Adsorption of naproxen enantiomers on chemically modified cellulose

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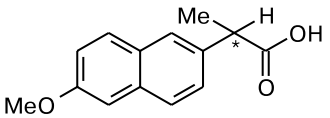
Adsorption of naproxen enantiomers on the Chiralcel OJ-H chiral stationary phase, being tris(4-methylbenzoate) cellulose coated on silica gel, was studied. The thermodynamic characteristics of adsorption were measured. The adsorption isotherms are described by the Langmuir equation within the concentration range under study.

Key words: enantioselective adsorption, enantiomers, naproxen, chiral stationary phase, nonsteroidal anti-inflammatory drugs.

The understanding of mechanisms of enantioselective adsorption plays an important role in search for methods of chromatographic isolation of single enantiomers, which are necessary for the preparation of enantiomerically pure chiral pharmaceuticals.[1] Studies in this area have been extended considerably after the systems with the simulated moving bed (SMB) of the adsorbent were developed. These systems turned out to be very efficient for the separation of racemic mixtures.[2] In the present work, the regularities of adsorption in the chiral systems were studied under the conditions of normal-phase liquid chromatography using naproxen (**1**) as an example. Naproxen is a representative of the class of profens (derivatives of 2-arylmethylpropionic acid). Practical significance of compounds of this group is due to their wide use as nonsteroidal anti-inflammatory drugs. Of the two optical isomers of profens, almost only *S*(+)-enantiomers are characterized by high pharmacological activity,[3,4] whereas the *R*(−)-form even can result in undesirable side effects.[5]



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The majority of works on the enantioselective chromatographic separation of profens is devoted to analytical separation,[6—16] *i.e.*, separation of dilute solutions under the conditions corresponding to the linear region of the adsorption isotherm. These studies help to reveal most promising chiral stationary phases (CSPS) and provide better understanding of the mechanism of interaction of optically active adsorbates with a chiral selector. However, a concept on the macroscopic mechanism of adsorption can be obtained only on the basis of analysis of adsorption isotherms. Works devoted to detailed investigation of enantioselective adsorption under the nonlinear chromatography conditions are rare. The binary adsorption isotherms of the ketoprofen enantiomers on a modified cellulose were measured and the elution curves were calculated within the framework of detailed investigation of the preparative separation of the enantiomers.[17,18] The semipreparative separation of pairs of enantiomers of two 2-arylpropionic acids on the glycopeptide CSP was described.[19] The results of the semipreparative enantioseparation of naproxen using the Whelk-O1 stationary phase without measuring the adsorption isotherms were published.[20] The separation of the ibuprofen enantiomers by the SMB method was studied in both supercritical fluid[21] and standard[22] modes.

In the present work, we studied the adsorption of profens in the high-concentration range and obtained the data necessary for the development of an adequate adsorption model. These data are important for the development and optimization of separation on an industrial scale.[23] The chiral adsorbent was tris(4-methylbenzoyl) cellulose coated on silica gel (Chiralcel OJ-H). A similar column was used to separate ketoprofen in cited works,[17,18] and its choice was caused by the high loading capacity and mass transfer rate.[17,18,24] It was of interest to compare adsorption of these two profens on the same CSP.

Experimental

Experimental data were obtained on an Agilent 1100 liquid chromatograph with a diode-matrix detector, which was equipped with a thermostat of columns, an automatic sample injector, and a pump with a low-pressure gradient control device. Measurements were carried out on a Chiralcel OJ-H column (250×4.6 mm, Daicel). A hexane—ethanol (80 : 20)

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mixture with an additive of trifluoroacetic acid (TFA, 0.1 vol.%) served as the eluent. The eluent was supplied by a gradient control device consisting of two vessels, one of which was filled with a TFA acid solution in hexane and another contained ethanol. This system provided reproducibility of the eluent composition in all experiments. The flow rate of the eluent was 0.985 $cm^3\ min^{-1}$ and was monitored by filling a volumetric flask (50 cm^3). Hexane (analytically pure grade) and the naproxen enantiomers (Sigma Aldrich) were used as received, TFA (pure grade) was purified by distillation, and ethanol (highest purification grade) was dried by distillation over CaO.

Adsorption isotherms were determined by the modified Gluekauf method[25] at 22 °C. The measurements were carried out with stock solutions of the enantiomers with concentrations of 0.42 and 4.1 $g\ L^{-1}$, the sample volume was ranged from 5 to 50 μL, and each measurement was repeated at least three times.

