Journal of Chromatography, 478 (1989) 87–99
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 21 603

BAND BROADENING IN HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC SEPARATIONS OF ENANTIOMERS WITH SWOLLEN MICROCRYSTALLINE CELLULOSE TRIACETATE PACKINGS

II. INFLUENCE OF ELUENT COMPOSITION, TEMPERATURE AND PRESSURE

ANDREAS M. RIZZI
Institute of Analytical Chemistry, University of Vienna, Währingerstrasse 38, A-1090 Vienna (Austria)
(First received February 20th, 1989; revised manuscript received April 27th, 1989)

SUMMARY

The peak dispersion in high-performance liquid chromatographic columns packed with swollen crystalline cellulose triacetate was investigated as a function of the eluent composition, the temperature and the pressure. The results provide support for a model which assumes the existence of at least two types of adsorption sites, "quick"-type and "slow"-type sites, which differ with respect to the rate of the adsorption/desorption process. The observed dependence of the peak dispersion on the temperature and the eluent composition can be understood as resulting from three factors: (i) changes in the diffusion velocity by changes in the solvent viscosity; (ii) changes in the three-dimensional structure of the adsorbent due to changes in the swelling state of the CTA adsorbent and (iii) changes in the availability and accessibility of the adsorption sites due to differences in the strength of the competitive adsorption of the solvent components.

INTRODUCTION

Swollen microcrystalline cellulose triacetate (swcrCTA) has been widely applied for liquid chromatographic separations of enantiomeric compounds 1,2. High enantioselectivity for many groups of compounds and high loadability are the main advantages of this adsorbent material. However, the large peak broadening usually observed results in reduced efficiency and limits the use of this material for analytical purposes.

In Part I [3] the peak broadening process on swcrCTA was systematically investigated with respect to the capacity factor of the analyte, analyte structure, flow velocity and column loading. It was concluded that in columns packed with swcrCTA the most important contribution to the plate height arises from the slow mass exchange in the packed bed. This is attributed to a slow transport process at narrow adsorption sites: slow diffusion and orientation at narrow sites, in narrow channels or in cavity-like structures.

0021-9673/89/$03.50 © 1989 Elsevier Science Publishers B.V.

The steric structure of the analytes was found to determine the plate height. From the dependence of the plate height on the capacity factors and the structures of the analytes, the existence of at least two types of adsorption sites was proposed: "slow"-type and "quick"-type sites, which differ in the rate of the adsorption/desorption process (including the diffusion in the vicinity of these sites). Both types of sites contribute to solute retention. Since the plate height is predominantly determined by the "slow"-type sites, no correlation was found between plate height and capacity factor.

In this paper quantitative data are reported on the dependence of the plate height on the eluent composition, the temperature and the pressure. The data provide strong support for the model presented previously[3].

The knowledge of the dependences investigated here may contribute to a deeper understanding of the adsorption mechanism on CTA materials, which is not yet clear. In addition these data provide a basis for a rational optimization of separations on swcrCTA adsorbents.

EXPERIMENTAL

The chromatographic equipment and experimental conditions were identical to those described previously[3]. The CTA column referred to in this paper was the previous column I.

RESULTS AND DISCUSSION

Capacity factor as a function of temperature

A good linear correlation is observed between $\ln \kappa$ and $1/T$, as is seen in Fig. 1. Similar slopes are obtained for most of the analytes, including analytes with dissimilar structures like anthracene, 2,2,2-trifluoro-1-(9-anthryl)ethanol (TFAE) and Trög-

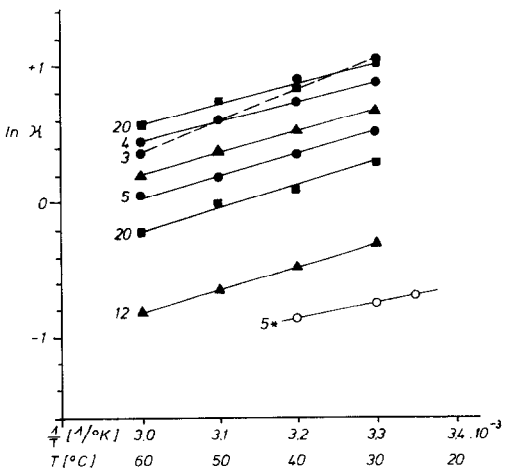


Fig. 1. Logarithm of the capacity factor as a function of the inverse of the temperature. Code numbers of solutes as in Table II. The open circles for compound **5** denote experimental data obtained from a octadecylsilica/ethanol–water system. Eluent: ethanol–water (96:4, v/v); flow-rate 1 ml/min.

er's base. An exception is toluene. The slopes are also similar to those obtained in reversed-phase systems using octyl-silica adsorbents.

