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ORIGINAL PAPER

A study of the interaction between enantiomers of zolmitriptan and hydroxypropyl-beta-cyclodextrin by capillary electrophoresis

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Abstract The enantioresolution of zolmitriptan was performed using cyclodextrin (CD)-modified capillary zone electrophoresis (CZE) with hydroxypropyl-β-CD (HP-β-CD) as the chiral selector. The influence of experimental conditions on the enantioseparation of zolmitriptan, such as pH, temperature, applied voltage, and concentrations of running electrolyte and CD, was systematically investigated, obtaining a baseline separation of two enantiomers by the use of a 25 mM sodium dihydrogen phosphate (SDPH) running electrolyte (pH 2.4) containing 30 mM HP-β-CD at 15 °C. Binding constants for each enantiomer–HP-β-CD pair at different temperatures, as well as thermodynamic parameters for binding, were calculated. A nonlinear van't Hoff plot was obtained, indicating that the thermodynamic parameters of complexation were temperature-dependent for zolmitriptan enantiomers. The significant contribution of the enthalpy difference to the Gibbs free energy change suggested a stereomeric barrier mechanism for chiral recognition.

Keywords Binding constant · Capillary zone electrophoresis · Enantioseparation · Zolmitriptan · Cyclodextrin

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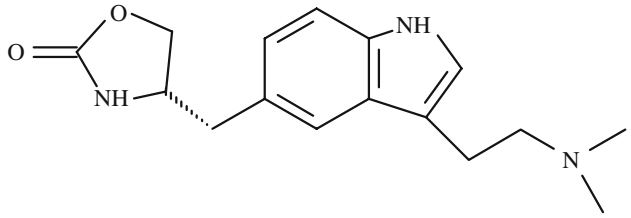
Introduction

Migraine affects 18% of women and 6% of men. The significant impact of migraine results in a huge burden for the individual, health services, and society. Successful treatment of acute migraine attacks can reduce the use of healthcare resources and improve health-related quality of life [1]. The introduction of the triptans in the 1990s revolutionized the treatment of migraine, and a second-generation triptan, zolmitriptan, is highly effective in the oral treatment of acute migraine with or without aura. Zolmitriptan is a novel serotonin 5-hydroxytryptamine receptor agonist that inhibits the peripheral trigeminovascular system and is able to access central sites in the brainstem involved in processing cranial pain [2, 3]. Zolmitriptan is synthesized as the (*S*)-stereoisomer, because it is pharmacologically more potent than the toxic (*R*)-stereoisomer. So separation of zolmitriptan enantiomers (Fig. 1) is necessary for quality control of this drug and its related pharmaceutical and biological study.

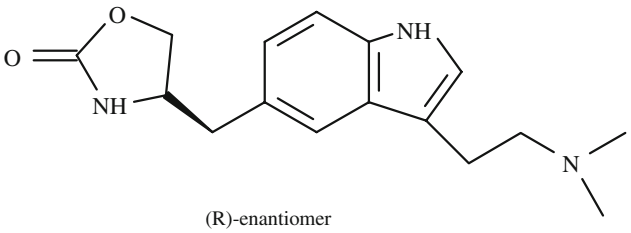
High-performance liquid chromatography (HPLC) has been widely used for the quantitative determination of triptans with UV [4–6], fluorescence [7–10], coulometric detection [11, 12], and mass spectrometry (MS) [13–19]. Until now, HPLC [6, 10, 20], capillary electrochromatography (CEC) [21], and capillary zone electrophoresis (CZE) methods with sulfated β-CD as chiral selector [22, 23] have been employed for enantioseparation of zolmitriptan enantiomers.

Capillary electrophoresis (CE) is a powerful technique for enantioseparation, because of high separation efficiency, low reagent consumption, and the simplicity of the system used. Inclusion into the cavity of cyclodextrins (CDs) represents the most frequently used approach for enantioseparation by CE. The chiral recognition mechanism is based on inclusion of a bulky hydrophobic part of the molecules into the hydrophobic cavity of the CD. Second-



Zolmitriptan

(4S)-4-[[3-[2-(dimethylamino)ethyl]-1H-indol-5yl]methyl]-2-oxazolidinone



(R)-enantiomer

Fig. 1 Chemical structures of zolmitriptan enantiomers

ary interactions are additionally required, including dipole–dipole interactions or hydrogen bonds between secondary hydroxyl groups around the cone opening and polar substituents close to the chiral center of the analyte.

