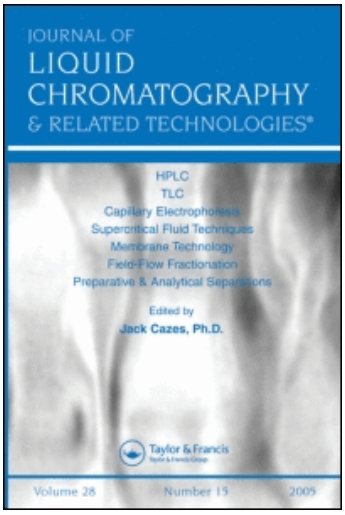

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DETERMINATION OF ADSORPTION ISOTHERMS BY THE INVERSE METHOD WITH A FIRST ORDER REVERSIBLE REACTION OCCURRING IN BOTH PHASES OF A LIQUID CHROMATOGRAPHIC COLUMN

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□ *The determination of rate constants and isotherm parameters by the inverse method is described for a first order reversible reaction. The application of the principle of microscopic reversibility is discussed for competitive isotherms. Experimental data are obtained from the enantiomerization of oxazepam.*

Keywords enantiomerization of oxazepam, inverse method, isotherm determination, reaction kinetics, reaction rate constants, reversible first order reaction

INTRODUCTION

The determination of rate constants from on-column first order reversible reactions, especially enantiomerizations, has been the subject of extensive study, because it can be related to the stability of pharmaceuticals, and some instructive reviews are available.[1,2] Fitting of computer simulated chromatograms, computed on the basis of the plate theory[3] or empirical peak shape equations,[4] as well as direct calculation of the rate constants from a chromatogram equation[5] or deconvolution of the chromatogram by UV and chiroptic detection[6] were successfully applied. Mostly, apparent rate constants were determined in the linear range of the sorption isotherms, i.e., linear combinations of the rate constants in the mobile and in the stationary phase with the retention capacities as weighing factors.

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On the other hand, the determination of non-linear adsorption isotherms has been intensively investigated during the last decades,[7,8] especially for the design of simulated moving bed separations. In particular, the so-called inverse method has been successfully developed, in which the system of partial differential equations governing the chromatographic process is solved numerically, and the solution is fitted to the experimental chromatograms by non-linear regression adjustment of the parameters.[9–13]

The objective of the present paper is to determine isotherm parameters from substances performing reversible first order reactions occurring in both phases of the chromatographic system. This is achieved using the inverse method with reaction terms within the mathematical model and tested on the enantiomerization of oxazepam, a reaction that has been thoroughly studied on column.

Such data might be required for continuous chromatographic reactors,[14] in which recently even the complete transformation of a racemate into a pure enantiomer has been demonstrated.[15] While the usefulness of the inverse method for chromatographic reactors was shown by Stroehlein et. al.[16] this paper is focused on the implications of the principle of microscopic reversibility for possible simplifications of the model.

THEORETICAL

Reaction Kinetic Model and Model of the Chromatographic Process

The evaluation of rate constants and thermodynamic parameters from reaction chromatograms requires a model for the chromatographic process as well as a model for the reaction kinetic process.

In case of enantiomerizations, for the latter, only a few conditions are to be met:

The value of the equilibrium constant amounts to one, the forward and backward rate constant in the mobile phase is the same, and ratios of the respective rate constants in the stationary phase follow from the principle of microkinetic reversibility, which will be discussed later.

For the column model, the equilibrium dispersion model (ED) and the linear driving force model (LDF) were applied.

The equilibrium-dispersion model assumes an equilibrium between the stationary and the mobile phase attributing all peak spreading phenomena to a dispersion process, which is modeled like a diffusion. For a chromatographic process with an enantiomerization, the following

equations are used:

$$\begin{aligned} &D_R\frac{\partial^2 c_R}{\partial x^2} - u\frac{\partial c_R}{\partial x} - k_m(c_R - c_P) - \sum_i k_{iR}a_{iR\infty} \\ &\quad + \sum_i k_{iP}a_{iP\infty} = \frac{\partial c_R}{\partial t} + \left(\frac{\partial c_R}{\partial t}\frac{\partial}{\partial c_R} + \frac{\partial c_P}{\partial t}\frac{\partial}{\partial c_P}\right)\sum_i a_{iR\infty} \\ &D_P\frac{\partial^2 c_P}{\partial x^2} - u\frac{\partial c_P}{\partial x} + k_m(c_R - c_P) + \sum_i k_{iR}a_{iR\infty} \\ &\quad - \sum_i k_{iP}a_{iP\infty} = \frac{\partial c_P}{\partial t} + \left(\frac{\partial c_R}{\partial t}\frac{\partial}{\partial c_R} + \frac{\partial c_P}{\partial t}\frac{\partial}{\partial c_P}\right)\sum_i a_{iP\infty} \end{aligned} \tag{1}$$

