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

I. INFLUENCE OF CAPACITY FACTOR, ANALYTE STRUCTURE, FLOW VELOCITY AND COLUMN LOADING

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SUMMARY

The peak dispersion in high-performance liquid chromatographic columns packed with swollen crystalline cellulose triacetate was investigated as a function of the capacity factors of the analytes and their structures as well as of the flow-rate, column loading and degree of cross-linking of the adsorbent material. The main contribution to the plate height is attributed to the packed bed, arising from slow adsorption/desorption processes at certain, narrow parts of the surface. The results show the existence of at least two types of adsorption sites, which differ in the rate of the adsorption/desorption process: "quick"-type and "slow"-type sites. These types of sites are assumed to differ also in the types of interactions with the analytes. The narrow, "slow"-type sites are of decisive importance for chiral recognition.

INTRODUCTION

Triacetylated cellulose has been known for several years as a useful stationary phase for the chromatographic separation of optical isomers[1–16]. Nowadays, two different forms of cellulose triacetate (CTA) ar used in chromatography. First, CTA is used directly in a swollen crystalline state, called swollen microcrystalline cellulose triacetate (swcrCTA). This material is obtained by heterogeneous acetylation of microcrystalline cellulose and subsequent swelling in boiling alcohol, *e.g.*, ethanol[1,2,3,8]. It is assumed to form some type of "inclusion complexes" with several types of analytes[1,2]. The strength of the interaction, and therefore the retention on the stationary phase, is determined by the fit of the analyte to the chiral "cavities" of the swollen microcrystalline stationary phase. The geometric arrangement of the chiral environment in the "cavities" is determined by the microcrystalline structure of this material.

The second method of using CTA stationary phases is to coat the solved CTA material onto the surface of macroporous silica particles[14,17,18]. The silica particles

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may be modified by chemically bonded aminopropyl or other groups. Solved and reprecipitated CTA loses its microcrystalline structure. The chiral recognition and discrimination properties of solved and reprecipitated CTA are quite different from those of the microcrystalline form[11-14].

Both materials are commercially available in a pressure-stable form, suitable for high-performance liquid chromatography (HPLC). swerCTA particles are available with diameters down to 7–10 μm (refs. 3, 5 and 6) and can be used at pressures above 200 atm. These particle sizes are comparable with those of usual silica particles. Silica coated CTA materials are available in the particle sizes of ground silica and are highly pressure-stable due to the silica backbone.

CTA materials are widely utilized for chromatographic separations of enantiomers on the analytical and preparative scales[1-8,13]. The advantages of swerCTA materials are the high enantioselectivity which can be obtained for many chiral analytes of different structural types and the high loadability[1,5]. A severe drawback is the large peak broadening often observed with these separations which leads to a low efficiency. In spite of the given enantioselectivity, in many cases there is insufficient chromatographic resolution, reduced detection limits by high dilution and a reduced peak capacity of the columns.

The enantioselectivity values obtained with coated CTA materials differ from those obtained with swollen crystalline materials. The former are smaller in many cases[14]. There is much less peak broadening with coated materials, however[14,17,18].

The problem of peak broadening on swollen crystalline CTA supports has briefly been stressed[1,2,5,8,16]. However, no systematic investigations have been described which deal with the dependence of the peak width on swerCTA packings on the capacity factors of the analytes, the analyte structure, the eluent composition, the temperature and the pressure. In this paper, quantitative data are reported on the dependence of the theoretical plate height on the capacity factors of the analytes and on their structures, as well as on the flow-rate, the column loading and on the degree of cross-linking of the CTA material. The dependence on the eluent composition, the temperature and the pressure forms the subject of another paper[19].

THEORETICAL

In the last decade the theory of band spreading in chromatography has reached a level which allows one to understand and to predict with fair accuracy the peak broadening behaviour of analytes in normal and reversed-phase chromatography. The theory describes the dependence of the degree of peak broadening on parameters like the particle size, diffusion coefficient, solvent viscosity, temperature, flow velocity, capacity factor and the quality of packing (geometric arrangement of the particles in the packed bed)[20-25]. Today, it is clear that the plate height, H_i, results from at least four contributions, H_{di}, H_{ci}, H_{fi} and H_{bi} (refs. 20–22, 24, 26 and 27) which have different dependences on the flow velocity, u, the particle size, d_p, and the capacity factor, κ_i:

$$H_i = H_{di} + H_{ci} + H_{fi} + H_{bi} \tag{1}$$

The index i indicates the solute and the subscripts d, c, f and b indicate the

contributions associated with dispersion due to axial diffusion, convection, mass exchange in the streaming part of the mobile phase and mass exchange in the fixed bed. H_b includes diffusion in the stagnant mobile phase, in the stationary phase and at the surface.