A more detailed estimation of the temperature dependence for enantiomeric pairs reveals that the enantio- and stereoselectivity may either slightly increase or slightly decrease with temperature (Table I). This may be due to slight differences in the temperature dependence of the free enthalpy of adsorption for the two enantiomers at different binding sites, and/or to changes in the swelling state and thus in the structure of the narrow adsorption sites upon increase in temperature. Depending on the analyte structure, this may reduce the differences in adsorption strength between the enantiomers, or may even enhance it.

TABLE I

ENANTIOSELECTIVITY, α, AS A FUNCTION OF THE TEMPERATURE

Non-trivial structures of analytes are given in ref. 3. Code numbers are identical to those in ref. 3. Flow-rate: 1 ml/min.

Code	*Solute*	*Temperature (°C)*			
		30	*40*	*50*	*60*
Eluent: ethanol–water (96:4, v/v)					
11	Spirobiindanone	2.29	2.24	2.14	–
12	TFMA	2.66	2.75	2.79	2.77
20	Tröger's base	2.07	2.13	2.14	2.21
Eluent: ethanol–methanol–water (76.8:20:3.2, v/v)					
8	Phenyldioxolanone	–	1.93	1.88	1.86
11	Spirobiindanone	2.10	2.06	2.03	2.00
12	TFAE	2.20	2.20	2.33	2.34
20	Tröger's base	–	1.60	1.68	1.75

Plate height as a function of the temperature

The influence of temperature on the plate height, h, is shown in Fig. 2. The influence of the temperature differs from analyte to analyte, depending on the adsorption mode. For aromatic benzene compounds adsorbed onto the "quick"-type adsorption sites[3] (circles in Fig. 2) the temperature effect is insignificant. However, a very pronounced temperature effect is observed for analytes with high contributions of the "slow"-type adsorption[3] (triangles and squares in Fig. 2). The steep decrease in h with increasing temperature is predominantly due to a gain in the diffusion velocity at the "slow"-type sites. This is caused by the decreasing strength of interactions between the analyte and its environment and partially by the lower viscosity of the solvents at higher temperatures. The slopes of the plots in Fig. 2 are similar for the enantiomers of TFAE and Tröger's base.

The decrease in h cannot be attributed simply to the reduction in the capacity factors: this would not explain the very steep descent observed in Fig. 2. The gain in efficiency has to be attributed to the increase in the diffusion velocity at "slow"-type

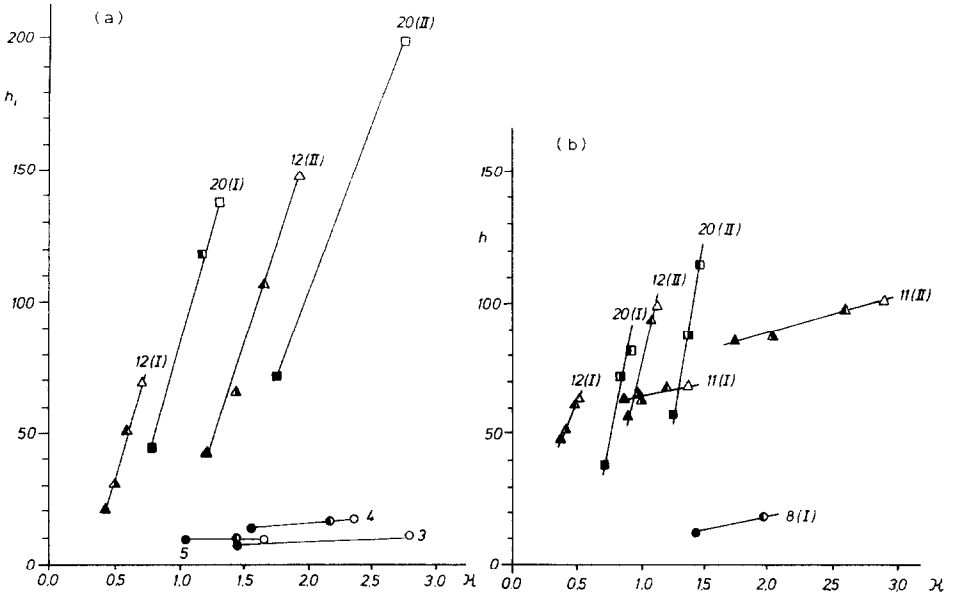


Fig. 2. Reduced plate height and capacity factor as a function of temperature. Temperatures: △, ○, □, 30; ◭, ◐, ◧, 40; ▲, ◑, ◨, 50; ▲, ●, ■, 60°C. Code numbers of solutes as in Table II; I and II indicate the first and second enantiomers eluted. Flow-rate: 1 ml/min. Solvents: (a) ethanol–water (96:4); (b) ethanol–methanol–water (76.8:20:3.2).

sites, as mentioned before. Comparison of the data in Fig. 2a and b shows that the effect of the temperature on the plate heights of TFAE and Tröger's base is decreased by adding methanol to the eluent. This finding is in accordance with expectation, since methanol reduces the contribution of the "slow"-type sites to the total adsorption of the analytes (*cf.*, next section).