The effective equilibrium concentrations at the adsorption centers $a_{i\infty}$ are expressed by the adsorption isotherms:

$$\begin{aligned} a_{iR\infty} &= A_{iR}(c_R, c_P) \\ a_{iP\infty} &= A_{iP}(c_R, c_P) \end{aligned}$$

The linear driving force model (LDF) assumes a non-equilibrium between mobile and stationary phase, where the mass transfer is linearly driven by the difference between the actual and the equilibrium surface concentration:

$$\begin{aligned} &D_R\frac{\partial^2 c_R}{\partial x^2} - u\frac{\partial c_R}{\partial x} - k_m(c_R - c_P) - \sum_i k_{fiR}(a_{iR\infty} - a_{iR}) = \frac{\partial c_R}{\partial t} \\ &\frac{\partial a_{iR}}{\partial t} = k_{fiR}(a_{iR\infty} - a_{iR}) - k_{iR}a_{iR} + k_{iP}a_{iP} \\ &D_P\frac{\partial^2 c_P}{\partial x^2} - u\frac{\partial c_P}{\partial x} + k_m(c_R - c_P) - \sum_i k_{fiP}(a_{iP\infty} - a_{iP}) = \frac{\partial c_P}{\partial t} \\ &\frac{\partial a_{iP}}{\partial t} = k_{fiP}(a_{iP\infty} - a_{iP}) + k_{iR}a_{iR} - k_{iP}a_{iP} \end{aligned} \tag{2}$$

Adsorption Isotherms

In this paper, the following isotherm equations are used:
Langmuir-Isotherm:

$$c_{si} = q_s\frac{K_i c_i}{1 + K_1 c_1 + K_2 c_2}$$

Toth-Isotherm:

$$c_{si} = q_s \frac{K_i c_i}{(1 + (K_1 c_1 + K_2 c_2)^{\nu})^{1/\nu}}$$

Bi-Langmuir-Isotherm:

$$c_{si} = q_{s1} \frac{K_{1i} c_i}{1 + K_{11} c_1 + K_{12} c_2} + q_{s2} \frac{K_{2i} c_i}{1 + K_{21} c_1 + K_{22} c_2}$$

Introducing efficient surface concentrations a and retention capacities

$$a = Fc_s \quad a_S = Fq_s \quad b = Fq_s K$$

the isotherm equations are transformed as follows:

Langmuir-Isotherm:

$$a_i = \frac{b_i c_i}{1 + b_1 \frac{c_1}{a_s} + b_2 \frac{c_2}{a_s}} \tag{3}$$

Toth-Isotherm:

$$a_i = \frac{b_i c_i}{\left(1 + \left(b_1 \frac{c_1}{a_s} + b_2 \frac{c_2}{a_s}\right)^{\nu}\right)^{1/\nu}} \tag{4}$$

Bi-Langmuir-Isotherm:

$$a_i = \frac{b_{1i} c_i}{1 + b_{11} \frac{c_1}{a_{s1}} + b_{12} \frac{c_2}{a_{s1}}} + \frac{b_{2i} c_i}{1 + b_{21} \frac{c_1}{a_{s2}} + b_{22} \frac{c_2}{a_{s2}}} \tag{5}$$

The requirements of microkinetic reversibility and the choice of adsorption isotherms. Schurig, Bürkle and Karfunkel[17] were the first who expressed the importance of including the principle of microkinetic reversibility in the modeling of enantiomerizations in the linear chromatographic mode.