The following discussion of the band spreading process in swcrCTA packings starts from the plate-height equation given by Huber[22]

$$h_i = \frac{\varphi_d}{v_i} + \frac{\varphi'_c}{1+\varphi''_c v_i^{-\frac{1}{2}}} + \varphi_f v_i^{\frac{1}{3}} \left(\frac{\kappa_i^*}{1+\kappa_i^*}\right)^2 + \varphi_b v_i \cdot \frac{\kappa_i^*}{(1+\kappa_i^*)^2} \tag{2}$$

where h_i is the reduced plate height and v_i is the reduced flow velocity

$$h_i = H_i/d_p \tag{3a}$$

$$v_i = u \cdot \frac{\varepsilon_m}{\varepsilon_f} \cdot d_p/D_{mi} \tag{3b}$$

where u is the linear flow velocity of the mobile phase, D_{mi} is the diffusion coefficient of the solute in the mobile phase, ε_m is the fraction of the column volume occupied by the mobile phase and ε_f is the fraction occupied by the flowing part of the mobile phase. The five geometry factors, φ_d, φ'_c, φ''_c, φ_f and φ_b, are constants for a given column depending on the geometry of the particles and the packing. The parameter κ_i^* is defined as the mass distribution coefficient between the fixed bed, b, and the flowing fluid, f

$$\kappa_i^* = \frac{Q_i^{(b)}}{Q_i^{(f)}} = \frac{\varepsilon_m}{\varepsilon_f}(\kappa_i+1) - 1 \tag{4}$$

where κ_i is the mass distribution coefficient (capacity factor) between the stationary phase, s, and the mobile phase, m, and Q being the symbol for quantity.

It should be noted that h_f and h_b have different dependences on the capacity factor. Whereas $\left(\frac{\kappa_i^*}{1+\kappa_i^*}\right)^2$ is an increasing function with increasing capacity factor, κ_i, $\frac{\kappa_i^*}{(1+\kappa_i^*)^2}$ is a decreasing function with increasing κ_i^* values greater than one. Eqn. 2 describes experimental data on silica-based reversed-phase and normal-phase systems quite well[26–28].

This theoretical approach is, however, incapable of describing the effects actually observed with swcrCTA packing materials. Therefore eqn. 2 has to be extended. The measurements on swcrCTA, which are presented in detail later, reveal for many analytes dramatically elevated reduced plate heights. These plate heights are not correlated with the capacity factors of the analytes, but rather with their steric structures. This indicates that it is the mass exchange term in the chromatographic bed, h_b, which is essentially the main source of the increase in the plate height.

It is assumed that in the porous swollen crystalline packing material the motion of the analytes very near to narrow parts of the adsorbent is hindered. These narrow

parts may be of "cavity"-like or channel-like structure, or can be more generally described as adsorption sites where the adsorption/desorption process including the diffusion to these sites and the optimum positioning at these sites is slow.

For the following discussion it is assumed that the slow transport processes in the packed bed can be taken into account by splitting h_b into two contributions, h_b^{quick} and h_b^{slow}

$$h_b = h_b^{quick} + h_b^{slow} \tag{5}$$

where h_b^{quick} is assumed to be the h_b contribution observed normally, typical for small analytes not sterically hindered in their diffusion and motion near the surface or in narrow channels. It thus accounts mainly for the mass transport in the stagnant mobile phase in the large pores of the particles and is described by the term $\varphi_b v_i \cdot \frac{\kappa_i^*}{(1+\kappa_i^*)^2}$ in eqn. 2; its value is expected to be small, as is found usually with porous silica particles[26–29]. The parameter h_b^{slow} is an additional contribution accounting for the slow transport and adsorption/desorption process near to or at some of the available adsorption sites. These sites, at which the entire process of adsorption/desorption is slow, are referred to as "slow"-type sites. (Since these sites are assumed to be narrow they are assumed to be decisive for chiral recognition in many cases.) The value of h_b^{slow} for a particular analyte can be regarded approximately as the difference between its measured h value and that of a small, not sterically hindered molecule, *e.g.*, toluene.

In discussing the dependences of the reduced plate height on the capacity factor, κ_i, the temperature, T, the availability of the different types of adsorption sites (which is determined by the eluent composition) and on the flow velocity, u, in this and in the subsequent publication[19], respectively, it should be kept in mind that h_d, h_c, h_f and $h_b^{quick}$are also influenced by these parameters. However, the effects on these plate height contributions will be much smaller compared with that on h_b^{slow} and are therefore not always seen to have statistical significance within the precision of the data.

The influence of the particle size is not investigated here. Since h_b^{slow} is assumed to result predominantly from slow adsorption/desorption processes at the sites, and do not result from a slow diffusion velocity in the whole stagnant zone, the dependence of h_b^{slow} on d_p will probably differ from that of h_b^{quick}, which is known to be linearly dependent on d_p (see last term in eqn. 2; v is linearly dependent on d_p, therefore h_b^{quick} is also linearly dependent on d_p).

EXPERIMENTAL

Apparatus

Chromatographic experiments were carried out using an high-pressure liquid chromatographic pump (Model L-6200 intelligent pump; Merck-Hitachi, Tokyo, Japan), a syringe-valve injector (Model 7161; Rheodyne, Cotati, CA, U.S.A.) equipped with a 20-μl loop, a column oven (Model 655A-52, Merck-Hitachi) and an UV detector (Model L-4000, Merck-Hitachi) connected to an integrator (Model D-2000 chromato-integrator, Merck-Hitachi).

Columns

If not explicitly indicated otherwise, the data reported in the tables and figures refer to column I, prepacked with swcrCTA having a mean particle diameter of 10 μm (Hibar®, E. Merck, Darmstadt, F.R.G.), 250 mm × 10 mm I.D. Column II was prepacked with swcrCTA material of probably enhanced degree of cross-linking, mean particle diameter 7 μm (Macherey Nagel, Düren, F.R.G.), 250 mm × 4 mm I.D.

Reagents and samples

Absolute ethanol was of p.a. quality, methanol and cyclohexane of LiChrosolv® quality from E. Merck. Water used for the eluent preparation was distilled twice and purified by passing through a RP-8 column before eluent preparation. The eluent mixtures were premixed and degassed in an ultrasonic bath. The analyte samples were of the highest purity grade available or were received in a highly purified state as gifts from synthesis laboratories.