Initial retention characteristics were determined at 15, 22, 30, and 40 °C. For this purpose, solutions of the enantiomers with the concentration 0.021 $g\ L^{-1}$ and sample volume 1 μL were used. It was found in preliminary experiments that a tenfold increase in the concentration does not change the retention time. This proves that the measurements were carried out in the linear region of the adsorption isotherm. The dead volume was determined from the elution of 1,3,5-tri-*tert*-butylbenzene.[26,27] The extra-column volume, determined from the elution time of the sample in the system without a column, was 0.042 cm^3.

Computer simulation. Elution curves were calculated by the equilibrium—dispersion model of chromatography using the mass balance equation[28]

$$\frac{\partial c(z,t)}{\partial t} + F\frac{\partial q(z,t)}{\partial t} + u\frac{\partial c(z,t)}{\partial z} = D_a\frac{\partial^2 c(z,t)}{\partial z^2}, \qquad (1)$$

where c and q are the concentrations of the adsorbate in the mobile and stationary phases, respectively, which are the functions of the coordinate along the column (z) and time (t); F is the phase ratio, u is the linear velocity of the mobile phase, D_a is the apparent axial dispersion coefficient related to the number of theoretical plates N as $D_a = Lu/(2N)$ (L is the column length). The c and q values are related by the adsorption isotherm equation $q = f(c)$, providing an instant equilibration in each point of the column at any moment. The initial and boundary conditions should be specified to solve the system of equations. The initial condition $c(z,0) = 0$, $q(z,0) = 0$ implies that at the moment $t = 0$ the column contains the pure mobile phase. The assumption that the sample was introduced as a rectangular impulse served as the boundary condition at the inlet of the column:

$$c(0,t) = \begin{cases} c^0, & \text{if } 0 < t \le t_{imp}, \\ 0, & \text{if } t > t_{imp}, \end{cases}$$

where c^0 is the concentration of the introduced sample, and t_{imp} is the duration of introduction (impulse). The boundary condition at the outlet of the column at $t > 0$ and $z = L$ is $\partial c/\partial z = 0$.

When using the equilibrium—dispersion model, it is assumed that all factors affecting the dispersion of the chromatographic peak are taken into account by the dispersion coefficient. This is a substantial simplification. Nevertheless, the numerical studies[28—30] on the simulation of the chromatographic process demonstrated that the approach described can be applied when the regularities of mass transfer are not interesting but it is important to determine only the adsorption isotherm. Particularly, the equilibrium—dispersion model was used in the study of the enantioselective adsorption of ketoprofen[17,18] and methyl mandelate[26] on the Chiralcel OJ CSP.

Equation (1) was solved by the orthogonal collocation method on finite elements. The method has earlier been described in detail.[31,32]

Results and Discussion

Measurements in the linear region. The temperature plots of the initial retention factor (k') for the both enantiomers are straight lines in the coordinates of the van´t Hoff equation (Fig. 1), which for k' is written as follows:

$$\ln(k'/F) = -\Delta H^\circ/(RT) + \Delta S^\circ/R, \qquad (2)$$

where ΔH° and ΔS° are the standard enthalpy and entropy of adsorption, R is the universal gas constant, T is the temperature, and F is the ratio of volumes of the stationary and mobile phases in the column (in this work, $F = 0.43$). The standard thermodynamic characteristics of adsorption calculated by Eq. (2) are given in Table 1. These data show that the differences in the thermodynamics of interaction of the enantiomers with the CSP under study are low. Nevertheless, they provide almost complete separation of the enantiomers. This is illustrated in Fig. 2 showing the chromatograms of racemic mixtures of the enantiomers obtained at different temperatures.

Nonlinear measurements. The adsorption isotherms (Fig. 3) demonstrate weak nonlinearity. The adsorption of the ketoprofen enantiomers on the Chiralcel OJ CSP, which is identical to the Chiralcel OJ-H sorbent by the type of chiral selector, has been studied previously.[17] A hexane—isopropanol—acetic acid (90 : 10 : 0.5) mixture was used as the mobile phase. The authors[17] succeeded to study the adsorption equilibrium in a wide concentration range due to the use of the frontal method.

