

Study of band broadening in enantioselective separations using microcrystalline cellulose triacetate

I. The linear case

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ABSTRACT

Linear chromatographic data were obtained for the enantiomers of Tröger's base separated on microcrystalline cellulose triacetate with pure ethanol as the mobile phase. The efficiency was determined for flow-rates between 0.1 and 1.0 ml/min at temperatures of 303, 318, 333 and 348 K. The efficiency is poor (below 100 plates) at 303 K, but increases rapidly with increasing temperature. From studies of the effects of these two parameters, the band profile dispersion is attributed to flow anisotropy and adsorption-desorption kinetics. The experimental elution profiles are compared with calculated profiles as a check on the quality of the efficiency measurements.

INTRODUCTION

The utility of microcrystalline cellulose triacetate (CTA) as a chiral discriminator for liquid chromatography has been exploited for 20 years [1–7]. Because it is readily available and inexpensive for a stationary phase, it has become popular for enantioselective separations [8]. Al-

though it exhibits good mechanical stability, it provides for many separations an efficiency that is far below what is desirable for HPLC. The mobile phase flow-rate [4,6] and the column temperature [5–7] can have marked effects on the resolution for a given separation, and in this respect their contributions to the efficiency of the phase system have been studied as separate entities. The reason why the efficiency of well packed columns is so low still eludes our understanding [8].

The separation of the enantiomers of Tröger's base on CTA has already been studied [1,6–8]

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and constitutes a convenient benchmark of enantioselective separations. This separation is attractive to study because the modest resolution achieved results from the combination of an excellent separation factor and a relatively poor column efficiency at moderate temperatures. Consequently, we can expect a substantial enhancement of the resolution by manipulating properly the mobile phase linear velocity and the column temperature. In this work, we analyzed the contributions of the mass transfer resistances to the band profile dispersion, and especially the contribution of the adsorption–desorption kinetics, as functions of these operating parameters.

EXPERIMENTAL

Equipment

The chromatographic experiments were performed on an HP 1090 liquid chromatograph (Hewlett-Packard, Palo Alto, CA, USA) equipped with a temperature-controlled column oven, a multi-solvent delivery system, a diode-array UV detector and a computer data acquisition system.

Materials

The microcrystalline cellulose triacetate (CTA) was packed into the column (25 cm × 0.46 cm I.D.) using a slurry technique with ethanol as the push solvent. Prior to packing, the CTA was swollen by boiling in ethanol for *ca.* 30 min [1].

Tröger's base (TB) and 1,3,5-tri-*tert.*-butylbenzene (TTBB) were purchased from Fluka (Buchs, Switzerland) and ethanol (100% pure) from Midwest Grain Products (Weston, MO, USA).

Procedures

All chromatograms were obtained under isocratic conditions with ethanol as the mobile phase. Each injection contained 150 ng of each enantiomer of TB and 160 ng of TTBB. The UV detector was set at 220 nm. Chromatograms were obtained at 303, 318, 333 and 348 K for flow-rates of 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.8 and 1.0 ml/min. The efficiency data were extracted from the peak widths at half-height.

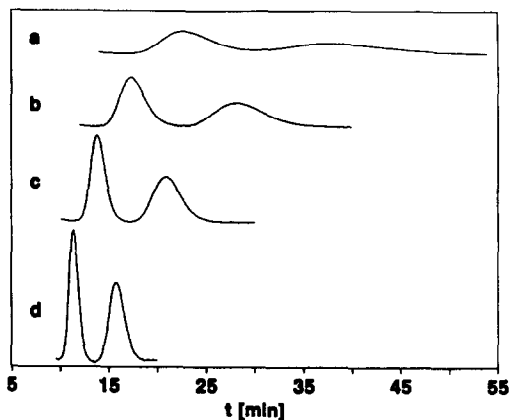


Fig. 1. Chromatograms of Tröger's base (TB) at (a) 303, (b) 318, (c) 333 and (d) 348 K. Experimental conditions: stationary phase, microcrystalline cellulose triacetate; mobile phase, pure ethanol; flow-rate, 0.4 ml/min.

RESULTS AND DISCUSSION

Retention and efficiency data

In Fig. 1 the elution profiles of Tröger's base (TB) are shown for the four temperatures studied. Simply by raising the temperature from 303 to 348 K, a dramatic improvement in the band profiles is observed. These results are in agreement with previously published results [5–7]. Both enantiomer band widths decrease sharply with increasing temperature, because of a combination of a decrease in their retention times and an increase in the column efficiency.