Procedure

All data refer to isocratic elution at constant temperature. After establishment of the thermal equilibrium, the constancy of retention data was about ±1%. The void volume for the 250 mm × 10 mm I.D. column was estimated as 15 ml from the retention volume of the system peaks of water, methanol or propanol injected. All the calculations of the capacity factors are based on a void volume of 15.00 ml for all solvent mixtures. UV detection was performed at 254 nm.

RESULTS AND DISCUSSION

The porous structure of the packed bed

Crystalline CTA in the swollen state has a certain porosity. A system of macropores and micropores exists in the particles. Within the microporous system, narrow structured channels and cavities are expected. This results in a size exclusion mechanism for small molecules together with an adsorption mechanism. The porosity of this material is therefore different for different analytes.

The results of the determination of the porosity depend on the type of molecules used for the measurement. Using solvent components, the porosity will be higher the smaller are these solvent molecules. In the case of cyclohexane, it is likely in addition that the available pore volume is determined not only by the size of this molecule but also by its hydrophobicity, since cyclohexane does not penetrate the near vicinity of polar adsorption sites. This is illustrated in Table I, where the column porosity values, ε_m, are evaluated from the system peaks of solvents injected in a mobile phase of ethanol. The usually accepted substance for evaluation of V_0, tri-*tert.*-butylbenzene, is partially excluded from the pores available to methanol and water.

This discussion makes clear that a defined particle porosity cannot be given, because this obviously depends on the analyte (and even the solvent) structure. To avoid uncertainties in the calculation of the capacity factors, in this paper an uniform value of 0.76 is chosen for the column porosity, ε_m.

Plate height as a function of the capacity factor

In Fig. 1 and Table II the reduced plate height, h, is given as a function of the

TABLE I

COLUMN POROSITY, ε_m, FOR VARIOUS SOLVENT COMPONENTS

Eluent: ethanol–water (96:4, v/v). Temperature: 50°C. V_{column} = 19.635 ml.

V_0 marker	V_0 (ml)	ε_m
Water	15.0	0.76
Methanol	14.5	0.73
Ethanol	14.0	0.71
1-Propanol	12.4	0.63
Cyclohexane	12.0	0.61
Tri-*tert.*-butylbenzene	12.0	0.61

capacity factors of the analytes. No simple correlation is found between the peak broadening and the retention behaviour. This contradicts predictions of the dependence of the peak width from capacity factors based on the usual chromatographic

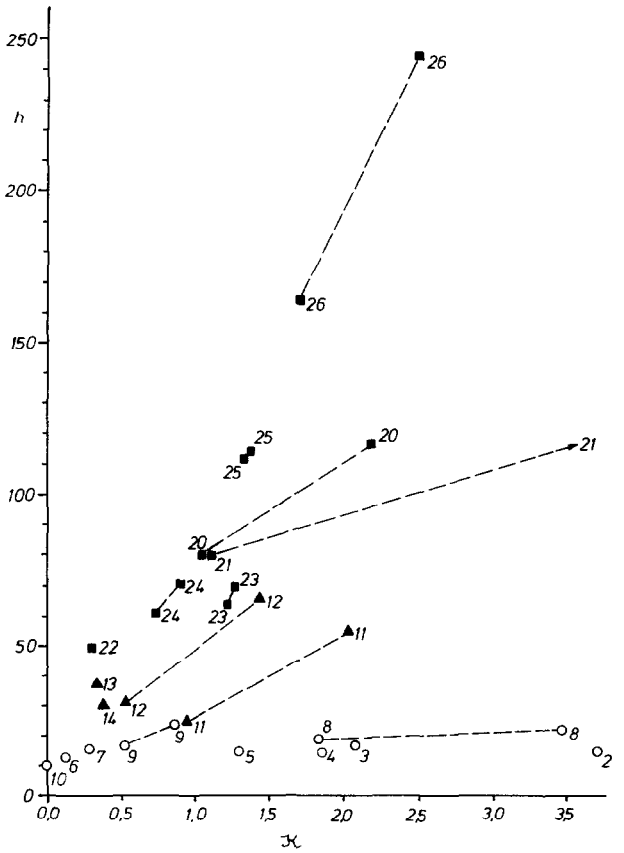


Fig. 1. Reduced plate height, h, for various non-chiral and chiral analytes as a function of their capacity factors. Enantiomers are connected by dotted lines. Symbols: ○, compounds with high efficiency; ▲ and ■, compounds with low or very low efficiency. Code numbers of the solutes as in Table II. Eluent: ethanol–water (96:4, v/v). Temperature: 50°C. Flow-rate: 1 ml/min.