In the nonlinear chromatographic mode, the requirements of microkinetic reversibility will be certainly met, if the adsorption processes are modeled by means of formal kinetics, which is now demonstrated at the case of

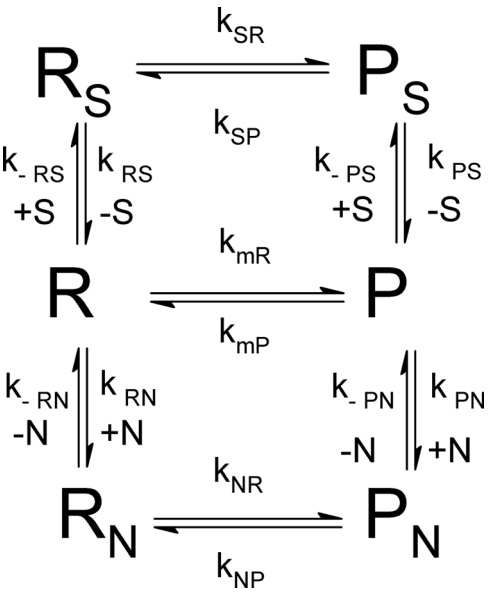

FIGURE 1 Scheme for a bi-Langmuir-adsorption accompanied by an enantiomerization.

a two-phase enantiomerization with a competetive bi-langmuir-isotherm assuming selective (enantiomeric) adsorption centers and non-selective (non-enantiomeric) adsorption centers (Fig. 1).

Then, we have the following "differential rate equations" and balance equations for the concentrations of the adsorption centers:

$$\frac{dc_R}{dt} = -k_{mR}c_R + k_{mP}c_P - k_{-RS}c_S c_R + k_{RS}c_{SR} - k_{-RN}c_N c_R + k_{RN}c_{NR}$$

$$\frac{dc_P}{dt} = -k_{mP}c_P + k_{mR}c_R - k_{-PS}c_S c_P + k_{PS}c_{SP} - k_{-PN}c_N c_P + k_{PN}c_{NP}$$

$$\frac{dc_{SR}}{dt} = k_{-RS}c_S c_R - k_{RS}c_{SR} - k_{SR}c_{SR} + k_{SP}c_{SP}$$

$$\frac{dc_{SP}}{dt} = k_{-PS}c_S c_P - k_{PS}c_{SP} - k_{SP}c_{SP} + k_{SR}c_{SR}$$

$$\frac{dc_{NR}}{dt} = k_{-RN}c_N c_R - k_{RN}c_{NR} - k_{NR}c_{NR} + k_{NP}c_{NP}$$

$$\frac{dc_{NP}}{dt} = k_{-PN}c_N c_P - k_{PN}c_{NP} - k_{NP}c_{NP} + k_{RR}c_{NR}$$

$$c_N = c_{N0} - c_{NR} - c_{NP}$$

$$c_S = c_{S0} - c_{SR} - c_{SP}$$

At equilibrium, all derivations with respect to time become zero leading to the following set of kinetic equilibrium constants:

$$\frac{k_{mR}}{k_{mP}} = \frac{c_P}{c_R} = 1$$
$$\frac{k_{-RS}}{k_{RS}} = \frac{c_{SR}}{c_R c_S}, \quad \frac{k_{-PS}}{k_{PS}} = \frac{c_{SP}}{c_P c_S}, \quad \frac{k_{SR}}{k_{SP}} = \frac{c_{SP}}{c_{SR}}$$
$$\frac{k_{-RN}}{k_{RN}} = \frac{c_{NR}}{c_R c_N}, \quad \frac{k_{PN}}{k_{PN}} = \frac{c_{NP}}{c_P c_N}, \quad \frac{k_{NR}}{k_{NP}} = \frac{c_{NP}}{c_{NR}}$$

By combining the latter, we finally get relations between the entantiomerization rate constants and the partition coefficients or (partial) retention capacities, respectively:

$$\frac{k_{SP}}{k_{SR}} = \frac{\frac{k_{-RS}}{k_{RS}} c_R c_S}{\frac{k_{-PS}}{k_{PS}} c_P c_S} = \frac{\frac{k_{-RS}}{k_{RS} a_{SS}} c_R}{\frac{k_{PS}}{k_{PS} a_{SS}} c_P} = \frac{b_{SR}}{b_{SP}} \frac{c_R}{c_P} = \frac{b_{SR}}{b_{SP}}$$
$$\frac{k_{NP}}{k_{NR}} = \frac{\frac{k_{-RN}}{k_{RN}} c_R c_N}{\frac{k_{-PN}}{k_{PN}} c_P c_N} = \frac{\frac{k_{-RN}}{k_{RN} a_{SN}} c_R}{\frac{k_{-PN}}{k_{PN} a_{SN}} c_P} = \frac{b_{NR}}{b_{NP}} \frac{c_R}{c_P} = \frac{b_{NR}}{b_{NP}} \tag{6}$$

These are the very same relations as in the linear case, as one would expect, because the ratio of the enantiomerization rate constants must be independent of the reactant concentrations, and the adsorption isotherm, on the other hand, must approach the retention capacity in case of low concentrations.