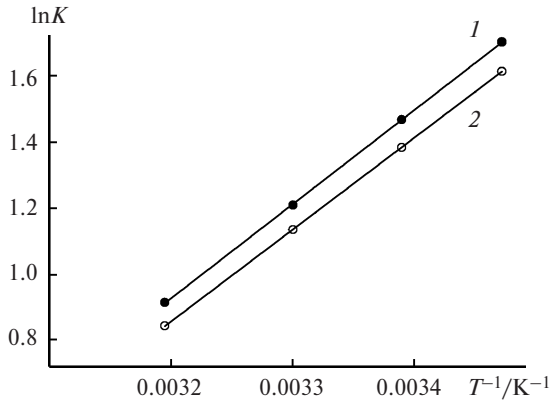

Fig. 1. Temperature plots of the logarithms of the retention factor for (*R*)- (*1*) and (*S*)-naproxen (*2*).

Table 1. Standard enthalpy and entropy of adsorption of naproxen enantiomers

Enantiomer	$-\Delta H^\circ$ /kJ mol^{-1}	$-\Delta S^\circ$ /J mol^{-1} K^{-1}	r*
S	17.9	54.4	0.99999
R	18.4	55.3	0.99998

* r is the correlation coefficient.

The obtained adsorption isotherms were nonlinear and described by the bi-Langmuir isotherm equation, which is the sum of two Langmuir terms and corresponds to the model of the surface bearing two types of adsorption sites.[28] It was assumed[17] that one group of the surface sites is nonselective, whereas the surface sites of another group are capable of enantioselective interaction, due to which optical isomers are separated.

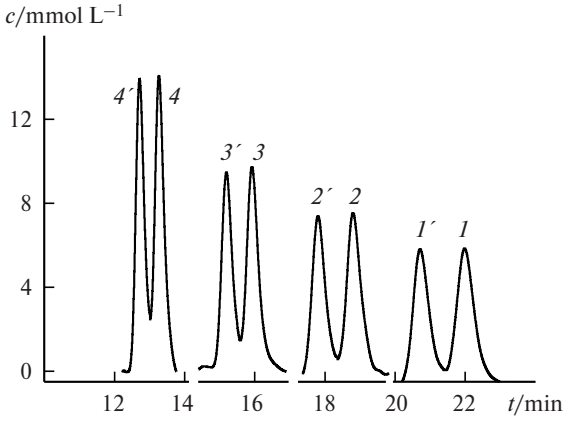

Fig. 2. Chromatograms of a racemic mixture of (*R*)- (*1*—*4*) and (*S*)-enantiomers of naproxen (*1´*—*4´*) at 15 (*1*, *1´*), 22 (*2*, *2´*), 30 (*3*, *3´*), and 40 °C (*4*, *4´*). The concentration of each enantiomer is 0.021 g L^{-1}, and the sample volume is 1 μL.

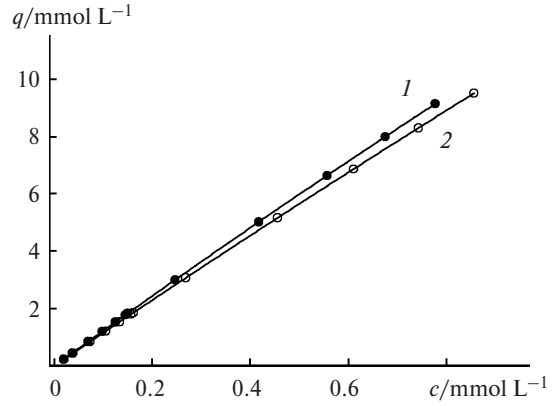

Fig. 3. Adsorption isotherms of (*R*)- (*1*) and (*S*)-naproxen (*2*). Points are experimental data, and lines are approximations by the Langmuir equation; in both cases, the determination coefficient is $r^2 > 0.9(9)_5$.

We failed to reliably approximate the obtained experimental results using the four-parametric model (bi-Langmuir), because the best fit coefficients turned out to be statistically insignificant. Evidently, the reason is that the range of equilibrium concentrations studied in this work is relatively narrow.