TABLE II

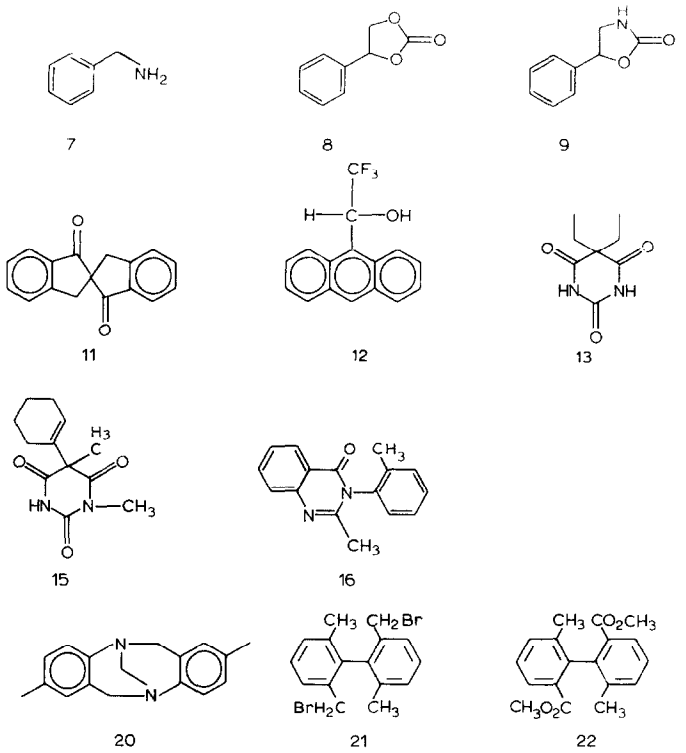
DEPENDENCE OF THE REDUCED PLATE HEIGHT, h, ON THE CAPACITY FACTORS, κ, AND THE STRUCTURES OF THE ANALYTES

Non-trivial structures of analytes are given in Scheme 1. I and II indicate the first and second isomers eluted, α denotes the enantioselectivity coefficient. Eluent: ethanol–water (96:4, v/v). Temperature: 50°C. Flow-rate: 1 ml/min. Z = Benzyloxycarbonyl; FMOC = fluorenylmethyloxycarbonyl; Asn, Phe, Pro and Trp denote the amino acids asparagine, phenylalanine, proline and tryptophan, respectively.

Code No.	*Solute*		κ	h	α
High efficiency					
1	1,3,5-Tri-*tert.*-butylbenzene		<0	8	
2	Benzene		3.70	15	
3	Toluene		2.08	17	
4	Anthracene		1.85	16	
5	Nitrobenzene		1.30	15	
6	Resorcinol		0.12	13	
7	Benzylamine		0.29	16	
8	4-Phenyl-1,3-dioxolan-2-one	I	1.84	19	1.89
		II	3.47	22	
9	5-Phenyltetrahydrooxazol-2-one	I	0.53	17	1.62
		II	0.86	24	
10	2-Deoxyadenosine		0.0	9.8	
Low efficiency					
11	2,2′-Spirobiindan-1,1′-dione	I	0.95	25	2.14
		II	2.03	55	
12	2,2,2-Trifluoro-1-(9-anthryl)ethanol	I	0.53	31	2.72
	(TFAE)	II	1.44	66	
13	Barbital		0.34	38	
14	Z-Asn-methyl ester	L	0.38	30	
Very low efficiency					
20	Tröger's base	I	1.05	80	2.09
		II	2.19	117	
21	*o,o′*-Dimethyl-*o,o′*-di(bromomethyl)-	I	1.11	80	
	biphenylene	II	6.08	150	
22	*o,o′*-Dimethyl-*o,o′*-di(methoxycarbonyl)-	I	0.30	50	1.53
	biphenylene	II	0.46		
23	Z-Trp-methyl ester		1.22	64	1.04
			1.27	70	
24	FMOC-Pro-methyl ester	L	0.73	62	1.23
		D	0.90	71	
25	FMOC-Phe-methyl ester	D	1.33	112	1.03
		L	1.37	114	
26	FMOC-Trp-methyl ester	D	1.70	164	1.47
		L	2.50	245	

theory[22,26,28] and has to be explained by taking account of additional factors (*cf.*, eqn. 5).

In principle, these finding can be discussed on the basis of two different, but similar models. Both models assume that the large band spreading observed for many substances arises from slow diffusion of these solutes in the near surroundings



Scheme 1.

(environment) of narrow structured adsorption sites. These narrow sites do not necessarily have a "cavity"-like structure.

(i) The first model assumes that the adsorption sites are essentially of a single type. The type and strength of the interaction between the sites and the analytes depend mainly on the analyte structure and configuration. Similarly, the rate of the adsorption/desorption process of the analytes at the sites depends only on the structure (bulkiness) of the analytes. Thus the peak broadening is more or less a function only of the analyte structure. This model would be sufficient to explaining the band broadening data observed for different analytes.

(ii) The second model assumes that two main types of adsorption (binding) sites are operative. They differ essentially in the accessibility for the analytes and in the type and strength of the interaction with the analytes. One type of adsorption sites can be accessed easily: there the adsorption/desorption process is rapid. They are referred as "quick"-type sites. The other type of binding sites has a narrow environment; the adsorption/desorption process at these sites is hindered for bulky analytes and therefore slow. These sites are called "slow"-type sites. (Within this type of sites a certain distribution in the narrowness is likely.) In this model the peak broadening is a function not only of the bulkiness of the analyte structure alone but also of the type of bindings predominantly formed with the adsorbent, *i.e.*, the type of binding site predominantly adsorbed on. Within this model, the overall plate height of a solute is

determined by the relative contribution of the narrow, "slow"-type adsorption sites to the total retention and by the individual diffusion velocity of the solute at these "slow"-type sites. For both factors the analyte structure is decisive.

Evidence in support of this second model is provided by the finding that non-polar aromatic compounds, in spite of their molecular size, always show low plate height values, and, secondly, by the observed dependence of the plate height and stereoselectivity data on the solvent composition, which is discussed in Part II[19]. A single type of site cannot account for these observations. This model is thus used to discuss the experimental data given in this paper.