The conclusion is that all adsorption isotherms are suitable, the ratio of which is equal to the ratio of the linear retention capacities, bearing in mind that in enantionmerizations the ratio of the mobile phase concentrations is 1. In multi-center isotherms this rule has to be applied separately for each of the centers.

To our knowledge, no noncompetetive isotherms do fulfill this condition, i.e., the simple Langmuir isotherm yields

$$\frac{\frac{b_1 c_1}{1+b_1\frac{c_1}{a_s}}}{\frac{b_2 c_2}{1+b_2\frac{c_2}{a_s}}} \neq \frac{b_1 c_1}{b_2 c_2}$$

whereas the Toth isotherm (5) as a competitive isotherm fulfills the requirements:

$$\frac{\frac{b_1 c_1}{(1+(b_1 c_1+b_2 c_2)^\nu)^{\frac{1}{\nu}}}}{\frac{b_2 c_2}{(1+(b_1 c_1+b_2 c_2)^\nu)^{\frac{1}{\nu}}}} = \frac{b_1 c_1}{b_2 c_2}$$

Parametric Studies. In enantiomerizations, it is expected that the reactant/product peak area ratio remains 1, if the inlet reactant/product peak area ratio is 1, i.e., if a racemat is injected.

However, independently of the column model, we found that there are slight deviations from the expected ratio which did not disappear by increasing the number of finite elements, interior collocation points or decreasing the time intervals in the numerical calculations. (cf. Table 1).

This will be discussed now with respect to the ED-model with a competitive Langmuir-isotherm (5).

Explicitely (which is necessary to discuss the consequences), the model equations (1) can be rewritten for this case:

$$D\frac{\partial^2 c_R}{\partial x^2} - u\frac{\partial c_R}{\partial x} - k_m(c_R - c_P) - k_{NR}a_{NR\infty} + k_{NP}a_{NP\infty} - k_{SR}a_{SR\infty} + k_{SP}a_{SP\infty} = \frac{\partial c_R}{\partial t} + \frac{\partial a_{NR\infty}}{\partial t} + \frac{\partial a_{SR\infty}}{\partial t}$$

$$D\frac{\partial^2 c_P}{\partial x^2} - u\frac{\partial c_P}{\partial x} + k_m(c_R - c_P) + k_{NR}a_{NR\infty} - k_{NP}a_{NP\infty} + k_{SR}a_{SR\infty} - k_{SP}a_{SP\infty} = \frac{\partial c_P}{\partial t} + \frac{\partial a_{NP\infty}}{\partial t} + \frac{\partial a_{SP\infty}}{\partial t} \tag{7}$$

Integrating all equation terms via $\int_0^\infty dt$, the equations (7) become

$$D\frac{\partial^2 m_R}{\partial x^2} - u\frac{\partial m_R}{\partial x} - k_m(m_R - m_P) - k_{NR}m_{aNR} + k_{NP}m_{aNP} - k_{SR}m_{aSR} + k_{SP}m_{aSP} = 0 \tag{8}$$

$$D\frac{\partial^2 m_P}{\partial x^2} - u\frac{\partial m_P}{\partial x} + k_m(m_R - m_P) + k_{NR}m_{aNR} - k_{NP}m_{aNP} + k_{SR}m_{aSR} - k_{SP}m_{aSP} = 0$$

TABLE 1 Results from the Simulation of Enantiomerization Chromatograms due to the ED-model using the Following Parameters: $t_0 = 1$, $N_R = N_P = 1000$, $b_{NR} = 0.8$, $b_{SR} = 0.2$, $b_{NP} = 0.8$, $b_{SP} = 1.2$, $Km = 1$, $a_{SN} = 5$, $a_{SS} = 5$, $m_{R0} = m_{P0} = 0.5^*$; Data calculated with parameters a_{SN} and a_{SS} altered to $a_{SN} = 2$, $a_{SS} = 2$

k_m	k_S	k_N	m_R	m_P	$m_R + m_P$
0	0	0	0.500	0.500	1.000
1	0	0	0.500	0.500	1.000
0.1	0.5	0.875	0.495	0.505	1.000
0.1*	0.5*	0.875*	0.488*	0.512*	1.000*

And, by addition of (8) with each other, it can easily be seen that $\frac{\partial(m_R+m_P)}{\partial x} = 0$, which means that $(m_{R0}+m_{P0}) = (m_R+m_P)$, as it should be in any case.

