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Physical Chemistry

The Effect of Temperature on the High-Performance Liquid Chromatographic Separation of Enantiomers on Covalently Immobilized Polysaccharide-Based Chiral Stationary Phase

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In the present work the effect of temperature on high-performance liquid chromatographic separation of ketoprofen and zaltoprofen enantiomers was studied. Thermodynamic parameters, such as Gibb's free energy, the standard molar entropy and the standard molar enthalpy of analyte transfer from the mobile to the stationary phase were calculated. The same thermodynamic quantities responsible for separation of enantiomers were also evaluated. In the case of the studied compounds, both the adsorption and separation were favored by enthalpic term and disfavored by entropy. © *2023 Bull. Georg. Natl. Acad. Sci.*

cellulose tris(3-chloro-5-methylphenylcarbamate), chiral selector, enantiomers, enthalpy, entropy, Gibb's free energy, mobile phase, thermodynamic parameters

The role of temperature in separation of enantiomers is very important. The methodology for calculating thermodynamic quantities of analyte transfer from the liquid to the solid phase based on the temperature dependence of analyte retention and separation selectivity is well established and shortly summarized below [1-10]. Temperature dependence of retention has been used for the calculation of standard molar Gibbs´s energy (ΔG0), the standard molar enthalpy (ΔH0) and the standard molar entropy (ΔS0) of solute transfer from the mobile to the stationary phase according to the Gibbs-Helmholtz and Van't Hoff´s equations

$$\Delta G^0 = \Delta H^0 - T\Delta S^0 = -RT\ln K, \qquad (1)$$

where T is the absolute temperature, R is the universal gas constant and K is the thermodynamic equilibrium constant. The relationship between the equilibrium constant K and the retention factor of analyte k in chromatography is expressed with Eq. (2) as follows:

$$k = K\phi, \qquad (2)$$

where ϕ represents the phase ratio of the column, calculated by the volume of the stationary phase divided by the volume of the mobile phase in the column. The latter factor equals the void volume of the column.

The Van't Hoff equation can be used to determine the thermodynamic quantities of analyte transfer from a mobile to a stationary phase.

$$\ln k = -\frac{\Delta H^0}{R} \cdot \frac{1}{T} + \frac{\Delta S^0}{R} + \ln \phi. \qquad (3)$$

The differences between the molar enthalpy of phase transfer of two enantiomers of a given chiral compound, as well as between the molar entropy of phase transfer of the same enantiomers are conveniently obtained by plotting the natural logarithm of the separation factor $\alpha = \frac{K_S}{K_R}$ (arbitrary considering KS > KR), versus the reciprocal of the absolute temperature T [1,4,5].

$$\ln \alpha = -\frac{\Delta_{S,R}\Delta H^0}{R} \cdot \frac{1}{T} + \frac{\Delta_{S,R}\Delta S^0}{R}, \qquad (4)$$

(ΔS,RΔH0) and (ΔS,RΔS0) are the differences between the standard molar enthalpy and the standard molar entropy of phase transfer between the enantiomers, respectively.

At a certain temperature, so called the isoenantioselective temperature (Tiso), the enthalpic and entropic terms compensate each other:

$$\Delta_{S,R}\Delta G^0 = 0 \qquad (5)$$

and the enantiomers are not separated.

$$T_{iso} = \frac{\Delta_{S,R}\Delta H^0}{\Delta_{S,R}\Delta S^0}. \qquad (6)$$

Although widely applied, following limitations of this approach have to be mentioned: 1) van't Hoff´s approach was developed for a true equilibrium and in chromatographic process we do not have a true but just a pseudo-equilibrium; 2) ΔG0 is applicable to isobaric system at a constant temperature. Its analogue for the isochoric system at a constant temperature is the standard Helmholtz free energy F. However, a chromatographic column represents an open system with pressure gradient. Therefore, it is neither isobaric nor isochoric [8, 11-15]; 3) On chiral stationary phase (CSP) there are at least two kind of adsorption sites (nonenantioselective and enantioselective) [8, 14, 15]. Therefore, thermodynamic characteristics determined from van't Hoff's approach provide some overall information and not the true thermodynamic quantities for each kind of adsorption site (nonenantioselective and enantioselective) separately. Despite of these disadvantages, the overall thermodynamic quantities derived from van't Hoff´s approach provide reasonable ideas regarding the chiral recognition mechanism, help explain some uncommon experimental observations, and have a certain practical value, too.

In the present study the temperature dependence of retention (k) and of separation factor (α) was studied for ketoprofen and zaltoprofen enantiomers on polysaccharide based chiral column with covalently immobilized cellulose tris(3-chloro-5-methylphenylcarbamate) chiral selector. Based on these measurements the thermodynamic quantities for enantiomer adsorption from a liquid to a solid phase, as well as for separation of enantiomers were calculated.

Materials and Methods

Chemicals. The chiral test compounds, racemic ketoprofen and racemic zaltoprofen, as well as (S)-(+)-ketoprofen and (S)-(+)-zaltoprofen were commercially available from Sigma-Aldrich (St. Louis, MI, USA). Formic acid and HPLC quality solvents, such as propan-2-ol and n-hexane were acquired from Carl Roth (Karlsruhe, Germany).

