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Thermodynamics of the adsorption of Tröger's base enantiomers from ethanol on cellulose triacetate

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ABSTRACT

The adsorption isotherms of the (−)- and (+)-enantiomers of Tröger's base on microcrystalline cellulose triacetate with ethanol as solvent were measured at 30, 40, 50 and 60°C using frontal analysis. The isotherms of the first eluted (−)-enantiomer can be described satisfactorily with the Langmuir equation at all temperatures. By contrast, the isotherms of the longer retained (+)-enantiomer exhibit a pronounced inflection point in the lower temperature range. This point nearly vanishes at 60°C where the isotherm is almost linear in the concentration range investigated. All these isotherms can be satisfactorily described by a quadratic isotherm equation. The standard thermodynamic functions were derived from these equilibrium data. The enthalpies and the isosteric heats of adsorption are not constant in the temperature region studied. The adsorption enthalpies of both enantiomers increase with increasing temperature. At constant temperature, the isosteric heat of adsorption of the (−)-enantiomer depends only slightly on the stationary phase concentration whereas that of the (+)-enantiomer increases strongly with increasing concentration.

INTRODUCTION

The direct chromatographic resolution of racemic mixtures is being actively studied for analytical [1,2] and preparative [3] purposes. Several practically useful types of stationary phases have been reported [4], including microcrystalline cellulose triacetate (CTA), one of the first phases used for enantioseparations by preparative chromatography. Several racemates have been successfully separated with this material [5–14]. CTA is produced by the heterogeneous acetylation of microcrystalline cellulose [5]. It has good mechanical properties and is relatively inexpensive compared with other chiral phases.

However, CTA is not stable in all solvents. Its enantioselectivity is achieved only in a swollen state. Methanol, ethanol, 2-propanol and mixtures of these alcohols with water or with hydrocarbons are the most useful mobile phases. Hesse and Hagel [5] concluded that sorption on CTA is not achieved by adhering to one glucose ester moiety, but by insertion between two such moieties. They emphasized the crucial contribution from the crystalline structure and coined the term "inclusion chromatography" to describe the retention mechanism. CTA was extensively studied and characterized by Koller *et al.* [7].

Tröger's base (TB) is a classical racemate. Its enantioseparation was performed on lactose as early as 1944 [9]. In a recent study, the unusual retention behavior of TB on CTA observed by several workers [5,10,14] was explained as the consequence of different isotherm shapes for both TB enantiomers [15]. Whereas the isotherm of the (−)-enantiomer could be described by Langmuir's equation, the isotherm of the longer retained (+)-enantiomer exhibits an inflection point and is accounted for by a quadratic equation. Using the adsorption isotherms measured for both pure enantiomers and the ideal adsorbed solution theory to calculate the competitive iso-

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therms, the elution bands could be predicted satisfactorily on the basis of the equilibrium dispersive model of chromatography [15].

The main object of this work was an investigation of the influence of temperature on the adsorption equilibrium of both TB enantiomers on CTA and a study of the standard thermodynamic functions describing the interactions between both enantiomers and the stationary and the mobile phases. A comparison of these functions should help to improve our understanding of enantioseparations on CTA. As demonstrated previouly for bovine serum albumin as stationary phase [16], such a thermodynamic study can contribute to understanding the mechanism of chromatographic chiral separations.

THEORY

Adsorption isotherms

Simple considerations of statistical thermodynamics give the following general adsorption isotherm equation [17,18]:

$$q = q_s \frac{C(b_1 + 2b_2C + 3b_3C^2 + \ldots + nb_nC^{n-1})}{1 + b_1C + b_2C^2 + b_3C^3 + \ldots + b_nC^n} \quad (1)$$

where the product nq_s is the saturation capacity of the adsorbent and the temperature-dependent coefficients b_i are related to the partition functions for an individual molecule adsorbed on the ith monomolecular layer [17].

The first- ($b_{2+} = 0$) and second-order ($b_{3+} = 0$) equations of the type proposed (eqn. 1) are the Langmuir and the quadratic isotherm equations, respectively [18]. The adsorption of only one molecule on each adsorption site of the saturated monolayer is assumed in the former model and that of two molecules in the latter.

Recently, we demonstrated the applicability of the Langmuir and the quadratic isotherm equations to describe the adsorption behavior of the (−)- and (+)-enantiomers of TB on CTA at 40°C [15].

Thermodynamics of adsorption

A thermodynamic analysis of adsorption processes can be found in several textbooks (*e.g.*, refs. 18 and 19). The standard thermodynamic functions K, ΔG, ΔH and ΔS at infinite dilution can be determined from the slope of the initial tangent of the isotherm. They can also be derived from the retention (capacity) factors, k'. The degree of agreement between these two sets of results is a measure of the validity of the isotherm at low concentrations. Disagreement between them is often caused by few residual, strong adsorption sites on the stationary phase surface.