The existence of different adsorption "principles" (generating different degrees of selectivity) for swcrCTA adsorbents[2,13] and different adsorption sites in coated CTA adsorbents[30] have been proposed before. It might also be concluded from the data reported recently by Roussel *et al.*[31] where for some types of racemic analytes the elution order of the enantiomers was found to be dependent on the analyte concentration. The assumption that in a more or less crystalline arrangement of the adsorbent the steric environment around different binding sites differs in narrowness has an high degree of probability.

It is clear from this discussion that the large plate height values observed for many solutes arise from the packed bed and can therefore be attributed as being a contribution to h_b.

Considering the plate height data in greater detail, the following pattern can be observed from Table II.

Some compounds (No. **1** and acidic compounds not included in Table II) are excluded from parts of the mobile phase volume due to their steric structure (tri-*tert.*-butylbenzene) or to their charges (organic acids). They are eluted slightly before the column void volume. Their h values are between 7 and 10, and thus lie above those obtainable with rigid silica particles of mean diameter 10 μm (h = 4–5). Nevertheless, packings of swollen CTA with h values of 7–10 have to be judged as excellent.

For non-polar aromatic compounds (benzene, toluene, naphthalene, anthracene), the reduced plate height values are found between 15 and 18. For these types of compounds no significant increase in plate height with increasing capacity factors is observed. [Such an increase is predicted by the chromatographic theory because of the dependence of h_f on the capacity factor, κ_i (*cf.*, eqn. 2)[22,26,27]. However, the effect expected from theory is too small to be observed with statistical significance within this investigation.] It is characteristic for this adsorbent that larger aromatic molecules are adsorbed less strongly than is benzene. No significant correlation between h and the size of this type of analytes is found. Within the chosen model, it is concluded that non-polar aromatic compounds are thus predominantly adsorbed onto adsorption sites with rapid adsorption kinetics; h_b^{slow} is negligible for these compounds.

Similar h values between 10 and 20 are also found for aromatic compounds with (sterically) rather small polar side-functions (compounds **5**–**10**, shown by circles in Fig. 1). This means that adsorption occurs either mainly onto the "quick"-type sites or, more probably, that the motion and steric orientation of parts of the molecules at the narrow sites is little hindered and therefore not slow.

Many analytes in Table II (compounds **11**–**26**, shown by triangles and squares in Fig. 1) have strongly increased values of h. Fig. 1 shows that in these cases h and thus

h_b^{slow} is not uniquely correlated with the capacity factor but depends on the structures of the analytes. It is not completely clear which structural features are decisive for the adsorption kinetics. It seems that the total size of the whole molecule is decisive only in those cases where the molecule has a rigid structure (spirocompounds, Tröger's base). In the other cases, it is probably the steric feature of the polar group which is decisive. Planar analytes show relatively low h_b values. (Maybe the aromatic ring acts like an anchor at "quick"-type sites.) However, the number of different molecules investigate d is too small for a subtle discussion of structure–efficiency relationships. In all cases of chiral analytes the more strongly retained enantiomer shows an higher value for the plate height. This is evidence in support of the assumption that the h_b^{slow} contribution is related to a process of adsorption and is not due to slow diffusion in a bulk environment.

Interpretation of these data —which is supported by the dependence of the plate height on the solvent composition reported in Part II[19]— leads to the assumption that "slow"- and "quick"-type sites differ in the type and strength of the interactions with the analytes, predominantly in the strength of the interaction with polar and non-polar groups of the analytes. These differences may be caused by differences in the polarizability of the sites and in their ability to undergo hydrogen bonding[30,32,33]. If the adsorption includes the motion into narrow structures, the narrow environment built by the adsorbent (including the eluent layer) interacts with the analyte and may represent a steric hindrance to the motion of the analyte or parts of it. Such sites may be therefore of the "slow"-type. Dependent on the steric structure of the analyte, the interactions are of different strengths (causing chiral separation if the strength differs between enantiomeric compounds). In narrow sites, the different strengths of interaction may cause differences also in the velocity of motion, and consequently in the plate height. The slope of the plate height *vs.* capacity factor is not the same for all analytes, since (i) the steric hindrance in the narrow, "slow"-type sites is not equally strong for all compounds and (ii) the contribution of adsorption at the "slow"-type sites relative to that at the "quick"-type sites is dependent on the types of interactions being formed and therefore on the structures of the analytes.

From these data, it has become clear that the loss in efficiency can essentially be attributed to h_b^{slow}, which may be influenced to a certain extent by the swelling state of crystalline CTA. This point is discussed elsewhere[19]. The "quality of the packing procedure" does not influence h_b. It usually determines the h_c and h_f contributions which result from mixing processes in the streaming part of the mobile phase[20–24] and which are predominant in the most frequently used systems with rigid particles[26,27,29]. Since in swcrCTA packings the h_f term is found to contribute to a minor extent for most of the interesting solutes, one has to conclude that the problem of low efficiency in swcrCTA separations cannot be solved by improving the quality of the packing procedure.

Plate height as a function of the flow velocity

The dependence of the plate height on the flow velocity, u, for different analytes is given in Fig. 2. The following patterns can be observed.