If we assume a Langmuir isotherm for the respective j-th adsorption center

$$a_{ji\infty} = \frac{b_{ji}c_i}{1 + b_{jR}\frac{c_R}{a_{sj}} + b_{jP}\frac{c_P}{a_{sj}}}$$

this can be rearranged to yield

$$a_{ji\infty} + b_{jR}\frac{c_R a_{j\infty}}{a_{sj}} + b_{jp}\frac{c_P a_{ji\infty}}{a_{sj}} = b_{ji}c_i \tag{9}$$

Any of the time dependent concentrations – i.e., all exept a_{sj} - can be expressed by

$$c = m\Psi \text{ and } a = m_a\Psi,$$

and this is applied to the left side of the aforementioned equation, which is then integrated via $\int_0^\infty dt$. After another rearrangement, the following "area isotherms" are produced:

$$m_{aNR} = \frac{b_{NR}m_R}{1 + b_{NR}\frac{m_R}{a_{SN}}\int_0^\infty \Psi_{mR}\Psi_{NR}dt + b_{NP}\frac{m_P}{a_{SN}}\int_0^\infty \Psi_{mP}\Psi_{NR}dt}$$

$$m_{aNP} = \frac{b_{NP}m_P}{1 + b_{NR}\frac{m_R}{a_{SN}}\int_0^\infty \Psi_{mR}\Psi_{NP}dt + b_{NP}\frac{m_P}{a_{SN}}\int_0^\infty \Psi_{mP}\Psi_{NP}dt}$$

$$m_{aSR} = \frac{b_{SR}m_R}{1 + b_{SR}\frac{m_R}{a_{SS}}\int_0^\infty \Psi_{mR}\Psi_{SR}dt + b_{SP}\frac{m_P}{a_{SS}}\int_0^\infty \Psi_{mP}\Psi_{SR}dt} \tag{10}$$

$$m_{aSP} = \frac{b_{SP}m_P}{1 + b_{SR}\frac{m_R}{a_{SS}}\int_0^\infty \Psi_{mR}\Psi_{SP}dt + b_{SP}\frac{m_P}{a_{SS}}\int_0^\infty \Psi_{mP}\Psi_{SP}dt}$$

While because of the principle of microkinetic reversibility (cf. Eqn. 6)

$$k_{NR}b_{NR} = k_{NP}b_{NP}$$
$$k_{SR}b_{SR} = k_{SP}b_{SP}$$

the denominators of the "area isotherms" (10) terms are not equal, and therefore

$$k_{NR} m_{aNR} \neq k_{NP} m_{aPR}$$
$$k_{SR} m_{aSR} \neq k_{SP} m_{aSP}$$

which means that, if there occurs a reaction in the stationary phase and if there is separation, then differences in the peak area must occur. In short, this seems to be a consequence of the fact that in nonlinear chromatography the ratio of substance in the mobile phase and the stationary phase does not remain constant along the length of the column.

However, due to the fact that the reaction peaks are very broad, the differences occur only if high sample concentrations are applied and remain small even then.

EXPERIMENTAL

Chromatograms from the enantiomerization of oxazepam were recorded at 300 nm using the DGS 20 P binary gradient system with variable wavelength detector and DIV-1 injection valve from D-Star Instruments (Manassas, USA), which was equipped with a K7 column oven from Techlab (Germany) and a ChiraDex 3×125 mm column from Merck (Darmstadt, Germany). A methanol-water eluent (40/60,v/v) was used with a 0.01 M TEAA buffer (pH 4.5). Methanol, TEA, acetic acid and water were purchased from Sigma Aldrich (Germany) as HPLC-grade substances, Oxazepam racemate with a purity >98% was also obtained from Sigma Aldrich (Germany).

100 µL injections were made ranging from 0.00625 g/dm^3 to 0.25 g/dm^3 oxazepam in the eluent including buffer. Attempts to use 0.5 g/dm^3 solutions failed since the chromatograms became too broad, and the solutions turned out to be oversaturated.