Chiral column. Cellulose tris(3-chloro-5-methylphenylcarbamate) covalently immobilized on silica (with a nominal particle diameter 5 micron and nominal pore diameter 100 nm) was an experimental column made in our laboratory. The schematic structures of cellulose tris(3-chloro-5-methylphenylcarbamate) is shown in Fig. 1. This chiral selector was prepared by reacting microcrystalline cellulose with 3-chloro-5-methylphenylisocyanate in dry pyridine as described in [16]. Column had the dimensions 4.6 × 250 mm. Chiral selector was covalently immobilized onto the surface of silica by using a proprietary photochemical method.

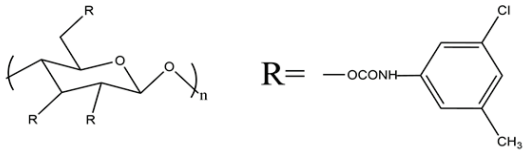


Fig. 1. Schematic structure of cellulose tris(3-chloro-5-methylphenylcarbamate) used as chiral selector in this study.

High-Performance liquid chromatography. HPLC experiments were performed with an Agilent 1200 HPLC instrument (Agilent Technologies, Waldbronn, Germany) equipped with a G1367C HiP ALS-SL autosampler, a G1316B TCC-SL temperature controller, G1311A quaternary pump, and G1314D VWD variable wavelength detector. The Chemstation software (version B.03.02-SR2) was used for instrument control, data acquisition, and data handling. If not stated otherwise, samples were dissolved in the mobile phase used for each respective separation at a concentration of 0.2 mg/mL. HPLC separations were performed with 1 mL/min mobile phase flow rate, while UV detection was performed at 254 nm, the temperature range was 10°C to 75°C with the 5° steps.

Results and Discussion

Two arylpropionic acid derivatives, which are non-steroidal anti-inflammatory drugs, namely ketoprofen and zaltoprofen, were selected for these experiments. Chromatograms of spiked mixture of enantiomers of both analytes at 10 and 75°C are shown on Fig. 2. As it can be seen, both enantiomers co-eluted at higher temperature but no reversal of enantiomer elution order is observed in the studied temperature range. The dependences of lnk vs. inverse temperature (1/T) were linear with high regression coefficients for both enantiomers of both analytes. This indicates that the adsorption mechanism does not change significantly within studied temperature range. The thermodynamic quantities derived from these dependences are summarized in Table. Based on these quantities one can conclude that a transfer of both enantiomers of both analytes from the mobile to the stationary phase is an exothermic (enthalpy driven) process that is disfavored by the entropy loss.

Based on the graphs of the natural logarithm of the separation factor versus the inverse absolute temperature (data not shown). the differential values of separation enthalpy and entropy for enantiomers can be calculated.

As it can be seen from Fig. 3 this dependence cannot be described with a single line for any of studied 2 compounds. This means that some kind of change in conformation of chiral analytes or chiral selector occurs at higher temperatures. Based on this behavior two sets of thermodynamic quantities were derived as they are represented in Table.

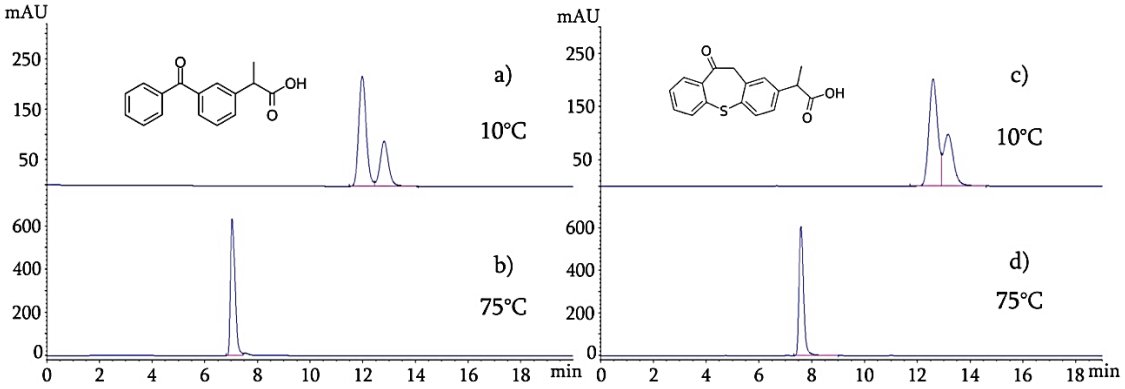
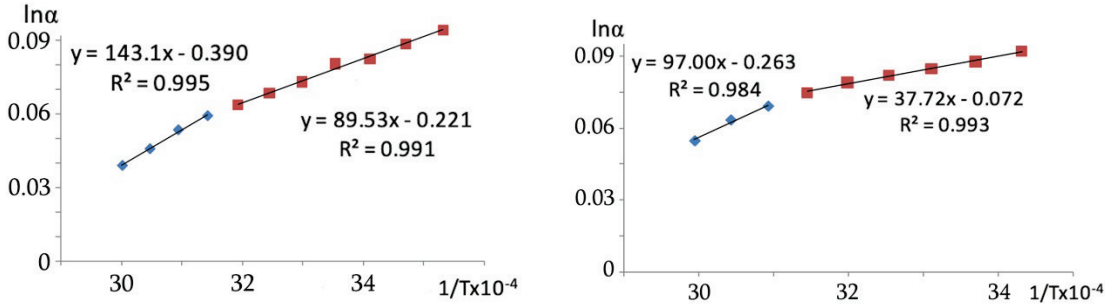