Plate height as a function of the solvent composition

The dependence of the plate height, h, on the solvent composition is shown in Figs. 3 and 4 and in Table II. Various contents of methanol, propanol, water and cyclohexane in ethanol were investigated, and different effects are observed for different solvent components. From the behaviour of the substances as represented by a plot of h *vs.* [κ, %(v/v) moderator] (Fig. 4) some conclusions about the adsorption mechanism for the solutes can be made.

(i) Methanol. The plate height, h, decreases significantly with increasing methanol content in the mixture. Similarly, the capacity factors of all compounds investigated decrease. This influence of the methanol content is illustrated by a plot of h *vs.* κ in Fig. 4a. The gain in efficiency probably results from three sources. (a) The addition of methanol reduces the mean viscosity of the eluent mixture (*cf.*, Table III) thus allowing an higher diffusion velocity of the analytes, which results in smaller values of h. (It mainly influences h_b^{slow}. A minor influence on h_e and h_f is expected to be insignificant in Fig. 4a.) (b) Each change in the solvent composition is assumed to change the swelling state of CTA. In this way the availability (important for the

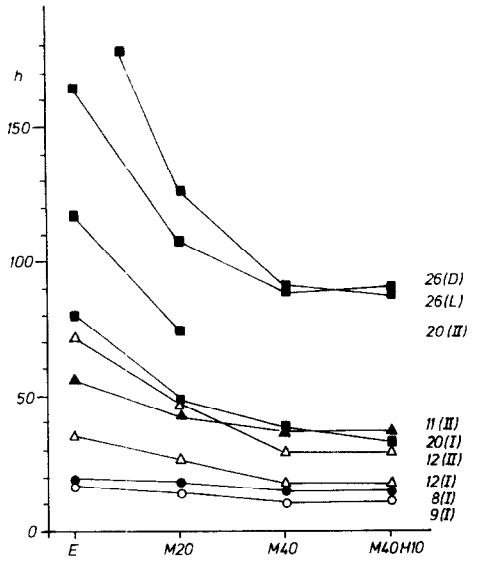
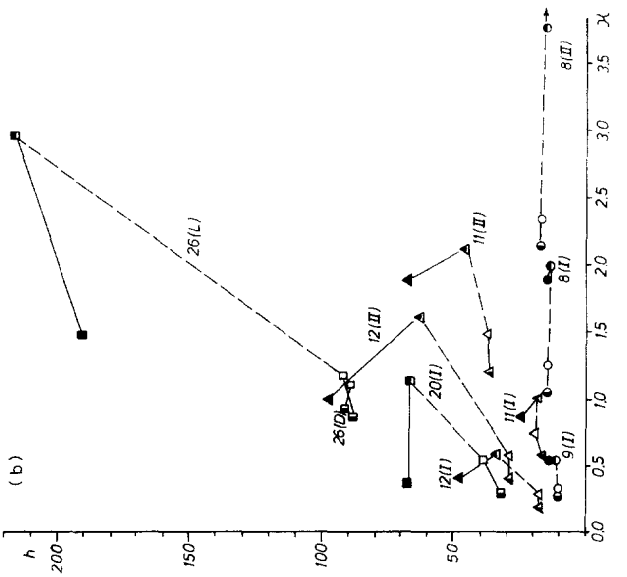


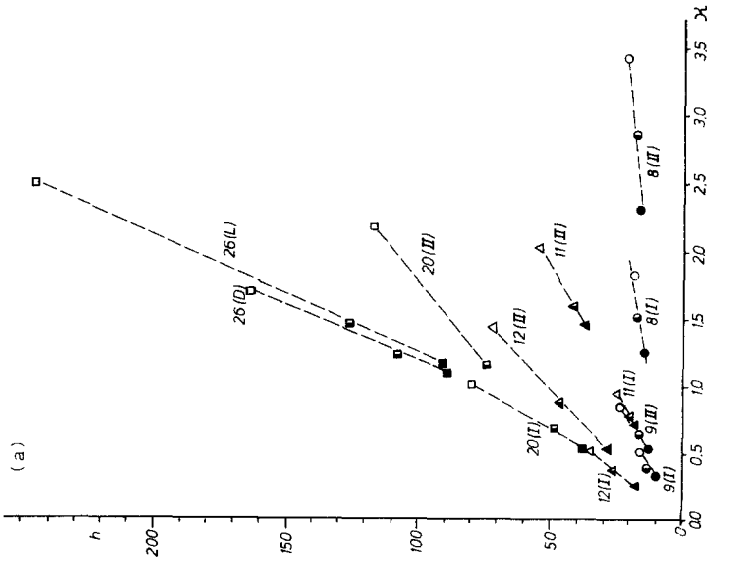
Fig. 3. Reduced plate height, h, as a function of the composition of the mobile phase. Code numbers of solutes as in Table II. Chromatographic conditions: temperature 50°C; flow-rate 1 ml/min. solvents: E = ethanol–water (96:4); M20 = methanol–ethanol–water (20:76.8:3.2); M40 = methanol–ethanol–water (40:57.6:2.4); M40H10 = methanol–cyclohexane–ethanol–water (40:10:48:2).

