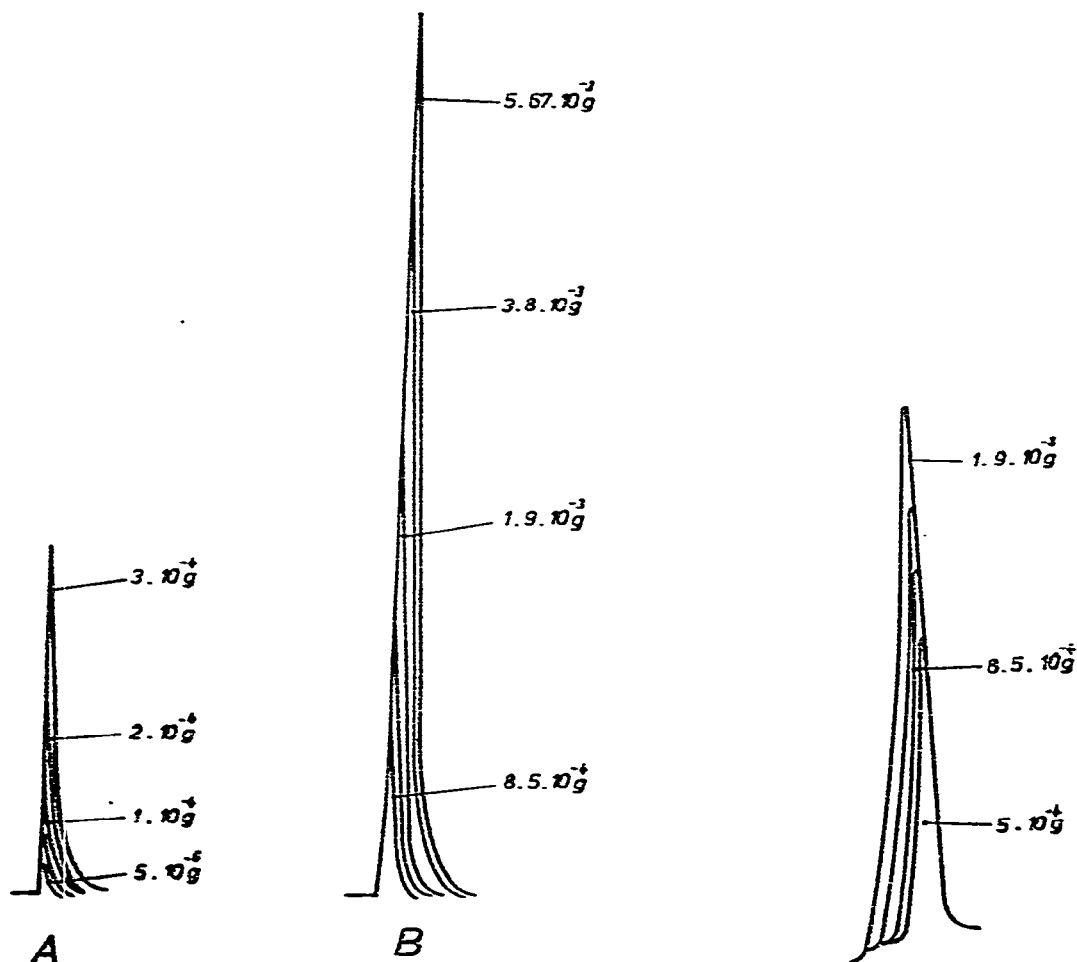


Fig. 10. Peak shapes of cyclohexanol (A and B). Mobile phase: heptane–2-propanol (97:3).

Fig. 11. Peak shapes of cyclohexanol. Mobile phase: heptane–chloroform (90:10)–2-propanol (99:1).

In systems for which the linear capacity of the column  $\theta_{0.1}$  is higher than  $10^{-2}$  g sample per g adsorbent and HETP remains low, the peaks were characterized by a nearly ideal symmetrical shape over the whole concentration range studied (Fig. 10). Similarly, a system containing two non-polar components (heptane-chloroform) and a relatively high amount (2%) of 2-propanol proved to be only a little inferior for cyclohexanol. When the concentration of 2-propanol in a heptane-chloroform-2-propanol mobile phase is decreased (1% or less), the capacity factor of cyclohexanol tends to increase rapidly with increasing sample size and a leading peak is obtained (a wider leading part, see Fig. 11).

In this context, the results obtained with a heptane-diethyl ether (65:35) system moderated by 1% 2-propanol are of interest. A change in the peak shape has been observed here for increasing cyclohexanol sample size (Fig. 12). Thus, a symmet-

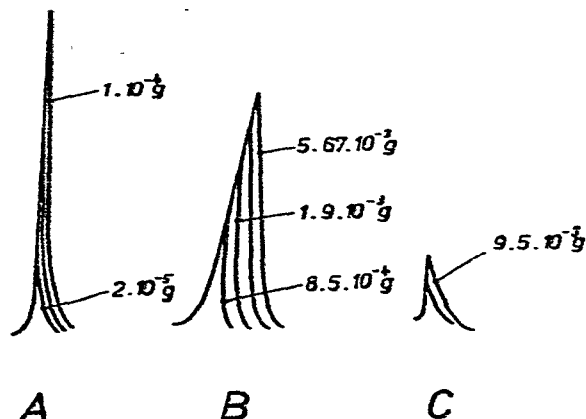


Fig. 12. Peak shapes of cyclohexanol. Mobile phase: heptane-diethyl ether (65:35)-2-propanol (99:1).

rical peak is obtained for the lowest sample sizes, being transformed into a classical adsorption tailing peak for increasing sample size and eventually a leading peak is obtained for the highest cyclohexanol concentrations. Similar changes in the peak shape with varying moderator concentration have been reported by Kirkland<sup>6</sup>.

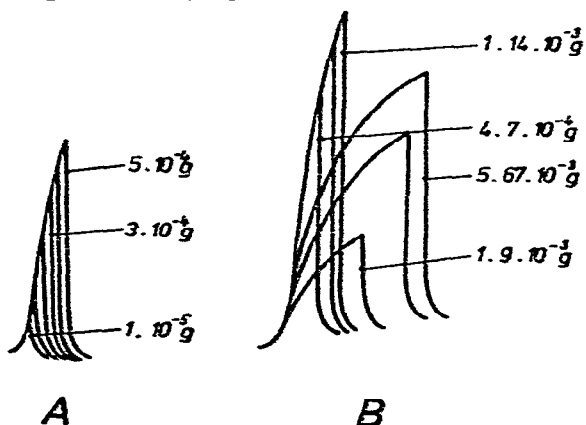


Fig. 13. Peak shapes of cyclohexanone. Mobile phase: heptane-2-propanol (99:1).

For cyclohexanone (in contrast to cyclohexanol) in a heptane-2-propanol (99:1) mobile phase a substantial increase in the capacity factor has been found for increasing sample size and the peaks obtained are also characterized by an unrepresentative shape (a wider leading part, see Fig. 13).

## CONCLUSION

The adsorption process on silica gel is strongly affected by the composition of the mobile phase and the sample character. Sample peak shape, the dependence of  $k'$  on the degree of coverage of the silica gel by the sample, and column efficiency (especially under high loadings) are affected in a specific manner by increasing sample size. Deviations from classical adsorption behaviour are most probably caused by hydrogen bond formation between silica gel, the polar component of the mobile phase, and the sample. The results discussed may be of importance in going from analytical to preparative HPLC.

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