The flow velocity corresponding to the minimum value of h, u_{min}, is found to be very low. Although linear velocities as small as 0.14 mm/s were applied, a real minimum was not seen. In the cases of TFAE (**12**) and Tröger's base (**20**) the curves

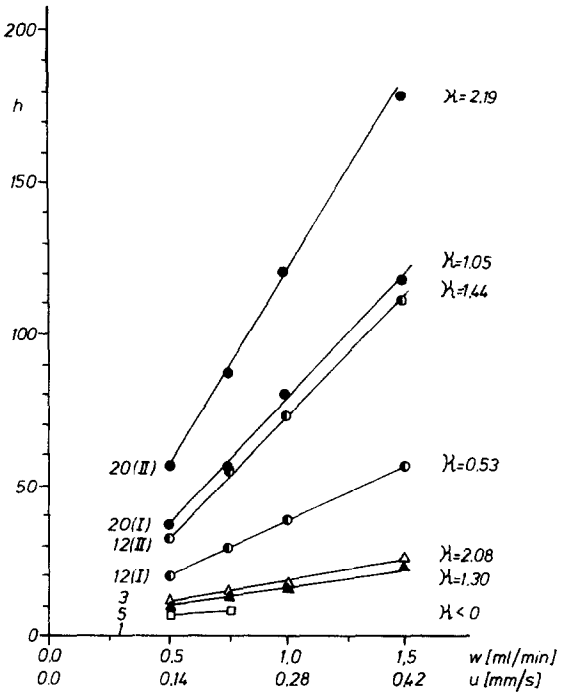


Fig. 2. Reduced plate height, h, as a function of the linear flow velocity, u. Code numbers of solutes as in Table II. Eluent and temperature as in Fig. 1.

observed result from extremely high contributions of h_b which cause the minimum in h to be shifted to very small values of u. The values of u_{min} and h_{min} depend on the magnitude of the h_b contributions and may therefore differ between different solutes. Undoubtedly, h_b is generally the main source of the small values of u_{min} and the large values of h_{min}. However, the extent of the observed shift of u_{min} for compounds **1**, **3** and **5** is surprising, since for these h_b is not as high. It is likely that some other factors may be responsible for the very small values of u_{min}. First, the net diameter of the particles may be enhanced in the swollen state. The v_{min} values correspond, therefore, to lower u_{min} values. Secondly, using fibre-shaped particles, the tortuosity factor may be enhanced. This reduces the value of φ_d in eqn. 2 and thus reduces the contribution of h_d. Thirdly, with partially compressible particles like swcrCTA, it might be possible that the contributions h_c and h_f are lower than with rigid particles of the same diameter.

Concerning h_{min}, rather high values compared with alkylsilica or silica columns are expected. It is clear that these values result from the larger h_b contributions and from significantly increased h_d contributions at small values of u_{min}. However, h_{min} may also be overestimated to some extent by underestimating the d_p value of swollen particles.

Due to the predominant influence of h_b, which is known to increase linearly with u, the total plate height also increases approximately linearly with the flow velocity. This is seen in Fig. 2. The slopes of the plots of h *vs.* u reflect the different diffusion velocities at the adsorption sites which depend on the steric structures of the analytes.

The slopes are not correlated with the capacity factor values and are different for enantiomers in the general case.

The increase in h with increasing u is not very strong for analytes mainly adsorbed onto the "quick"-type adsorption sites, as expected from the previous discussion. For these analytes, the increase in h_f with u may also be important. However, the precision of the data is not sufficient to quantify the contribution of h_f to the total increase in h with u for these compounds.

Plate height and peak symmetry as a function of the analyte concentration

Generally, the peak symmetry obtained on the packing material and column used is excellent. At moderate concentrations, the symmetry factors, a, defined in Table III, usually lie between 1.10 and 0.85.

Although small, the asymmetry seems to be correlated with the retention mechanism. Non-polar analytes, mainly adsorbed onto the "quick"-type adsorption sites, show a slight leading in most cases, whereas the analytes which also penetrate to the narrow "slow"-type adsorption sites show a slight tailing. Depending on the analyte structure, this tailing is more or less pronounced.

These results can be interpreted by the following model. (i) At the narrow, "slow"-type adsorption sites a competitive adsorption mechanism is operative. In addition, these narrow sites may be not totally homogeneous in spatial configuration and thus not completely homogeneous in adsorption energies. The presence of adsorption sites having slightly different binding strengths causes a slight convex curvature of the adsorption isotherm which may be the source of the slight tailing observed. Because of the smallness of the effect, no significant shift of the capacity factor with loading can be observed. (ii) Aromatic compounds are assumed to adsorb preferentially onto the "quick"-type adsorption sites (*cf.*, the discussion above). To some extent, the "quick"-type adsorption sites may allow a second-layer or multi-layer adsorption of other molecules of the same type onto the first adsorbed ring, thus inducing a slight concave form of the adsorption isotherm. This interpretation is based on the correlation of tailing and leading with the different adsorption and band

TABLE III

REDUCED PLATE HEIGHT, h, AND PEAK SYMMETRY FACTOR, a, AS A FUNCTION OF THE ANALYTE CONCENTRATION

Eluent: ethanol–water (96:4, v/v). Temperature: 50°C. Flow-rate: 1 ml/min. Injection volume: 20 µl. The symmetry factor, a, was determined according to ref. 34 at 1/10 of the peak height; values >1 indicate leading, those <1 indicate tailing.

Code	*Solute*					
4	Anthracene		Q_{inj} (µg)	0.2	2.0	20.0	
			h	20	20	22	
			a	1.04	1.09	1.15	(leading)
12	TFAE		Q_{inj} (µg)	0.15	1.50	30.0	
		I	h	37	35	44	
			a	0.86	0.94	0.74	(tailing)
		II	h	71	72	76	
			a	–	0.72	0.80	(tailing)

broadening behaviours of the analytes. It must be pointed out, however, that these effects are very small and the peak shape is found to be highly symmetrical, very often to an higher extent than in most cases of reversed-phase chromatography.