In Fig. 2 and Table I, the temperature dependence of the retention factors and the separation factor are reported. The adsorption enthalpies of TTBB, (–)-TB and (+)-TB are –4.60, –20.1 and –22.3 kJ/mol, respectively. To demonstrate the increase in column efficiency at a flow-rate of 0.4 ml/min, the plate numbers (Table II) increase from 798, 91 and 67 at 303 K to 1207, 652 and 459 at 348 K for (TTBB), (–)-TB and (+)-TB, respectively. This is a sevenfold increase for the TB enantiomers and a 50% increase for the least retained TTBB.

Previously, TTBB was used as an unretained marker [2], but on this column the compound showed a slight retention compared with an impurity in the TTBB sample. The choice to track the impurity as the void marker was made because at the different temperatures the void

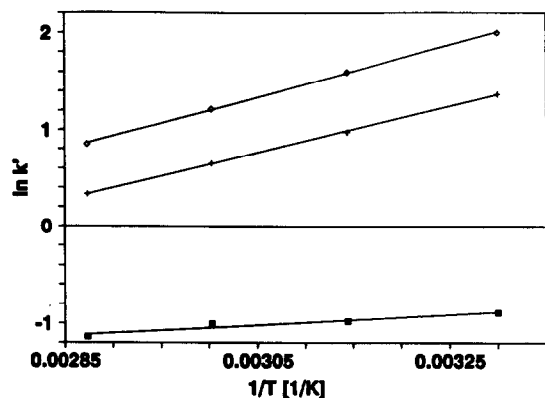


Fig. 2. Variation of the logarithm of the retention factors with the inverse of the absolute temperature. ■ = TTBB; + = (-)-TB; ◇ = (+)-TB.

TABLE I

CAPACITY FACTORS, k' , AND SELECTIVITIES, α

T (K)	k'_{TTBB}	$k'_{(-)\text{-TB}}$	$k'_{(+)\text{-TB}}$	$\alpha_{(-,+)}$
303	0.41	3.95	7.38	1.87
318	0.38	2.66	4.94	1.86
333	0.37	1.92	3.39	1.76
348	0.32	1.40	2.34	1.67
ΔH_{ads} (kJ/mol)	-4.60	-20.1	-22.3	

TABLE II

PLATE NUMBERS AT A FLOW-RATE OF 0.4 ml/min

T (K)	TTBB	(-)-TB	(+)-TB
303	798	91	67
318	894	181	131
333	1024	336	213
348	1207	652	459

volume for this unknown remained constant at 1.88 ml, whereas for TTBB a significant decrease in retention occurred with an increase in temperature (Table I). The fact that TTBB is slightly retained although similar in mass to the TB enantiomers (the molecular masses of TTBB and TB are 246 and 250, respectively) makes it likely that no size-exclusion phenomena enter into a comparison between the compounds.

In Figs. 3–6, the height equivalent to a theoretical plate, H (cm), is plotted *versus* the mobile

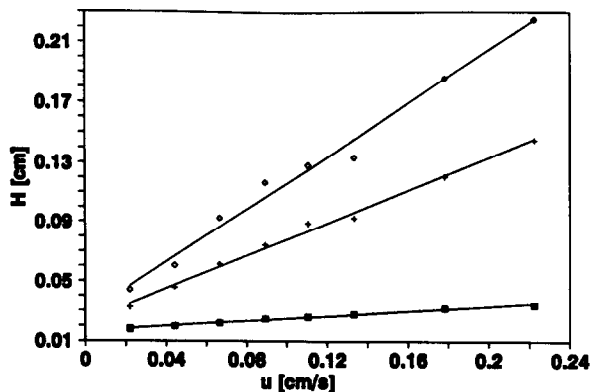


Fig. 3. Van Deemter plot at 333 K. ■ = TTBB; + = (-)-TB; ◇ = (+)-TB.

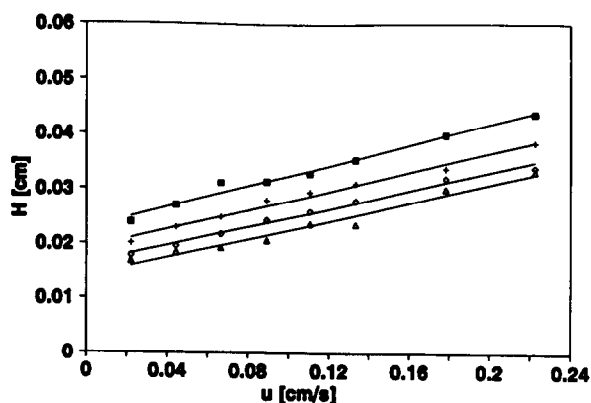


Fig. 4. Van Deemter plot of TTBB at (■) 303, (+) 318; (◇) 333 and (Δ) 348 K.