The thermodynamic equilibrium constant, K, is the ratio of the activities of a component in the stationary and the mobile phases

$$K = a_s/a_m \quad (2)$$

Assuming a temperature-independent concentration for both phases as the standard state, the equilibrium constant is the initial slope of the adsorption isotherm:

$$K = \lim_{c \to 0} (q/C) \quad (3)$$

In the case of the isotherm eqn. 1,

$$K = b_1 q_s \quad (4)$$

The equilibrium constant is related to the molar Gibbs free energy of adsorption, ΔG, by

$$\Delta G = -RT \ln K \quad (5)$$

The following equation, derived from the Gibbs–Helmholtz equation, permits the determination of the molar enthalpy of adsorption, ΔH, by plotting $\ln K$ against $1/T$:

$$\frac{\partial(\ln K)}{\partial(1/T)} = -\frac{\Delta H}{R} \quad (6)$$

From ΔG and ΔH the molar entropy of adsorption, ΔS, can be calculated by

$$\Delta S = -\frac{\Delta G - \Delta H}{T} \quad (7)$$

Further, from the adsorption isotherms measured at different temperatures it is possible to derive the isosteric heat of adsorption, ΔH_{st}, or heat of adsorption at constant adsorbate concentration, according to the following equation:

$$\left.\frac{\partial(\ln C)}{\partial(1/T)}\right|_q = \frac{\Delta H_{st}}{R} \quad (8)$$

The slope of a plot of $\ln C$ *vs.* $1/T$ at constant q yields ΔH_{st}. The determination of the isosteric heats of adsorption is useful at high concentrations, when the isotherm is no longer linear and ΔH_{st} depends on

the surface coverage. In the linear range of the isotherm, the isosteric heat of adsorption is identical with the molar enthalpy of adsorption defined in eqn. 6.

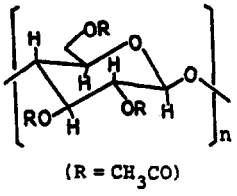
EXPERIMENTAL

Equipment

The determination of isotherms by frontal analysis (FA) and the measurement of elution profiles were performed using an HP 1090 liquid chromatograph (Hewlett-Packard, Palo Alto, CA, USA), equipped with a temperature-controlled column chamber, a solvent-delivery system, an automatic sample injector, a rapid-scan UV photodiode array detector and a data station. The column temperature could be controlled with an accuracy of *ca.* 1 K. The detector response was calibrated using the plateaux of the FA runs. For a wavelength of 300 nm the response was fitted with a third-order polynomial for TB concentrations up to 0.006 mol/l.

Materials

Stationary phase. A sample of microcrystalline cellulose triacetate (CTA, 15–25 μm), Fig. 1, was kindly supplied by Dr. J. N. Kinkel (Merck, Darmstadt, Germany). The application of this material to enantioseparations by preparative chromatography has been described [10,13]. A 10 × 0.46 cm I.D. stainless-steel column was packed using a slurry technique. Prior to packing, CTA was boiled in ethanol for 30 min to allow its swelling. After cooling and decanting, the suspension was treated in an ultrasonic bath for 5 min at ambient temperature and poured into the packing chamber. An 80-ml volume of ethanol was applied as pushing solvent at a pressure of 275 bar.



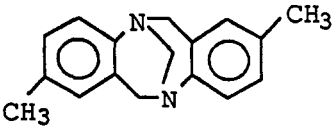


Fig. 1. Structures of microcrystalline cellulose triacetate and Tröger's base.

Following Koller *et al.* [7], the total column porosity was measured by injecting 1,3,5-tri-*tert.*-butylbenzene (TTB). As its retention time did not change significantly with the temperature, TTB could be considered as non-retained on CTA. The number of theoretical plates of the column for TTB was about 400. The total void volume measured for the column was 1.0 ml, leading to a total porosity $\varepsilon_T = 0.602$, corresponding to a phase ratio F of 0.661, with $F = (1 - \varepsilon_T)/\varepsilon_T$.

An additional volume of tubing (0.78 ml) caused a time delay for the step injections performed with the solvent-delivery system compared with injections of small samples made with the automatic sample injector.

Mobile phase and chemicals. All experiments were performed under isocratic conditions, using pure ethanol (Midwest Grain Products, 200 proof). A racemic mixture of Tröger's base (TB), $C_{17}H_{18}N_2$ ($M = 250.35$ g/mol), Fig. 1, was also supplied by Dr. Kinkel. TB enantiomers with purity >99.5% and 1,3,5-tri-*tert.*-butylbenzene were purchased from Fluka (Buchs, Switzerland). All these compounds were used as received.

Procedures

Prior to isotherm determinations, some small samples of both TB enantiomers were eluted. The classical parameters of linear chromatography were determined at different temperatures and are reported in Table I. Noteworthy are the high separation factors and the low column efficiency. Because of the considerable band broadening due to slow mass transfer, longer columns would be necessary to deliver the number of theoretical plates required for preparative separations. However, FA is the only chromatographic method allowing accurate isotherm measurements with low-efficiency columns [20]. As the method requires complete saturation of the stationary phase with the sample solution, shorter columns are advantageous to save material and therefore a 10-cm column was used.