Up to about 10 μg injected, the influence of the loaded mass on the plate height, peak symmetry or retention time of the peak maximum is statistically insignificant or unimportant (Table III). In the case of anthracene, the highest concentration given in Table III was about half the saturation concentration in ethanol.

Differences between different swollen crystalline cellulose acetate materials

Similar investigations to those described for the CTA material I have also been done for another CTA material, II, which is assumed to have an higher degree of cross-linking. Most likely, it is a cellulose 2.5-acetate. The main chromatographic differences between these two materials are as follows.

Material II yields strongly tailing peaks at 30°C. This tailing is eliminated nearly completely when working at 50°C.

With material I, the group of non-chiral, rather non-polar aromatic compounds (benzene, naphthalene, anthracene, toluene, ethylbenzene, chlorobenzene, nitrobenzene and anisole) have a very uniform dependence of h on κ (see Fig. 1). With material II, characteristic differences can be observed within this group as shown in Fig. 3. The larger analytes (anthracene, naphthalene, nitrobenzene, ethylbenzene) have similar behaviours to those on material I. The smaller ones (benzene, chlorobenzene and toluene) show a pronounced increase in h with κ. This means that hindered diffusion has to be assumed also for parts of the non-polar adsorption sites, especially for those which are accessible only to the smallest aromatic molecules. This may result from different steric structures and swelling states of the materials, due to a different degree of cross-linking.

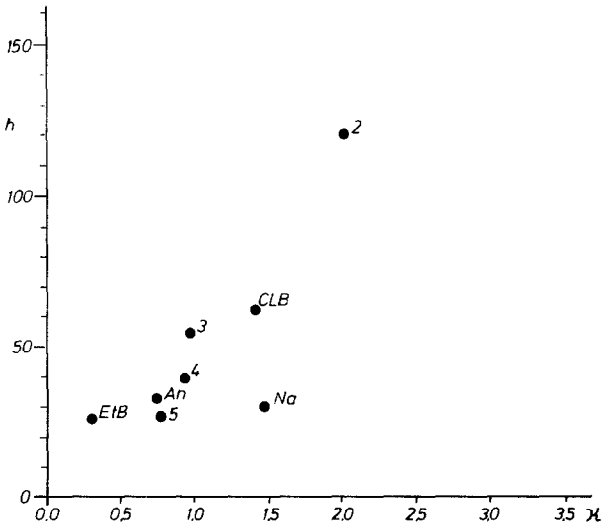


Fig. 3. Reduced plate height, h, as a function of the capacity factors, κ, for various non-chiral aromatic compounds using a cross-linked CTA material (II). EtB = Ethylbenzene; ClB = chlorobenzene; An = anisole; Na = naphthalene; code numbers for all other compounds as in Table II. Chromatographic conditions as in Fig. 1.

CONCLUSIONS

From the investigation of the dependence of the peak dispersion on the capacity factor and the structure of the analyte, one can primarily conclude that in columns packed with swcrCTA the most important contribution to the plate height arises from the slow mass exchange in the packed bed. Most probably, the source is a slow transport process at parts of the adsorption sites available, *e.g.*, diffusion and orientation at narrow sites, in narrow channels or cavity-like structures.

The steric structure of the analytes is found to be the decisive parameter which determines the plate height. The fact that relatively small h values can be found for some compounds, whereas for others very high values are observed, is interpreted by assuming the existence of at last two types of adsorption sites: "slow"- and "quick"-type sites. These types 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. The plate height, however, is predominantly determined by the "slow"-type sites. Since the relative contribution of the "slow"-type sites to the overall retention differs between different analytes depending on their structures, no correlation is found between plate height and capacity factor.

The different magnitudes of band spreading processes for analytes of different structures is illustrated together with the high enantioselectivity and the excellent peak symmetry obtainable with swcrCTA by the chromatograms in Fig. 4.

The dependence of the plate height on the flow-rate is approximately linear within the range of flow-rates applied. This is expected from the predominant contribution of the packed bed related mass-exchange term, h_b. The minima of the curves of h_i *vs.* u are shifted to very low flow velocities.

The axial diffusion contribution, h_d, and the mobile phase related contributions, h_c and h_f, cannot be determined individually. It is not yet clear whether for slightly

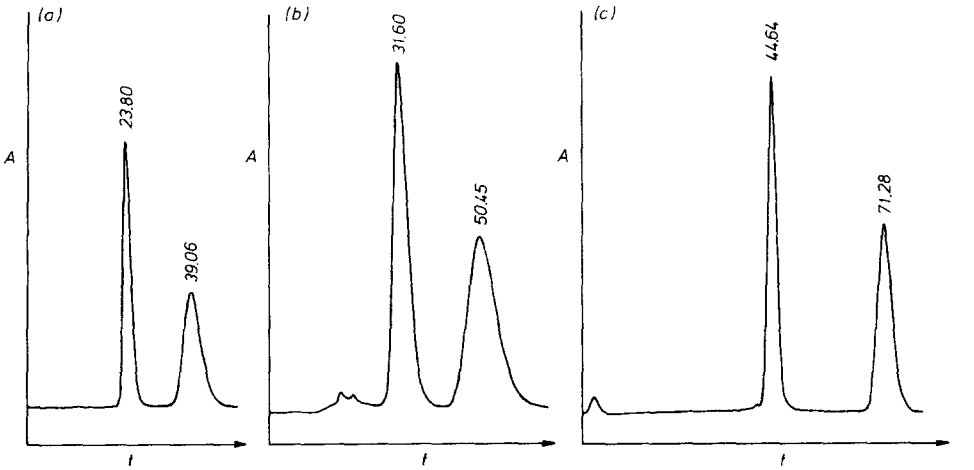


Fig. 4. Chromatograms of chiral analytes. (a) TFAE; (b) Tröger's base; (c) 4-phenyl-1,3-dioxolan-2-one. t = Time in min; A = UV absorption. Stationary phase: swcrCTA. Mobile phase: ethanol–water (96:4). Column: 250 mm × 10 mm I.D. Flow-rate: 1 ml/min. Temperature: 50°C. t in min.

compressible swcrCTA particles with enhanced tortuosity factors these contributions are strictly comparable with the corresponding terms for rigid silica particles. It is clear, however, that h_e and h_f are little enhanced in comparison to other packings with the same particle size. Thus, one has to conclude that the columns are well packed and that a better packing procedure will not decrease the peak broadening dramatically.