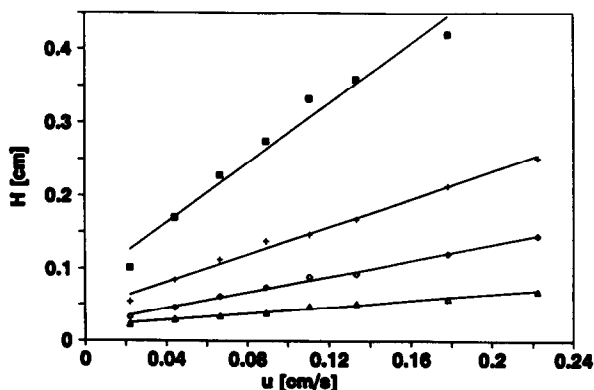


Fig. 5. Van Deemter plot of (-)-TB at (■) 303, (+) 318, (◇) 333 and (Δ) 348 K.

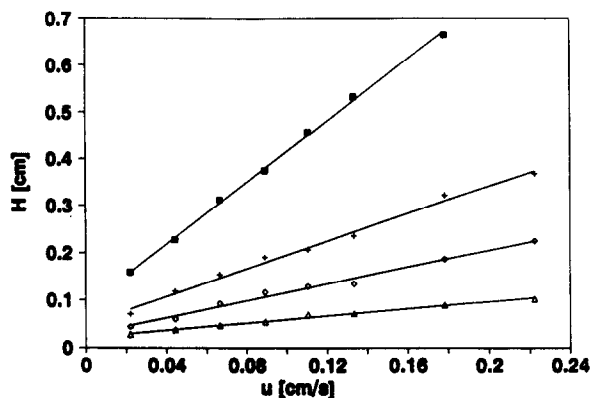


Fig. 6. Van Deemter plot of (+)-TB at (■) 303, (+) 318, (◇) 333 and (△) 348 K.

phase linear velocity, u (cm/s), in Van Deemter plots. Fig. 3 shows the efficiency for the temperature 333 K. For all temperatures, the ten-

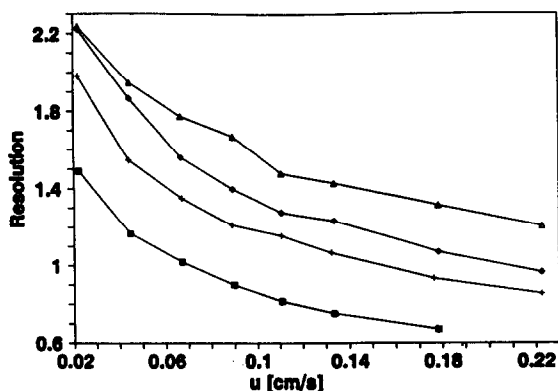


Fig. 7. Variation of the resolution of (-)- and (+)-TB with the mobile phase linear velocity at (■) 303, (+) 318, (◇) 333 and (△) 348 K.

ency is identical with that for the more retained (+)-TB, having the lowest efficiency, followed by the (-)-TB, having an intermediate efficiency, and finally TTBB, having the highest efficiency.

For Figs. 4–6, the variation of the column efficiency for each compound is plotted for the four temperatures. A pattern again develops in that as the temperature increases, the efficiency increases. As a result, the resolution (Fig. 7) is strongly influenced by both the mobile phase velocity and the column temperature. In order to achieve a comparable resolution, e.g., 1.5, a linear flow velocity of ca. 0.02 cm/s needs to be used at 303 K whereas 0.14 cm/s, a seven times higher velocity, can be used for 348 K.

Temperature dependence of the mass transfer resistances

In order to describe the change in the efficiency with both the flow velocity and temperature, a modified Van Deemter equation is chosen [9]:

$$H = A + C_s u \quad (1)$$

where

$$C_s = \frac{2k'}{(1 + k')^2 k_f} \quad (2)$$

k' is the retention factor and k_f the rate constant of the adsorption–desorption kinetics. The second term of the classical Van Deemter equation, which accounts for axial molecular diffusion, is eliminated because this term is very small under

TABLE III
FLOW ANISOTROPY TERM, A , AND KINETICS TERM, C_s

T (K)	A (cm)			C_s (s)		
	TTBB	(-)-TB	(+)-TB	TTBB	(-)-TB	(+)-TB
303	0.0229	0.0815	0.0858	0.0946	2.04	3.29
318	0.0191	0.0429	0.0487	0.0874	0.955	1.46
333	0.0164	0.0228	0.0273	0.0835	0.553	0.888
348	0.0141	0.0191	0.0218	0.0839	0.230	0.374
ΔH_{act} (kJ/mol) ^a				0.271	49.3	54.5

^a Activation energy for k_f (eqn. 2).

the present experimental conditions. In the measured range (0.1–1.0 ml/min), the experimental data failed to show a minimum (Figs. 3–6), in agreement with previous findings [4,6]. The inability to observe a measurable minimum for the van Deemter plots indicates the column efficiency is controlled by the contributions of the flow anisotropy and the mass transfer resistances (*i.e.*, the adsorption–desorption kinetics and the kinetics of diffusion across the particles). It was not possible to extend the range of our investigation. At flow-rates below 0.1 ml/min the pumps of the chromatograph failed to maintain a constant flow-rate, and above 1.0 ml/min the stability of the packing at the higher temperatures was in question.