At the same time, the plots in Fig. 3 are well approximated by the Langmuir isotherm equation, whose coefficients are given in Table 2. However, the type of the model equation cannot unambiguously be determined from the experimental points of the isotherm, because one can propose several expressions describing the experimental data with equal statistical accuracies. Therefore, additional criteria of adequacy for the model are needed. Some researchers use the numerical simulation of elution curves for the verification of the model.[28] Since not only the values of the isotherm function in each calculated curve but also the first derivative values should coincide for the theoretical and experimental chromatograms to coincide, this method is more sensitive to the isotherm type than the approximation method in which only coincidence of function value is needed. The chromatograms of the enantiomers are compared in Fig. 4 with the results of numerical simulation using the Langmuir isotherm and the coefficients from Table 2. A good agreement is seen, which proves applicability of the accepted adsorption model in the specified concentration interval. At the same time, the q* coefficients do not coincide for the both enantiomers, which means, possibly, the presence of the group of sites inaccessible for one of the enantiomers. In our opinion, the explanation associated with the understanding of the constants of the isotherms as apparent coefficients seems more probable. In fact, the surface of the polysaccharide CSP is heterogeneous[33] and characterized by a certain energy distribution among adsorption sites. Then the q* and b parameters will be average weighed values for the corresponding distribution functions $q^*(E)$ and $b(E)$, where E is the adsorption site energy. Based on the results on ketoprofen,[17] we can assume that the distribution function is bimodal for naproxen as well. At the same time, for elution chromatography the peak shape is predicted with a high accuracy in the framework of the model of the surface containing one type of adsorption sites under the condition that the concentrations of these sites differ for different enantiomers.

Table 2. Coefficients of the Langmuir adsorption isotherm[a]

Enantiomer	q*/mmol L^{-1}	b/L mmol^{-1}
S	221±2	0.0525±0.0004
R	189±2	0.0653±0.0008

[a] $q = q^*bc/(1 + bc)$, where q* is the concentration of adsorption sites, and b is the adsorption coefficient.

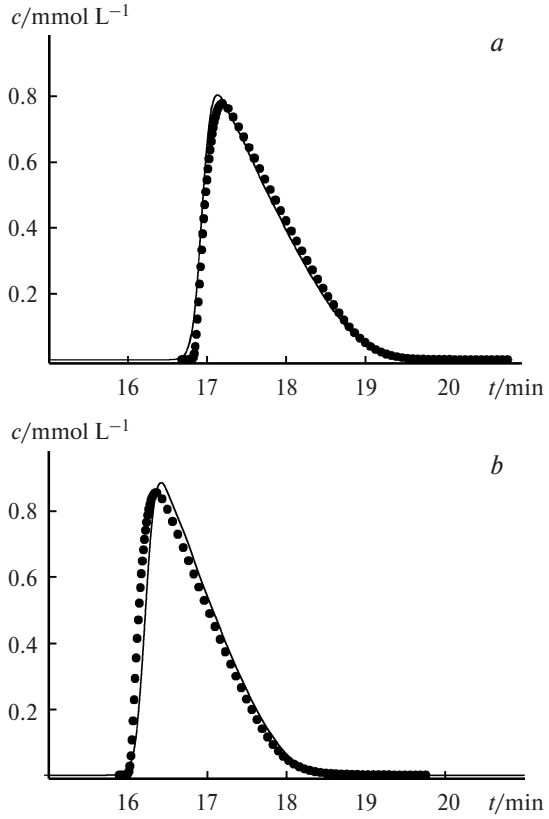

Fig. 4. Comparison of the experimental (points) and calculated (line) chromatograms of (*R*)- (*a*) and (*S*)-naproxen (*b*). The sample concentration is 4.1 g L^{-1}, and the volume is 50 μL.

Thus, adsorption of the naproxen enantiomers on the Chiralcel OJ-H CSP from a hexane—ethanol solution with a TFAA additive in the region of adsorbate concentrations ≤0.8 mmol L^{-1} is satisfactorily described by the Langmuir adsorption isotherm, which is confirmed by the results of simulation of the chromatograms. Therefore, enantioselective interactions do not modify the formal adsorption model but only change its numerical parameters. Note that the apparent saturation adsorption capacity is lower for the more strongly retained enantiomer and the observed order of elution is provided by the difference in the adsorption coefficients. According to the moderate values of the adsorption enthalpies and an insignificant difference between them, the enantiorecognition mechanism is not related to any certain type of the specific interaction (electrical orientation, hydrogen bonding, π—π interaction) between naproxen and modified cellulose and is determined by steric factors.

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