The dependence of h_b on the particle diameter has not been investigated here; h_b^{quick} is assumed to be linearly dependent on d_p, whereas this is not expected for h_b^{slow}. Since h_b^{slow} is in most cases the predominant plate height contribution, knowledge of the influence of the particle size on this term will be of considerable practical importance.

The peak symmetry is excellent even at high analyte concentrations. No significant increase in plate height can be observed up to 10 μg of analytes injected in 20 μl.

A detailed study of the plate height dependence on the eluent composition, the temperature and the pressure (which all influence the swelling state of the adsorbent) will be presented in the following paper[19].

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REFERENCES

1 G. Hesse and R. Hagel, *Chromatographia*, 9 (1976) 62.
2 G. Hesse and R. Hagel, *Liebigs Ann. Chem.*, (1976) 996.
3 K. R. Lindner and A. Mannschreck, *J. Chromatogr.*, 193 (1980) 308.
4 K. Schlögel and M. Widhalm, *Chem. Ber.*, 115 (1982) 3042.
5 H. Koller, K.-H. Rimböck and A. Mannschreck, *J. Chromatogr.*, 282 (1983) 89.
6 G. Blaschke, H.-P. Kraft and H. Markgraf, *Chem. Ber.*, 116 (1983) 3611.
7 K. Schlögel and M. Widhalm, *Monatsh. Chem.*, 115 (1984) 1113.
8 A. Mannschreck, H. Koller and R. Wernicke, *Kontakte (Darmstadt)*, 1985/1 (1985) 40.
9 E. Francotte, R. M. Wolf, D. Lohmann and R. Mueller, *J. Chromatogr.*, 347 (1985) 25.
10 E. Francotte, H. Stierlin and J. W. Faigle, *J. Chromatogr.*, 346 (1985) 321.
11 K.-H. Rimböck, M. A. Cuyegkeng and A. Mannschreck, *Chromatographia*, 21 (1986) 223.
12 G. Blaschke, *J. Liq. Chromatogr.*, 9 (1986) 341.
13 J. Scharf, K. Schlögel, M. Widhalm, J. Lex, W. Tuckmantel, E. Vogel and F. Pertlik, *Monatsh. Chem.*, 117 (1986) 255.
14 T. Shibata, I. Okamoto and K. Ishii, *J. Liq. Chromatogr.*, 9 (1986) 313.
15 A. Hussenius, R. Isaksson and O. Matsson, *J. Chromatogr.*, 405 (1987) 155.
16 M. Krause and R. Galensa, *J. Chromatogr.*, 441 (1988) 417.
17 Y. Okamoto, M. Kawashima, K. Yamamoto and K. Hatada, *Chem. Lett.*, (1984) 739.
18 A. Ichida, T. Shibata, I. Okamoto, Y. Yuki, H. Namikoshi and Y. Toga, *Chromatographia*, 19 (1984) 280.
19 A. Rizzi, *J. Chromatogr.*, 478 (1989) 87.
20 J. C. Giddings, *Dynamics of Chromatography*, Part I, Marcel Dekker, New York, 1965.
21 J. F. K. Huber, *J. Chromatogr. Sci.*, 7 (1969) 85.
22 J. F. K. Huber, *Ber. Bunsenges. Phys. Chem.*, 77 (1973) 179.
23 G. J. Kennedy and J. H. Knox, *J. Chromatogr. Sci.*, 10 (1972) 549.
24 C. Horvath and H. J. Lin, *J. Chromatogr.*, 149 (1978) 43.
25 J. H. Knox and H. P. Scott, *J. Chromatogr.*, 282 (1983) 297.

26 J. F. K. Huber, J. Quaadgras and A. Rizzi, *8th Int. Symp. Column Liquid Chromatography, New York, May 1984*, Abstract No. 3pG3.
27 J. F. K. Huber, J. Quaadgras and A. Rizzi, in preparation.
28 E. Katz, K. L. Ogan and R. P. W. Scott, *J. Chromatogr.*, 270 (1983) 51.
29 J. F. K. Huber and A. Rizzi, *J. Chromatogr.*, 384 (1987) 337.
30 I. W. Wainer and M. C. Alembik, *J. Chromatogr.*, 358 (1986) 85.
31 C. Roussel, J.-L. Stein, F. Beauvais and A. Chemlal, *J. Chromatogr.*, in press.
32 I. W. Wainer, M. C. Alembik and E. Smith, *J. Chromatogr.*, 388 (1987) 65.
33 I. W. Wainer, R. M. Stiffin and T. Shibata, *J. Chromatogr.*, 411 (1987) 139.
34 L. R. Snyder and J. J. Kirkland, *Introduction to Modern Liquid Chromatography*, Wiley, New York, 1979, p. 222.