The eddy diffusion term, A , in eqn. 1 should be constant. However, we observe that it varies with increasing temperature (Table III). This can be attributed to the particle size distribution of the packing material (15–25 μm), and to its irregularity. We find also that, for a given temperature, the two enantiomers have nearly the same A value, but that the value corresponding to TTBB falls slightly below in each instance. These differences may arise from the inaccuracy of eqn. 1. The flow anisotropy is diffusion dependent as the molecule must diffuse into and out of adjacent streams of fluid traveling at different velocities [10]. Further, neglecting the classical B/u term in the Van Deemter equation enhances the flow-rate dependence of A . However, fitting the data to more elaborate constructions for this term failed to yield worthwhile parameters.

From Tables I and III and eqn. 2, values of the apparent adsorption–desorption coefficients, k_f , were calculated. As the measurements were made at high velocity, this coefficient is more accurate than A . In Fig. 8, the data are plotted as $\ln k_f$ versus $1/T$. The experimental data lie along reasonably straight lines, and from the slopes of these lines the activation energies are calculated to be 0.271, 49.3 and 54.5 kJ/mol for TTBB, (–)-TB and (+)-TB, respectively. With energies this large the contribution from the adsorption–desorption kinetics cannot be overlooked in the case of the two enantiomers.

It is striking that whereas the activation energy

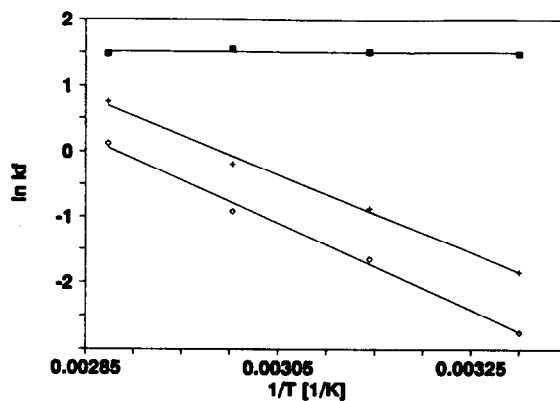


Fig. 8. Variation of $\ln k_f$ (eqn. 2) with $1/T$. ■ = TTBB; + = (–)-TB; (◇) = (+)-TB.

for TTBB (Table III) is eighteen times smaller than its adsorption enthalpy (Table I), the activation energies are 2.5 times larger than the adsorption enthalpies for the two TB enantiomers. The unusually large values of these activation energies suggest that the contribution to the mass transfer resistance which controls band broadening in the present instance is the kinetics of adsorption–desorption or of the formation of the chiral discrimination complex.

The band profiles obtained are very close to Gaussian. The retention times and standard deviations measured on these profiles are in excellent agreement with the values calculated using the transport-dispersive model [11], the values of k_f determined in this work and the experimental adsorption isotherm [7] in its linear range, or those derived from the model of Van Deemter *et al.* [9].

CONCLUSIONS

The structure of microcrystalline cellulose is extremely complex [6], which has so far prevented an understanding of the mechanisms of retention and enantioselective separations on this stationary phase. No satisfactory suggestions have yet been made. The study of the kinetics of phase equilibrium during the chromatographic separation shows that band broadening is due to a small contribution of flow anisotropy, similar for the two enantiomers under isothermal conditions, and a major contribution due to mass

transfer resistances. The activation energy of this latter contribution suggests that it involves more of the kinetics of the retention mechanism, presumably a form of adsorption–desorption, than of mass transfer kinetics by intraparticle diffusion.

By better segregation of the contributions to dispersion, more accurate simulations of the band profiles can be ascertained, especially when the kinetics vary greatly for the components of the system. Also, by being able to predict accurately the elution profiles, optimization of the operating parameters for a minimum analysis time with a given degree of resolution becomes that much more valuable. It is certainly satisfactory to observe that, as predicted by theory, the band profiles are still nearly symmetrical at very low column efficiencies.

SYMBOLS

A	flow anisotropy term (cm)
B	axial diffusion coefficient (cm^2/s)
C	mobile phase concentration of the solute (mol/l)
C_s	kinetics term (s)
H	height equivalent to a theoretical plate (cm)
k'	retention factor
k_f	apparent adsorption–desorption coefficient (s^{-1})
T	temperature (K)
u	mobile phase linear velocity (cm/s)
α	selectivity
ΔH_{act}	activation energy (kJ/mol)
ΔH_{ads}	adsorption enthalpy (kJ/mol)

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