Flow rates of 0.3, 0.5 and 0.7 cm^3/min and temperatures of 15, 20, 25 and 30°C were applied.

RESULTS AND DISCUSSION

The determination of the model parameters was done by fitting chromatograms computed by the method of orthogonal collocation on finite elements (OCFE) with 50 elements and 5 interior collocation points to the experimental chromatograms.[18,19] As recommended by Seidel-Morgenstern[7] – the retention capacities were evaluated from

measurements in the linear isotherm range, i.e., at 0.00625 g/dm^3. However, since the injector loop is already quite large with respect to a 3×125 mm column, the injection profile was not anymore a rectangular plug, but an exponentially decaying pulse. The apparent rate constants (cf. Table 2) which were also obtained from these measurements, are in good agreement to those published by Cabrera et al.[20] and Lange et al.[21]

Then, the remaining isotherm parameters were numerically evaluated from the chromatograms of the 0.25 g/dm^3 series using different isotherm models.

The Fisher parameter[23]

$$F = \frac{(n-p)\sum (c_{\text{exp}} - \overline{c_{\text{exp}}})^2}{(n-1)\sum (c_{num} - c_{\text{exp}})^2}$$

(n = Number of points, p = number of parameters, c_{num}: simulated concentrations, c_{exp}: experimental concentrations) was also obtained to quantify the goodness of fit.

The first attempt to evaluate those parameters was based on the ED model with a bi-Langmuir isotherm (cf. Eqns. (1), (5)). This, however, led to unreasonable large dispersion coefficients.

Therefore, the LDF-model (Eq.(2)) was used with a competitive Langmuir isotherm (F = 39), a competitive Toth-isotherm (F = 60) and a competitive bi-Langmuir (F = 69) isotherm.

Though the Bi-Langmuir-isotherm yields the highest F-value, it is still comparable with the Toth-isotherm. Therefore, with the available experimental information, the Toth isotherm seems to be the appropriate model for the column adsorption process. Fig. 2 shows the best and the worst fit for the 0.25 g/dm^3 series.

TABLE 2 Thermodynamic and Reaction Kinetic Parameters from the Oxazepam Enantiomerization using the Toth Isotherm

T (°C)	b_1	σ_{b1}	b_2	σ_{b2}	k_{app} min^{-1}
15	2.46E + 00	1.43E-03	1.39E + 00	2.31E-03	4.72E-02
20	2.15E + 00	1.29E-03	1.24E + 00	2.07E-03	9.19E-02
25	1.95E + 00	1.12E-01	1.14E + 00	1.94E-01	1.76E-01
30	1.69E + 00	1.91E-01	1.03E + 00	2.95E-01	3.15E-01
T°C	σ k_{app} min^{-1}	a_S µmol/mL	σ_{aS} µmol/mL	ν	σ_ν
15	6.77E-02	2.51E00	1.4E-01	8.00E-01	8.53E-02
20	3.44E-02	2.62E00	2.6E-01	7.89E-01	8.53E-02
25	1.90E-02	2.72E00	2.5E-01	8.52E-01	8.79E-02
30	9.44E-02	3.40E00	10E-01	7.29E-01	2.79E-02

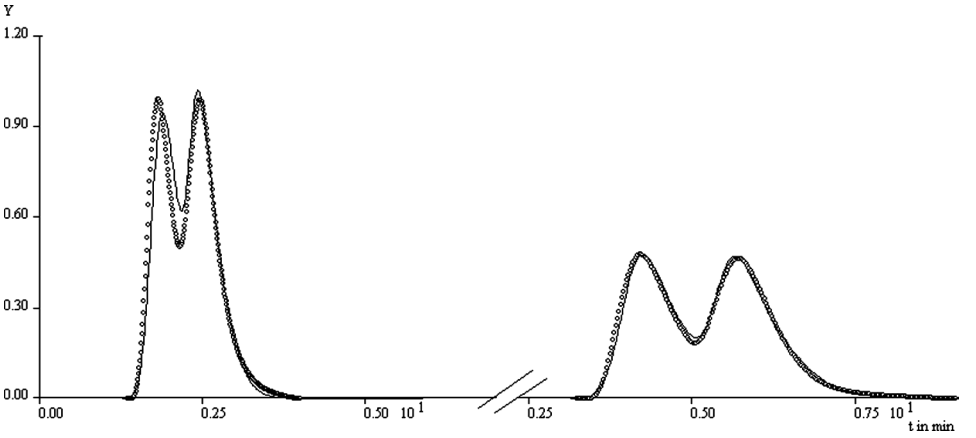

FIGURE 2 Worst and best fit of reaction chromatograms from the oxazepam enantiomerization using the Toth isotherm.