TABLE I

PARAMETERS DETERMINED FROM INJECTIONS OF SMALL SAMPLE SIZES (LINEAR CHROMATOGRAPHY)

T (K)	k' (−)	K (−)	N_P (−)	k' (+)	K (+)	N_P (+)	α
303	2.50	3.78	29	5.13	7.76	20	2.05
313	1.96	2.96	44	4.24	6.41	31	2.17
323	1.63	2.47	63	3.34	5.05	47	2.04
333	1.19	1.80	105	2.40	3.63	71	2.02

In FA, successive step changes in the eluent composition at the column inlet are made. Solving the integral mass balance equation from each breakthrough curve gives one point of the isotherm. Owing to the self-sharpening of the breakthrough fronts, the concentration steps ought to be positive for isotherms of the Langmuir type, whereas if the isotherm curvature is the opposite, decreasing concentration steps should be used.

In our previous study, we observed an inflection point for the isotherm at 40°C of the (+)-enantiomer in the concentration range 0.0012–0.0024 mol/l [15]. To be sure to determine this feature accurately, the concentration of the solutions used for the FA experiments was chosen to be 0.0048 mol/l for both enantiomers. As the duration of the experiments was several hours, during which the solution is degassed with helium, the concentration of the enantiomer solution increases slightly during the runs, owing to solvent losses. Ten concentration steps were performed to determine ten points for each isotherm. The flow-rate in all the FA experiments was 0.5 ml/min. The column temperature was varied between 30 and 60°C in intervals of 10 K. The primary chromatographic data were transferred to the VAX 8700 computer of the University of Tennessee Computing Center for further processing.

The amount adsorbed during each step of an FA run was calculated from the integral mass balance equation

$$t_R = t_0\left[1 + F\cdot\frac{q(C_E) - q(C_0)}{C_E - C_0}\right] \quad (9)$$

where $q(C_0)$ is the amount adsorbed at equilibrium before the step change from concentration C_0 to concentration C_E at column inlet and t_0 is the retention time of a non-retained component. Eqn. 9 allows the determination of the new point of the isotherm, $q(C_E)$, from the retention time of the breakthrough front, t_R. The retention time of each concentration front could be determined with a precision of better than 1.5%.

The FA experiments were carried out using the automatic solvent-delivery system to generate the concentration steps. Only steps of increasing concentration were performed for the (−)-enantiomer of TB. Owing to the observed inflection point of the TB (+)-isotherm at 40°C [15], steps of decreasing concentration were also recorded. The combined analysis of the adsorption and desorption steps permits a more accurate determination of isotherms which have an inflection point. Depending on the concentration range, either the adsorption or the desorption front is sharper and allows a more

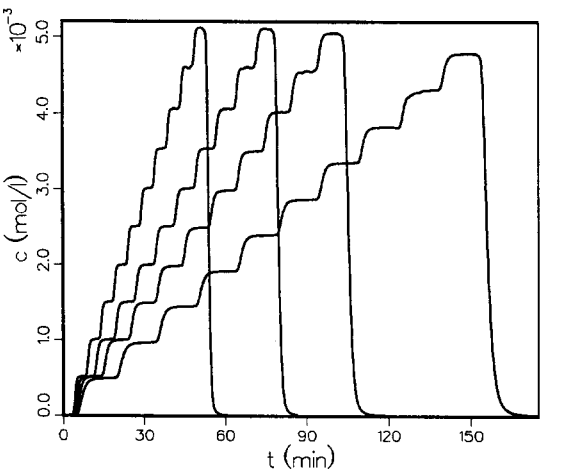


Fig. 2. Frontal analysis to determine the isotherms of the (−)-enantiomer of TB at 30, 40, 50 and 60°C (right to left).

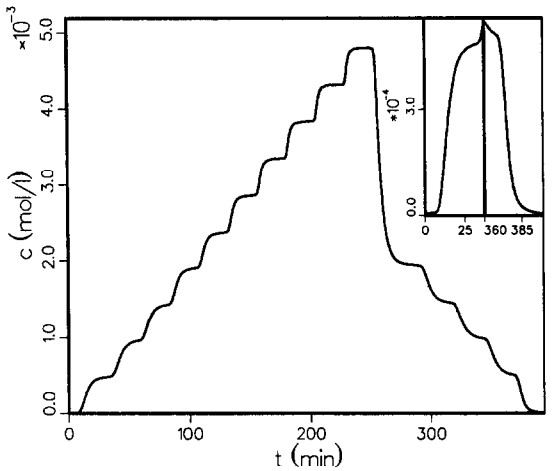


Fig. 3. Frontal analysis to determine the isotherm of the (+)-enantiomer of TB at 30°C. Inset: adsorption and desorption fronts for the lowest concentration range.