capacity factors and the selectivity) and the accessibility (important for the plate height) of the different types of adsorption sites is changed too. The importance and the direction of this influence on the plate height is not yet well understood. (c) With this type of packing material the elution power of an eluent component depends not only on the types of interactions with the solute and adsorbent, but also on its steric size. At narrow and therefore "slow"-type sites methanol is thus expected to be a stronger competitor for the adsorption of the analytes than is ethanol. Enhanced competition for the "slow"-type sites by methanol reduces the retention of the analytes and the relative influence of the slow kinetic. This results in a net decrease in h for those analytes, where, due to their structures, the adsorption onto "slow"-type sites results in high h_b^{slow} values.

Factors (b) and (c) are not independent of each other. The decreases in h_b^{slow} are found to be approximately proportional to the decrease in κ (Fig. 4a). It is therefore probable that the gain in efficiency upon the addition of methanol is mainly due to the effects mentioned in (b) and (c). The decrease in enantioselectivity for many analytes upon addition of methanol (Table IV) supports the assumption of changed relative contributions of "slow"- and "quick"-type adsorption sites, since it is likely that the narrow, "slow"-type sites are decisive for the chiral recognition. The finding that the addition of methanol also reduces the retention of non-polar aromatic hydrocarbons may indicate the importance of changes in the swelling state of CTA.

(ii) Cyclohexane. When increasing the content of cyclohexane in the mixture, the plate height remains approximately constant whereas the capacity factors generally decrease (Table II and Fig. 4b). It should be noted that cyclohexane as well as


(b)
h
200
150
100
50
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
x
26(L)
26(D)
12(II)
11(II)
20(I)
11(I)
12(I)
9(I)
8(I)
8(II)


(a)
h
200
150
100
50
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
x
26(L)
26(D)
20(II)
12(II)
11(II)
8(II)
8(I)
11(I)
9(II)
20(I)
12(I)
9(I)