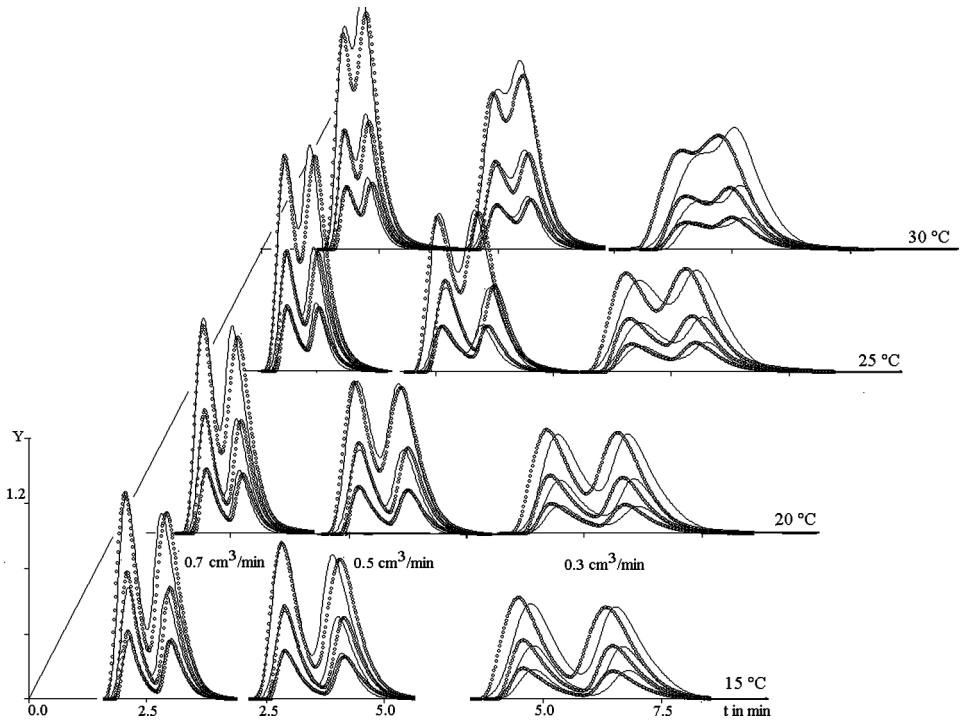

FIGURE 3 Simulation of oxazepam enantiomerization chromatograms (0.0625 g/L, 0.125 g/L and 0.25 g/L) using the averaged date from Table 2 (Lines: simulated chromatograms, circles: experimental chromatograms).

The retention capacities and the apparent rate constants, which exhibit Nernst or Arrhenius behavior, respectively, were averaged over the flow rates. The efficient saturation concentrations and the Toth parameters, which do not exhibit an unequivocal temperature dependence, where averaged over the whole series (cf. Table 2), and chromatograms were calculated by OCFE for the 0.0625 g/dm^3, the 0.125 g/dm^3 and the 0.25 g/cm^3 series (cf. Fig. 3).

CONCLUSIONS

The determination of adsorption isotherms from substances carrying out an on-column reversible first order reaction requires the application of competitive adsorption isotherm equations. The nonlinear isotherms lead to small deviations in the 1:1 composition of reaction chromatograms from enantiomerizations.

The inverse method was used for the determination of isotherm parameters from enantiomerization chromatograms of oxazepam. Problems were occurring from an insufficient solubility of the samples, which were circumvented by determining as many parameters as possible under linear (diluted) adsorption conditions. The large errors in the parameters are at least partially due to the necessary volume overloading. Nevertheless, a reasonable simulation of the experimental chromatograms was possible.

SYMBOLS

a	effective concentration (stationary phase)
a_s	effective saturation concentration
b	retention capacity
c	concentration
c_s	surface concentration
D	dispersion coefficient
F	phase ratio
K	adsorption isotherm parameter
k	rate constant
k_f	mass transfer coefficient
m	zeroth moment (concentration-time area)
q	surface saturation concentration
t	time
u	linear flow rate
x	spatial coordinate
ν	Toth heterogeneity parameter
Ψ	peak shape density function

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