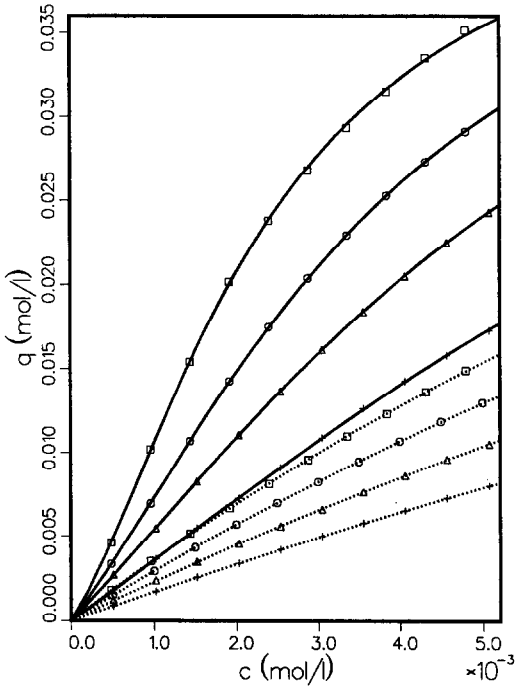


Fig. 4. Isotherms of the (−)- and (+)-enantiomers of TB on CTA at 30, 40, 50 and 60°C (top to bottom). The lines are calculated with (dotted lines) the Langmuir isotherm for the (−)- and (solid lines) with the quadratic isotherm equation for the (+)-enantiomer; parameters as in Table II. The symbols indicate the experimental data.

accurate determination of the breakthrough retention time and hence of the isotherm point.

Fig. 2 shows the FA chromatograms recorded at the four temperatures investigated for the (−)-isomer. The chromatograms were recorded beginning with 30°C, and in order of increasing temperature. The slight increase in the plateau concentrations mentioned above can be seen. This effect was taken into account in the data analysis. In Fig. 3 the FA analysis at 30°C for the (+)-enantiomer is shown. The inset shows that the adsorption front for the first concentration step is more dispersed than the corresponding desorption front. This indicates an increase in the isotherm slope, dq/dC, in this concentration region and hence that the isotherm is convex downward.

RESULTS AND DISCUSSION

Modeling of the isotherms

The equilibrium isotherms obtained for the adsorption of the enantiomers of TB on CTA at the four temperatures investigated are shown in Fig. 4. The lines were calculated by fitting the data points to the Langmuir isotherm $[q = q_s b_1 C/(1 + b_1 C)]$ for the (−)-enantiomer and to the quadratic isotherm $[q = q_s C(b_1 + 2b_2 C)/(1 + b_1 C + b_2 C^2)]$ for the (+)-enantiomer. In each instance, the selected model describes the experimental isotherms very well. The Langmuir model was not able to account for the (+)-enantiomer isotherm. The values obtained for the parameters of the equation and the corresponding standard deviations are given in Table II. These values were calculated by minimizing the following objective function, using Marquardt's method [21]:

$$\sigma\ (\%) = 100 \sqrt{\frac{1}{N_D - P} \sum_{i=1}^{N_D} \left(\frac{q_i^{ex} - q_i^{th}}{q_i^{ex}} \right)^2} \qquad (10)$$

where N_D and P are the numbers of data points and of model parameters, respectively.

There are two major differences between the isotherms of the two enantiomers as shown in Fig. 4. First, in the concentration range investigated, the adsorption capacity of CTA for the (+)-enantiomer is almost double that for the (−)-enantiomer. Second, the isotherms of the (+)-enantiomer exhibits an inflection point.

The parameters reported in Table II allow an

TABLE II

PARAMETERS OF THE ISOTHERM EQUATIONS

Enantiomer	T (K)	q_s (mol/l)	b_1 (l/mol)	b_2 (l^2/mol^2)	σ (%)
(−)-	303	0.08001	47.59	0	0.50
	313	0.08504	36.00	0	0.28
	323	0.08176	29.17	0	0.79
	333	0.08848	19.73	0	0.38
(+)-	303	0.02386	316.37	173 572	0.65
	313	0.02587	244.29	63 459	0.33
	323	0.02771	183.86	26 430	0.33
	333	0.03116	113.38	8129	0.37

estimation of the saturation capacity of the stationary phase. However, this estimation, which relies on the extrapolation of isotherm data to high concentrations, cannot be very accurate, as the highest concentration investigated in this study is more than an order of magnitude smaller than the solubility concentration of TB in ethanol. The values obtained