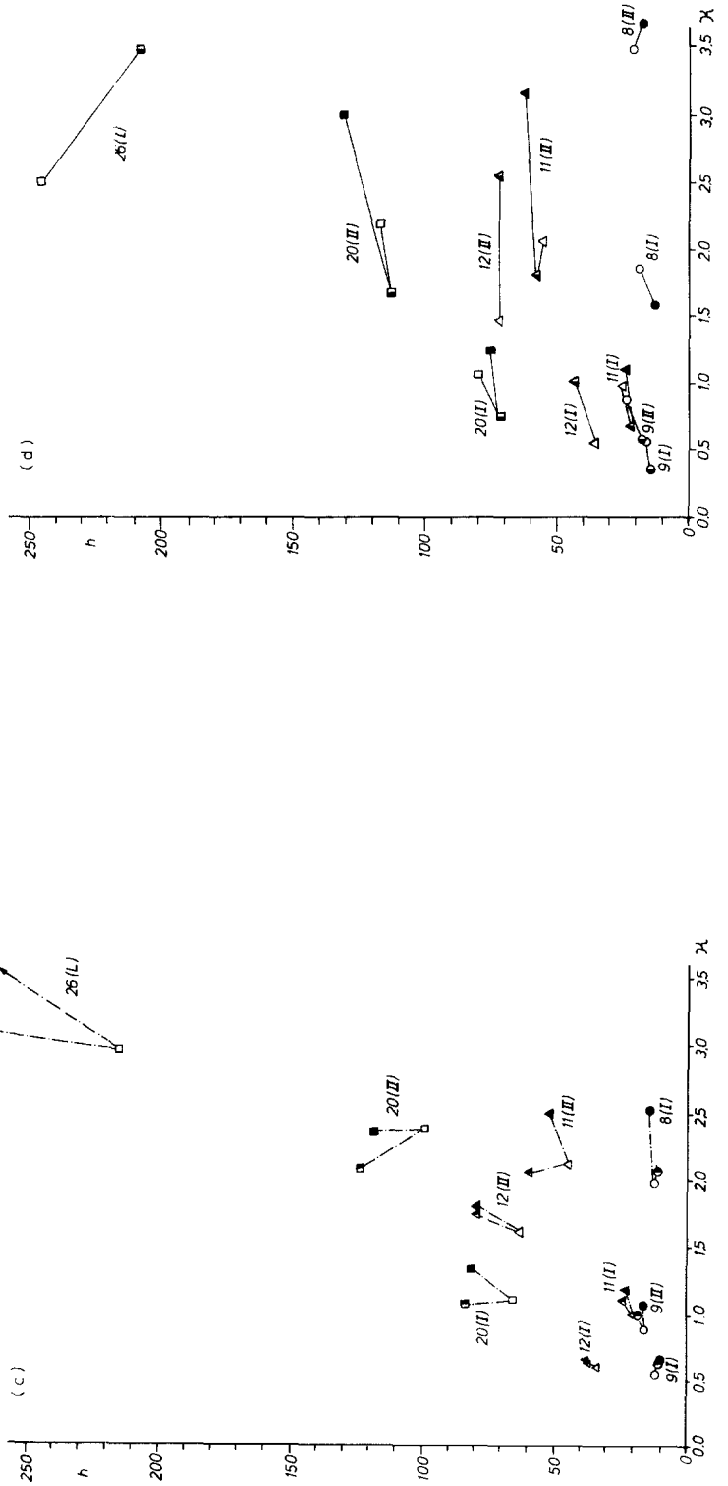


Fig. 4. Reduced plate height, h, and capacity factor, κ, as a function of the type and concentration of a second solvent component added to the ethanolic mobile phase: (a) methanol; (b) cyclohexane; (c) propanol; (d) water. Code numbers of solutes as in Table II. Symbols for the solvent systems: (a) ○, △, □, E = ethanol–water (96:4); ●, ▲, ■, M20 = methanol–ethanol–water (20:76.8:3.2); ●, ▲, ■, M40 = methanol–ethanol–water (40:57.6:2.4). (b) ○, △, □, M40; ●, ▲, ■, M40H10 = methanol–cyclohexane–ethanol–water (40:10:48.2); ●, ▲, ■, E; ●, ▲, ■, H20 = cyclohexane–ethanol–water (20:76.8:3.2). (c) ○, △, □, E; ●, ▲, ■, 1-P30 = 1-propanol–ethanol–water (30:67.2:2.8); ●, ▲, ■, 2-P30 = 2-propanol–ethanol–water (30:67.2:2.8). (d) ○, △, □, E; ●, ▲, ■, W10 = ethanol–water (86.4:13.6); ●, ▲, ■, W20 = ethanol–water (79.8:23.2). Chromatographic conditions: temperature 50°C; flow-rate 1 ml/min.

TABLE II

REDUCED PLATE HEIGHT, h, AND CAPACITY FACTOR, κ, AS A FUNCTION OF THE COMPOSITION OF THE MOBILE PHASE

Non-trivial structures of analytes are given in ref. 3. Solvent systems: E = Ethanol–water (96:4); M20 = methanol–ethanol–water (20:76.8:3.2); M40 = methanol–ethanol–water (40:57.6:2.4); M40H10 = methanol–cyclohexane–ethanol–water (40:10:48:2); 1-P10 = 1-propanol–ethanol–water (10:86.4:3.6); 1-P30 = 1-propanol–ethanol–water (30:67.2:2.8); 2-P30 = 2-propanol–ethanol–water (30:67.2:2.8); W10 = ethanol–water (86.4:13.6); W20 = ethanol–water (76.8:23.2); H20 = cyclohexane–ethanol–water (20:76.8:3.2).

Code No.	*Solute*		*Ethanol–methanol and ethanol–methanol–cyclohexane (system back pressure above 70 atm)*							
			E		*M20*		*M40*		*M40H10*	
			κ	h	κ	h	κ	h	κ	h
1	Tributylbenzene		<0		<0	11	<0	13	<0	15
3	Toluene		2.08	17	1.92	11	1.71	12		
4	Anthracene		1.85	20	1.49	16	1.13	13	0.97	14
5	Nitrobenzene		1.30	15	1.21	9	1.10	9		
6	Resorcinol		0.12	13	0.07	9	0.04	8	0.02	8
8	Phenyldi-	I	1.84	19	1.53	18	1.26	15	1.05	15
	oxolanone	II	3.47	22	2.89	18	2.32	17	2.13	17
9	Phenyltetrahydro-	I	0.53	17	0.40	14	0.33	10	0.28	11
	oxazolone	II	0.86	24	0.66	17	0.55	13	0.53	14
11	Spirobi-	I	0.95	25	0.78	20	0.73	19	0.57	16
	indanone	II	2.03	55	1.59	42	1.46	37	1.19	37
12	TFAE	I	0.53	35	0.37	27	0.26	18	0.19	17
		II	1.44	72	0.88	47	0.55	29	0.40	29
20	Tröger's base	I	1.05	80	0.69	49	0.53	38	0.30	$\leqslant 33^a$
		II	2.19	117	1.15	74	0.77	–	0.30	$\leqslant 33^a$
26	FMOC-Trp	D	1.70	164	1.23	108	1.10	89	0.91	91
	methyl ester	L	2.50	245	1.46	126	1.16	91	0.85	88