Fig. 2. Separation of enantiomers of ketoprofen (R:S=1:2) (a,b) and zaltoprofen (R:S=1:2) (c,d) on the column with immobilized chiral selector. The mobile phase was n-hexane/propan-2-ol/formic acid 90/10/0.1 (v/v/v) at 10 and 75°C with a flow rate of 1 ml/min. UV signal was recorded at 254 nm.

Table. Thermodynamic parameters for ketoprofen and zaltoprofen

Chiral Analyte	ΔH_S cal/mol	ΔH_R cal/mol	$\frac{\Delta S^0}{R} + \ln\phi$ cal/mol	$\frac{\Delta S^0}{R} + \ln\phi$ cal/mol	$\Delta\Delta H_{S,R}$ cal/mol	$\Delta\Delta S_{R,S}$ cal/mol	T_{iso}, K
Ketoprofen	-2086.76	-2379.28	-5.70	-6.53	-177.91 (283-313 K)	-0.44 (283-313 K)	404 (283-313 K)
Ketoprofen					-284.37 (318-333 K)	-0.78 (318-333 K)	365 (318-333 K)
Zaltoprofen	-2280.32	-2504.47	-6.18	-6.83	-74.96 (283-308 K)	-0.14 (283-308 K)	535 (283-308 K)
Zaltoprofen					-192.76 (313-323 K)	-0.52 (313-323 K)	370 (313-323 K)



(a) (b)

Fig. 3. Dependence of the natural logarithm of the separation factor (a) on the inverse absolute temperature for ketoprofen (a) and zaltoprofen (b).

Similar to the adsorption processes also separation of enantiomers of both analytes were favored by enthalpy terms and disfavored by entropy terms in both temperature ranges. Since the temperature dependence of enantioseparation looked quite similar for both analytes one can conclude that a structural change occurred most likely in chiral selector and not in chiral analyte.

The isoenantioselective temperatures above which the reversal of enantiomer elution order would occur is in the range 365-404K and 370-535K for ketoprofen and zaltoprofen, respectively. Thus, in both cases these temperatures are above the range that can be used in HPLC. Accordingly, there is no possibility to achieve a temperature-dependent reversal of enantiomer elution order for studied analytes on the studied column and in the studied mobile phase.

For both compounds the differential values of thermodynamic parameters determined based on retention data correlated better with the same quantities determined based on selectivity data in higher temperature range.

ფიზიკური ქიმია

ტემპერატურის გავლენა ენანტიომერების დაყოფაზე კოვალენტურად იმობილიზებულ პოლისაქარიდზე დაფუძნებული ქირალური სტაციონარული ფაზის გამოყენებით მაღალეფექტურ სითხურ ქრომატოგრაფიაში

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მაღალეფექტური სითხური ქრომატოგრაფიის მეთოდით შევისწავლეთ კეტოპროფენისა და ზალტოპროფენის ენანტიომერების დაყოფის თერმოდინამიკური პარამეტრები. ანალიზები ჩატარდა სხვადასხვა ტემპერატურაზე ჰექსანი/იზოპროპანოლი/ჭიანჭველმჟავა 90/10+0.1% (მოცულობითი თანაფარდობა) მოძრავი ფაზის და სილიკაგელზე კოვალენტურად იმობილიზებული ქირალური სელექტორის, ცელულოზა ტრის(3-ქლორ-5-მეთილფენილკარბამატის) გამოყენებით. ორივე შესწავლილი ქირალური ნივთიერებისთვის, ენთალპიური წევრი ხელს უწყობს ადსორბციას, ხოლო ენტროპიული წევრი, პირიქით, უარყოფითად მოქმედებს მასზე. ადსორბციის ანალოგიურად, გამოკვლეული ორივე ნივთიერების შემთხვევაში ენთალპიური წევრი ხელს უწყობს ენანტიომერების დაყოფას, ხოლო ენტროპიული წევრი ხელს უშლის მას. იზოენანტიოსელექტიური წერტილების მნიშვნელობა როგორც კეტოპროფენის, ისე ზალტოპროფენის შემთხვევაში აღემატება სითხურ ქრომატოგრაფიაში გამოყენებული ტემპერატურების ზედა ზღვარს და, ამგვარად, ენანტიომერების ელუირების რიგის მართვა ტემპერატურის მიხედვით ამ კონკრეტული ნივთიერებებისთვის შესწავლილ ქრომატოგრაფიულ პირობებში შეუძლებელია.

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