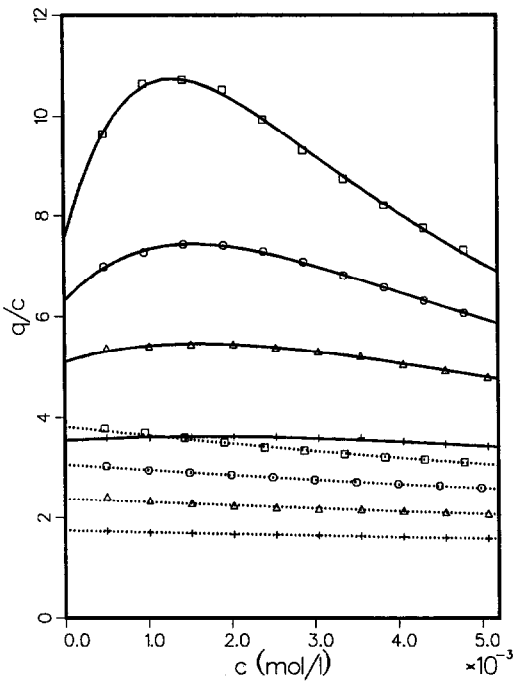


Fig. 5. Plot of the ratio q/C of the isotherms in Fig. 4 *versus* the liquid phase concentration C. Symbols as in Fig. 4.

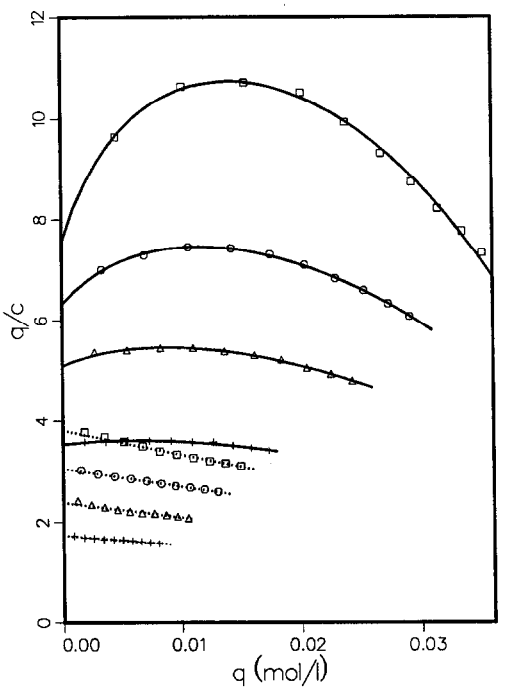


Fig. 6. Plot of the ratio q/C of the isotherms in Fig. 4 *versus* the stationary phase concentration. Symbols as in Fig. 4.

for the parameter q_s of the (−)-enantiomer are almost independent of temperature, suggesting a saturation capacity between 0.08 and 0.09 mol/l. For the (+)-enantiomer the extrapolated values of the saturation capacities are lower. They increase from 0.048 to 0.062 mol/g when the temperature increases from 30 to 60°C. Owing to the complicated course of the (+)-enantiomer isotherm, these values must be considered as uncertain. An accurate determination of the saturation capacity of the stationary phase requires additional measurements in a higher concentration range.

The existence of an inflection point of the TB (+)-isotherms is illustrated in Figs. 5 and 6, where the ratio q/C for each isotherm is plotted against the mobile and the stationary phase concentration, respectively. For the Langmuir equation, a plot of q/C *vs.* q yields a straight line. The plots corresponding to the data for the (−)-enantiomer obviously fulfil this condition very well. The (+)-enantiomer shows a completely different behavior, giving plots that exhibit a marked maximum. The inflec-

TABLE III

MAXIMA OF THE SLOPES dq/dC OF THE ISOTHERMS FOR THE (+)-ENANTIOMER AND CORRESPONDING LIQUID PHASE CONCENTRATIONS AND LOADINGS

T (K)	C (mol/l)	q (mol/l)	dq/dC_{max}
303	0.00079	0.0082	11.63
313	0.00095	0.0070	7.77
323	0.00101	0.0055	5.75
333	0.00106	0.0038	3.64

tion point of the isotherm is most pronounced at 30°C. This effect vanishes gradually with increasing temperature. At 60°C, the isotherm is almost linear. The parameter b_2 in eqn. 1 allows for the description of the inflection point. It is about 21 times smaller at 60°C than at 30°C. As can be seen in Figs. 5 and 6, the location of the inflection point shifts between 30 and 60°C in the direction of higher liquid phase concentrations (Fig. 5) and of lower stationary phase concentrations (Fig. 6). To demonstrate this effect more precisely, we report in Table III the mobile and stationary phase concentrations for the maxima of the slope dq/dC of the TB (+)-isotherm.

TABLE IV

THERMODYNAMIC PARAMETERS

Enantiomer	T (K)	K (−)	ΔG (kJ/mol)	ΔH (kJ/mol)	ΔS (J/mol · K)
(−)-	303	3.807	−3.37		
	308	3.410[a]	−3.14	−17.27	−45.9
	313	3.061	−2.91		
	318	2.698[a]	−2.62	−20.96	−57.7
	323	2.385	−2.33		
	328	2.037[a]	−1.94	−27.88	−79.1
	333	1.746	−1.54		
(+)-	303	7.549	−5.09		
	308	6.901[a]	−4.94	−14.07	−29.6
	313	6.320	−4.80		
	318	5.669[a]	−4.59	−18.09	−42.5
	323	5.095	−4.41		
	328	4.234[a]	−3.94	−32.73	−87.8
	333	3.533	−3.49		

[a] $K(T) = \exp[\ln K_b - (\Delta H/R)(1/T - 1/T_b)]$, where b designates one of the interval borders.