			Ethanol–propanol (back pressure between 30 and 40 atm)							
			E		*1-P10*		*1-P30*		*2-P30*	
			κ	h	κ	h	κ	h	κ	h
1	Tributylbenzene		<0	11	<0		<0	14	<0	19
3	Toluene						1.84	9	2.40	13
4	Anthracene		1.99	12	1.85	11	1.93	13	2.06	12
5	Nitrobenzene						1.31	8	1.60	10
6	Resorcinol				0.13	9	0.16	9	0.17	8
8	Phenyldi-	I	1.98	13	1.98	12	2.06	11	2.52	14
	oxolanone	II	3.75	15	3.71	14	3.83	13	4.89	16
9	Phenyltetrahydro-	I	0.54	12	0.51	9	0.61	11	0.63	10
	oxazolone	II	0.88	16	0.83	14	0.99	18	1.07	16
11	Spirobi-	I	1.00	18	1.00	21	1.10	24	1.18	23
	indanone	II	2.10	45	2.05	48	2.02	60	2.49	54
12	TFAE	I	0.59	34	0.61	34	0.61	37	0.62	38
		II	1.60	63	1.68	67	1.73	79	1.78	79
15	Hexobarbital	I			0.89	30	0.99	$\leqslant 38^a$	1.05	36
		II			1.29	35	1.39	$\leqslant 47^a$	1.45	45
20	Tröger's base	I	1.11	66	1.09	67	1.07	84	1.31	82
		II	2.36	99	2.23	101	2.06	124	2.34	118
26	FMOC-Trp	L	2.94	215			3.07	262	3.93	295
	methyl ester									

TABLE II *(continued)*

Code No.	*Solute*		*Ethanol–water and ethanol–cyclohexane (back pressure between 30 and 40 atm)*					
			W10		*W20*		*H20*	
			κ	h	κ	h	κ	h
1	Tributylbenzene		<0	9			<	13
3	Toluene		2.01	10			0.81	10
4	Anthracene		2.97	13			1.29	16
5	Nitrobenzene		1.23	8			0.50	10
6	Resorcinol		0.09	7			0.14	12
8	Phenyldi-	I			1.57	13	1.87	15
	oxolanone	II			3.66	17	4.06	18
9	Phenyltetrahydro-	I	0.34	15			0.55	15
	oxazolone	II	0.57	18			1.03	21
11	Spirobi-	I	0.67	22	1.08	24	0.86	25
	indanone	II	1.80	58	3.14	62	1.87	68
12	TFAE	I	0.99	43			0.40	48
		II	2.54	72			0.97	97
15	Hexobarbital	I	0.60	33	0.87	54		
		II	0.99	43	1.51	71		
20	Tröger's base	I	0.74	72	1.24	75	0.36	$\leqslant 67^a$
		II	1.67	113	2.99	131	0.36	$\leqslant 67^a$
26	FMOC-Trp methyl ester	L	3.45	207			1.54	190

[a] Incompletely resolved peak.

TABLE III

RELATIVE VISCOSITY (η/η_{water}) AND KINEMATIC VISCOSITY (η/ρ) AND DENSITY DATA FOR PURE AND AQUEOUS SOLVENTS AT 20°C[4]

η denotes the viscosity coefficient, ρ the density.

	η/ρ *(cSt)*	η/η_{water}	ρ *(g/cm³)*
Pure solvent			
Ethanol	1.525	1.201	0.789
Methanol	0.740	0.585	0.792
1-Propanol	2.773	2.223	0.803
2-Propanol	3.100	2.428	0.785
Cyclohexane	1.256	0.980	0.78
Water	1.004	1.000	1.000
Aqueous ethanol (%, v/v)			
96	1.674	1.339	0.801
90	1.885	1.539	0.818
86	2.021	1.671	0.828
80	2.229	1.877	0.843
76	2.361	2.011	0.853

TABLE IV

ENANTIOSELECTIVITY, α, AS A FUNCTION OF THE CONCENTRATION OF METHANOL AND CYCLOHEXANE IN THE ETHANOLIC MOBILE PHASE

Chromatographic conditions: flow-rate 1 ml/min; temperature 50°C. Solvent systems as in Table II.