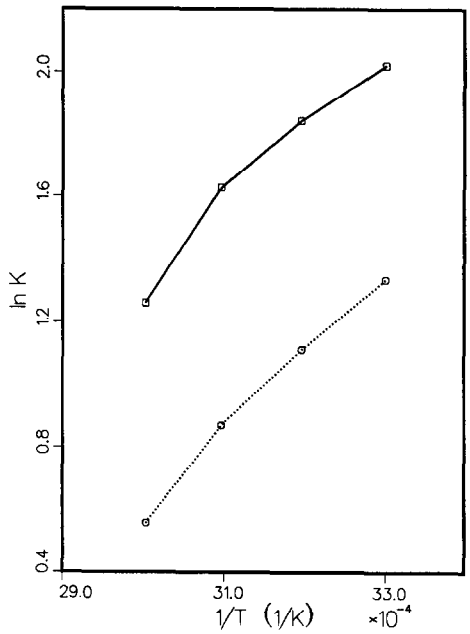


Fig. 7. Temperature dependence of the equilibrium constants of the (dotted line) (−)- and (solid line) (+)-enantiomers.

Thermodynamic analysis

The values calculated for K, ΔG, ΔH and ΔS at infinite dilution are summarized in Table IV. The difference between the adsorption equilibrium constants, K, determined from the retention times of small sample injections in the linear range of the isotherms (Table I) and calculated from the parameters of the experimental isotherms according to eqn. 4 is less than 3.6%, which is satisfactory.

The determination of the adsorption enthalpies at infinite dilution according to eqn. 6 is illustrated in Fig. 7. Obviously the data cannot be represented by a straight line and the adsorption enthalpies depend on the temperature. For this reason, the three temperature intervals were evaluated separately. It was assumed that within each interval the adsorption enthalpy is constant. To calculate the mean Gibbs free energies and adsorption entropies for each temperature interval, an equilibrium constant K was estimated for the average temperature, using the adsorption enthalpies determined from the interval borders.

The absolute values of ΔH obtained (Table IV) in the intervals 30–40 and 40–50°C are smaller for the

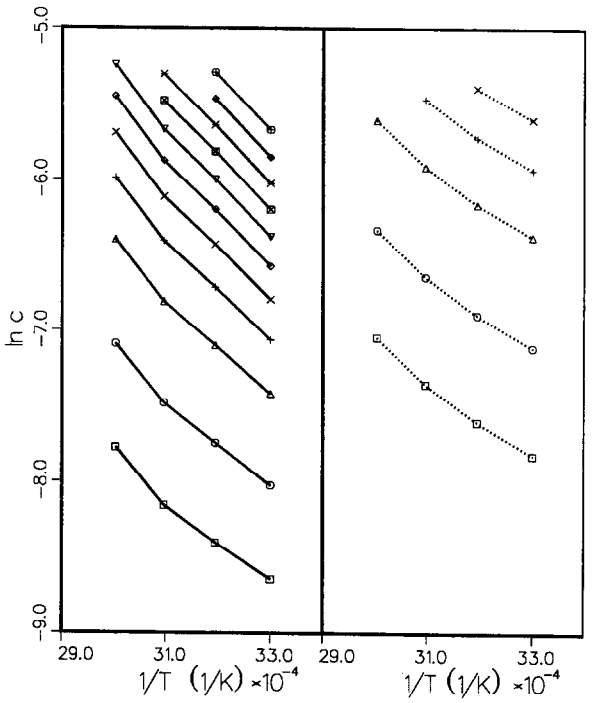


Fig. 8. Plot of ln C *versus* $1/T$ at constant stationary phase concentration for the (dotted lines) (−)- and (solid lines) (+)-enantiomers. The stationary phase concentrations increase from bottom to top: $q = 0.0015$, 0.003, 0.006, 0.009, 0.012 (for both enantiomers) and 0.015, 0.018, 0.021, 0.024, 0.027, 0.030 mol/l [the (+)-enantiomer].

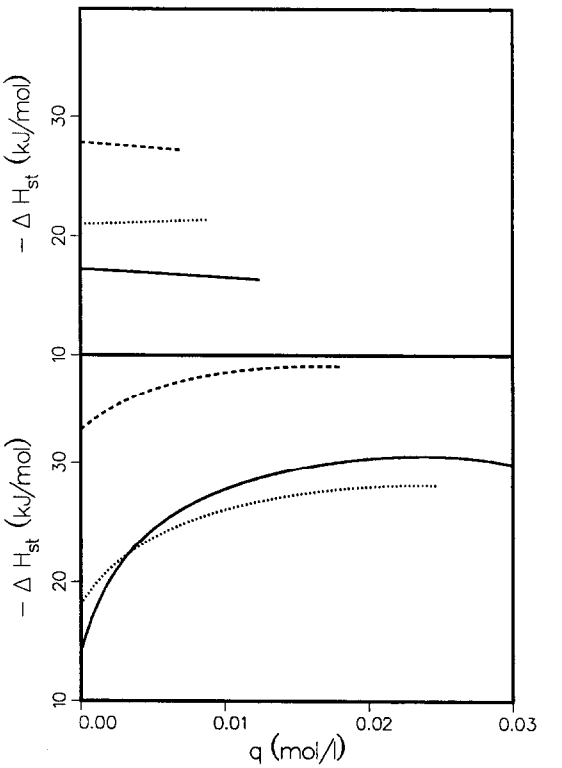


Fig. 9. Plot of the isosteric heat of adsorption *versus* the stationary phase concentration for the (top) (−)- and (bottom) (+)-enantiomers. The lines belong to different temperature intervals: (solid) 30–40, (dotted) 40–50 and (dashed) 50–60°C.

(+)-enantiomer than for the (−)-enantiomer. The opposite holds true for the interval 50–60°C. For both enantiomers, the adsorption entropies decrease very rapidly with increasing temperature (Table IV). Comparing the values for the (−)- and (+)-enantiomers, the same observations as mentioned above for the adsorption enthalpies can be made in the three temperature intervals.

The plot of ln C *vs.* $1/T$ at constant q, given in Fig. 8, demonstrates the determination of the isosteric heat of adsorption for different surface coverages. According to eqn. 8, the slopes of these curves, proportional to the isosteric heat of adsorption, depend on temperature. Again, for each of the three temperature intervals, the isosteric heats of adsorption were estimated separately. The results for both enantiomers are summarized in Fig. 9 as plots of $-\Delta H_{st}$ *versus* q. In these plots, the adsorption enthalpies at infinite dilution (Table IV) are included, as the limit of the isosteric heats of adsorption for zero stationary phase concentrations.

Fig. 9 shows the completely different behavior of the two enantiomers. The loading dependence of the isosteric heat of adsorption is not very pronounced for the (−)-enantiomer; in fact it is barely significant. By contrast, the isosteric heat of adsorption of the (+)-enantiomer increases strongly with increasing mobile phase concentration, up to *ca.* 0.01 mol/l. This concentration range includes the inflection points of all the TB (+)-isotherms whose shape is strongly effected by it (Table III). The increase in the isosteric heat of adsorption observed is most pronounced between 30 and 40°C and becomes less important at higher temperatures, as does the change in isotherm curvature around its inflection point (Figs. 5 and 6). At high concentrations, the isosteric heat of adsorption of the (+)-enantiomer is less

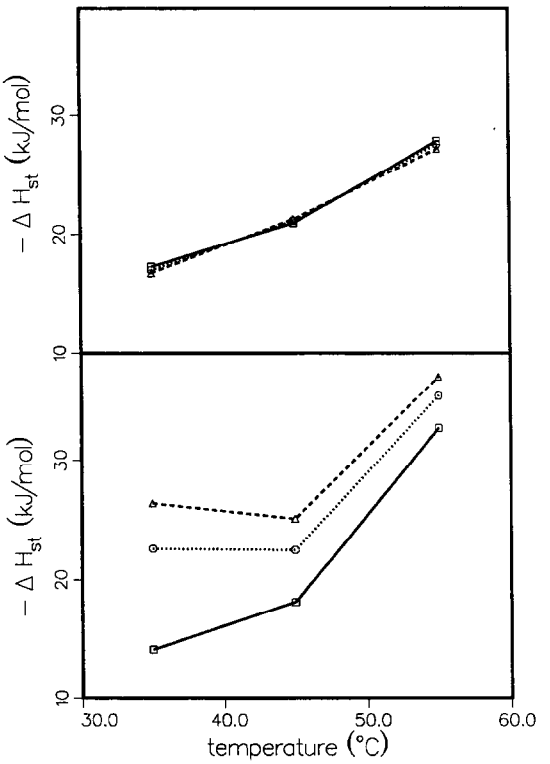


Fig. 10. Plot of the isosteric heat of adsorption *versus* the average temperatures of the three intervals for the (top) (−)- and (bottom) (+)-enantiomers. The lines belong to different loadings: (solid) 0, (dotted) 0.0035 and (dashed) 0.007 mol/l.

dependent on the concentration for all the temperature intervals studied.

Fig. 10 illustrates the dependence of the isosteric heat of adsorption on the temperature at infinite dilution and for two stationary phase concentrations in the region of the inflection point of the (+)-isotherm. For the (−)-enantiomer, ΔH_{st} increases nearly linearly with increasing temperature in the considered range and is independent of the stationary phase concentration. The results for the (+)-enantiomer show that at infinite dilution the isosteric heat of adsorption, or adsorption enthalpy, increases with increasing temperature. At the two finite stationary phase concentrations, ΔH_{st} is almost constant between 35 and 45°C but increases markedly for the highest temperature. Further, the striking difference between the two enantiomers is the distinct increase in the isosteric heat of adsorption of the (+)-enantiomer with increasing stationary phase concentration at constant temperature.