Code No.	*Solute*	*E*	*M20*	*M40*	*M40H10*	*H20*	*W10*
8	Phenyldi-oxolanone	1.88	1.89	1.84	2.03	2.18	–
9	Phenyltetrahydro-oxazolone	1.62	1.64	1.65	1.87	1.88	1.68
11	Spirobiindanone	2.14	2.04	2.01	2.08	2.17	2.69
12	TFAE	2.72	2.38	2.17	2.11	–	2.57
15	Hexobarbital	1.45	1.46	1.39	1.25	1.11	1.65
16	Methaqualon	1.53	1.67	1.74	1.86	1.57	1.61
20	Tröger's base	2.08	1.67	1.43	$\leqslant 1.20^a$	$\leqslant 1.20^a$	2.26
22	*o,o'*-Disubstituted biphenylene	1.58	1.42	$\leqslant 1.20^a$	$\leqslant 1.20^a$	–	2.00
26	FMOC-Trp methyl ester	1.47	1.18	1.06	1.07	–	–

[a] Unresolved peak: the value given is the limit, where a partial resolution would be seen.

methanol is a stronger displacer than is ethanol on swerCTA adsorbents. The non-polar and rigid cyclohexane is assumed to be a stronger competitor than ethanol for the adsorption of analytes onto non-polar sites which are concluded to be mainly of the "quick"- type. These non-polar sites are responsible for the strong adsorption of non-polar aromatic compounds (benzene, toluene, naphthalene) (*cf.*, Fig. 1 in ref. 3). Since the competitive effect of cyclohexane mainly affects the "quick"-type sites, its addition induces a decrease in κ but has only a minor influence on the plate height. In cases where the relative importance of "quick"-type sites is reduced significantly, the plate height even increases, due to the enhancement of the relative importance of the adsorption onto the "slow"-type sites (Fig. 4b). The increase in enantioselectivity for many analytes upon the addition of cyclohexane (Table IV) again provides support for this assumption of changed relative contributions of the "slow"- and "quick"-type adsorption sites. The divergent influence on the capacity factors of enantiomers (**8**) and changes in the elution order of enantiomers (**26**) indicate that changes in the swelling state may be important, too.

(iii) Propanol. Different effects on the capacity factors are observed when 1-propanol or 2-propanol is added. The addition of 1-propanol induces a small increase or a small decrease in the capacity factors. On the other hand a significant increase in κ is always observed with 2-propanol. This is probably due to the reduced competition from 2-propanol originating from steric effects but may also be due to small changes in the swelling state of the CTA material. In all cases the plate height increases (Fig. 4c) since the addition of propanol induces a large increase in the solvent viscosity. This seems to be the main effect with 1-propanol. In the case of 2-propanol the opposite effect to that discussed for methanol may also be of importance.

(iv) Water. The addition of water strongly influences the capacity factors of the solutes. In spite of a large increase in solvent viscosity, it has, however, only a slight influence on the efficiency (Fig. 4d).

The addition of water most probably has a strong influence on the swelling state of CTA, and thus on the availability and accessibility of adsorption sites. The relative importance of the "slow"-and "quick"-type sites for the adsorption of the analytes is therefore changed (as described above for methanol). A decrease in h, expected for this effect, may just be balanced by the effect resulting from an increased viscosity.

However, other explanations might be valid too. Since no measurements are available which focus on the changes in the swelling state and on the preferential adsorption of water or ethanol onto CTA, it is not clear whether the addition of water does influence the viscosity of the solvent layers in the "cavities" significantly, or not. The changes in retention of analytes are partially due to changes in the activity coefficients of the solutes in the mobile phase upon the addition of water. This is especially valid for the rise in the capacity factors at higher concentrations of water. In this case the relative importance of the "slow"- and "quick"-type sites for the adsorption of the analytes would remain unchanged. This might be an alternative explanation for the constancy of h.

Influence of column pressure and column stability

During the first period of use of a column, the back pressure at a given temperature increased continuously. After cleaning the bottom frit of the column, the back pressure was significantly reduced and became very stable. Changes in the solvent composition had no significant additional influence on the back pressure and on the plate height after changing back to the solvent used originally. This implies that changes in the swelling state of CTA were reversible within the range of solvent mixtures investigated.

The increase in back pressure during the first period was utilized to investigate the influence of the pressure on the retention and on the efficiency. Both the retention and the efficiency are influenced as shown in Table V. The capacity factor decreases

TABLE V

REDUCED PLATE HEIGHT, h, AND CAPACITY FACTOR VALUES, κ, AS A FUNCTION OF THE BACK PRESSURE OF THE COLUMN

Eluent: ethanol–water (96:4, v/v). Temperature: 50°C. Flow-rate: 1 ml/min. 1 atm = $1.0133 \cdot 10^5$ Pa.