All these results demonstrate that the interactions between the TB enantiomers, CTA and ethanol are complex. We have not investigated the interactions between ethanol and CTA or their temperature dependence. The experimental results could also be affected by the possibility of hydrogen bondings between TB and ethanol, which are also temperature dependent. A further interpretation of the observed behavior of the enantiomers of TB on CTA requires, besides adsorption measurements, additional characterization of the changes of the stationary phase at different temperatures and loadings using other methods.

Elution profiles

The consequences for the profiles of chromatographic bands of the non-linear behavior of the isotherm are well known [20]. They result from the changes in the slope of the adsorption isotherm with increasing concentration, and in the relationship

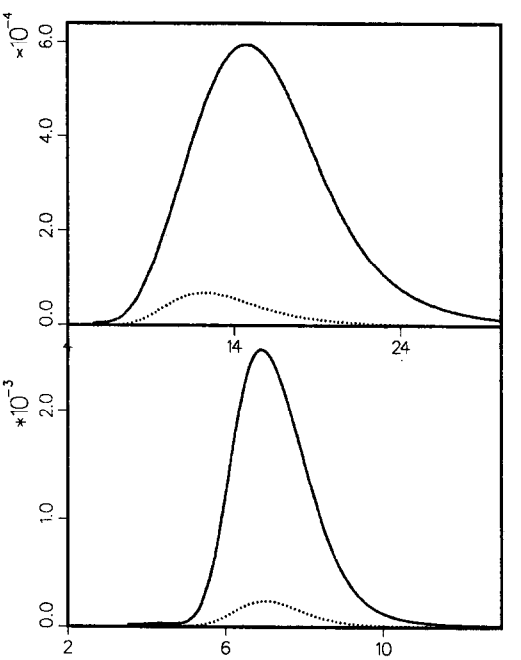


Fig. 11. Elution profile for the (+)-enantiomer at (top) 30 and (bottom) 60°C. Injected sample concentration, 0.06 mol/l; injected volumes, (dotted lines) 5 and (solid lines) 60 μl; flow-rate, 0.5 ml/min. The times of the peak maxima are 12.3 and 14.7 min (30°C) and 7.0 and 6.9 min (60°C).

between this slope and the retention time associated with the concentration. When the concentration increases, a decrease in the isotherm slope with increasing concentration leads to a decrease in the retention time, whereas an increase in this slope causes an increase in the retention time.

To demonstrate the influence of an inflection point in the isotherm on the band profile, we compare in Fig. 11 chromatograms recorded for two samples of different sizes of the (+)-enantiomer, at 30 and 60°C. Obviously, there is a significant increase in the retention time with increasing loading at 30°C. This effect can be fully explained with the shape of the adsorption isotherm in the relevant concentration range (compare Figs. 4–6), and results from the fact that $d^2q/dC^2 > 0$. At 60°C, the retention times of the two samples are very similar and the peaks are nearly symmetrical, illustrating the consequence of an almost linear adsorption isotherm. The possibility of predicting the elution chromatograms of TB on CTA with the equilibrium-dispersive model using the experimental adsorption isotherms was demonstrated previously [15].

The observed differences in the thermodynamic behavior of the two enantiomers studied are limited to a marked change in elution profiles at high concentrations. Their separation factor remains exceptionally constant, around 2. Nevertheless, these results indicate that the experimental conditions for the separation and purification of two isomers by preparative chromatography should be carefully selected. The knowledge of the adsorption isotherms is the most important prerequisite for the optimization of the temperature and the sample size. In the present instance, the selection of the temperature permits a modulation of the intensity of the displacement effect.

SYMBOLS

a	Activity
b_i	Parameter in isotherm eqn. 1 (l/mol)^i
C	Liquid (mobile) phase concentration (mol/l)
F	Phase ratio
ΔG	Molar free energy of adsorption (kJ/mol)
ΔH	Molar enthalpy of adsorption (kJ/mol)
ΔH_{st}	Isosteric heat of adsorption (kJ/mol)
k'	Retention factor
K	Thermodynamic equilibrium constant
N_P	Number of theoretical plates
N_D	Number of data
P	Number of parameters
q	Stationary phase concentration (loading) (mol/l)
q_s	Parameter in eqn. 1, related to the saturation loading (mol/l)
R	Universal gas constant (kJ/mol · K)
ΔS	Molar entropy of adsorption (J/mol · K)
t	Time (s)
t_R	Retention time (s)
t_0	Retention time of a non-retained component (s)

Greek letters

α	Separation factor, $\alpha = k'_2/k'_1$
ε_T	Total porosity

Superscripts

ex	Experimental value
th	Theoretical value

Subscripts

E	At column inlet
m	Mobile phase
s	Stationary phase

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