Code No.	*Solute*		*ΔP (atm)*			
			25–30		*90–95*	
			κ	h	κ	h
4	Anthracene		1.99	12	1.85	20
8	Phenyldi-	I	1.98	13	1.84	19
	oxolanone	II	3.75	15	3.47	22
9	Phenyltetrahydro-	I	0.54	12	0.53	17
	oxazolone	II	0.88	16	0.86	24
12	TFAE	I	0.59	34	0.53	35
		II	1.60	63	1.44	72
20	Tröger's base	I	1.11	66	1.05	80
		II	2.36	99	2.19	117
26	FMOC-Trp methyl ester	L	2.94	215	2.50	245

with increasing pressure, whereas the plate height increases. Most probably, some of the narrow structured sites ("cavities") become less accessible in a "compressed" state. This reduced accessibility leads to reduced capacity factors and a significant increase in h.

CONCLUSIONS

In this paper the influence of the temperature and the eluent composition on the theoretical plate height is investigated. The data show that an increase in temperature greatly improves the efficiency by affecting the diffusion velocity at the "slow"-type sites. Probably it also causes some changes in the swelling state of the packed bed.

Changes in the solvent composition affect the elution strength and the mean viscosity of the solvent as well as the swelling state of the adsorbent. The relative contribution of the "slow"-type sites for the overall adsorption is changed in these ways. With the addition of methanol and water, the importance of the "slow"-type sites generally decreases. These sites seem to be more polar than the "quick"-type sites and are very important for enantioselective recognition. The addition of methanol consequently leads to a decrease in the plate height of the analytes and in their retention. 1- and 2-propanol cause a small increase in the plate height, predominantly due to viscosity effects. They may also affect to some extent the swelling state of the packing. Cyclohexane is predominantly a competitor at the rather non-polar "quick"-type sites. Thus it increases the relative importance of the "slow"-type sites for several analytes, causing a small increase in the plate height connected with a decrease in retention.

Stereorecognition seems to occur mainly when adsorption onto the narrow "slow"-type sites is involved. The availability of these sites, and therefore their relative contribution to the overall retention, is determined by the competitive adsorption of solvents on this type of sites and by the swelling state. In both ways the eluent composition is a decisive factor for the enantioselectivity of the system. Methanol most often causes a decrease in enantioselectivity, whereas water and cyclohexane cause an increase.

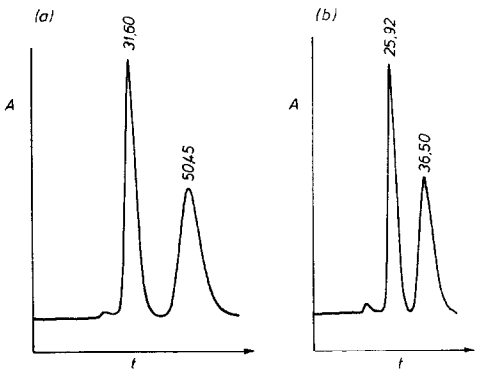


Fig. 5. Chromatograms of racemic Tröger's base obtained with different mobile phases. t = time in min; A = UV absorption. Stationary phase: swerCTA. Mobile phases: (a) ethanol–water (96:4); (b) methanol ethanol–water (20:67.2:12.8). Column: 250 mm × 10 mm I.D. Flow-rate: 1 ml/min. Temperature: 50°C.

For practical use it is important that changes in the swelling state induced by changes in the solvent composition are fully reversible within the range of solvent composition investigated.

There was a certain influence of pressure on the plate height. Obviously the packed bed is still compressible to a small extent which affects the adsorption kinetics onto the "slow"-type sites.

The chromatograms in Fig. 5 illustrate the influence of the eluent composition on the peak width and the chromatographic resolution of racemic Tröger's base.

The detailed study of the plate height dependence on the four variables structure [3], retention [3], eluent composition and temperature provides interesting information about the mechanisms of adsorption and chiral recognition on swollen crystalline CTA materials. The consequences for the optimization of separations of optical isomers by choosing an appropriate eluent composition, temperature and flow-rate for an improvement in the efficiency will be discussed in detail in a forthcoming paper [5].

ACKNOWLEDGEMENTS

This work was made possible by a grant from the Austrian Fond zur Förderung der Wissenschaftlichen Forschung (FWF), Project Number P6300C. The author deeply appreciates this support and thanks the Institute for Organic Chemistry of the University of Vienna, and Hoechst-AG for kindly donating chiral test substances.

REFERENCES

1 G. Blaschke, *J. Liq. Chromatogr.*, 9 (1986) 341.
2 T. Shibata, I. Okamoto and K. Ishii, *J. Liq. Chromatogr.*, 9 (1986) 313.
3 A. Rizzi, *J. Chromatogr.*, 478 (1989) 71.
4 *CRC Handbook of Chemistry and Physics*, CRC Press, Boca Raton, FL, 1980.
5 A. Rizzi, *J. Chromatogr.*, 478 (1989) 101.