CE is also a convenient technique for studying binding constants in separation systems without any adaptations and approximations, allowing the measurement of the binding constants under the exact conditions of enantioseparation [24–35]. CE also allows one to determine thermodynamic parameters [31, 32, 36–43] and to observe enantioselective recognition in the selector–selectand pairs in which other techniques do not provide any indications [40–43].

The inclusion complexation eventually leads to the improvement of drug solubility and stability, the enhancement of drug absorption, and the alleviation of local and systemic toxicity [44]. Chiral recognition of zolmitriptan with pyrrolidinylidenesulfamido-modified β-CDs has been studied by fluorescence-spectral titrations [45]. Hydroxypropyl-β-CD (HP-β-CD), a kind of water-soluble, safe, and bioadaptable drug carrier, is more desirable than other chemically modified CDs and has considerable pharmaceutical potential. The knowledge of binding constants and thermodynamic parameters of drug–HP-β-CD complexes helps to control the complexation equilibrium and thus to enhance drug bioavailability.

In this work, a CZE method with HP-β-CD as selector, instead of sulfated β-CD [23], was developed for the determination of zolmitriptan enantiomers and their binding constants with the chiral selector. Three linear plotting methods, which are actually applicable to zolmitriptan because of its completely ionized status under the operating conditions used (pK_a=9.6), were used and the van't Hoff relationship was applied to compute the temperature dependence of the thermodynamic parameters. To investigate the chiral recognition mechanism, the differences between the molar Gibbs energy, enthalpy and entropy of the two enantiomers were determined. In addition, some related parameters were calculated, including the optimum CD concentration for enantioseparation, electrophoretic mobility of the fully complexed analyte, and the isoenantioselective temperature.

Experimental

Apparatus and conditions

All experiments were performed with an Agilent 3D CE system with air-cooling and diode array detector (Agilent Technologies, Palo Alto, CA, USA). A 48.5-cm (40.0 cm to the detector) × 50-μm-id uncoated fused silica capillary (Sino Sumtech, Handan City, Hebei, China) was utilized. Other conditions were capillary temperature 15 °C, applied voltage 25 kV, UV detection at 220 nm, and sample injection at 50 mbar for 5 s. The new capillary was washed with 1.0 M sodium hydroxide (15 min), water (20 min), and the running electrolyte (10 min) in turn. Between consecutive analyses, the capillary was flushed with 1.0 M sodium hydroxide (0.5 min), water (5 min) and the running electrolyte (5 min) sequentially to guarantee good reproducibility.

Chemicals

Zolmitriptan and (*R*)-enantiomer standards were kindly donated by Orient Lüyuan Co., Ltd. (Beijing, China). HP-β-CD was purchased from Acros Organics (97%, New Jersey, USA). Sodium dihydrogen phosphate dihydrate, phosphoric acid, and formamide were analytical grade from Beijing Chemical Factory (Beijing, China). Redistilled water was used for the preparation of solutions.

For the preparation of running electrolytes and standard solutions, please see Electronic supplementary material.

Results and discussion

Optimization of CD-modified CZE conditions

Optimization was performed to find operational conditions leading to satisfactory enantioseparations in short run times and permitting the study of the compound behavior. The experimental conditions were systematically optimized by

investigating the effects of main CE parameters on separation as below.

Influence of pH

The pH of running electrolyte is the most important factor to optimize for the chiral resolution. A series of sodium dihydrogen phosphate (SDHP) running electrolytes at pH ranging from 2.0 to 4.5 were investigated in detail with 20 mM HP-β-CD in the running electrolyte. With increasing pH, the resolution (Rs) of enantiomers first increased and then decreased, while the resolution was not good enough at pH 2.0. The pH is responsible for the stabilities of the diastereomeric complexes formed between enantiomers and the chiral selector. It also affects the degree of ionization of the analytes and electroosmotic flow (EOF). Considering inaccuracies of the measurements and the time to achieve equilibrium on the silica, pH 2.4 afforded the maximum experimental resolution and is suitable for this chiral separation.

Effect of CD type and concentration

β-CD and HP-β-CD, which are both cheaper than sulfated β-CD, were tested under the same experimental conditions. HP-β-CD was chosen as the chiral selector because of better solubility and higher resolution (Fig. 2) than those of β-CD. The influence of HP-β-CD concentration, in the range 5–40 mM, on enantioseparation was investigated, showing that the resolution reached a maximum (2.21) at 30 mM and then decreased as shown in Fig. 3.

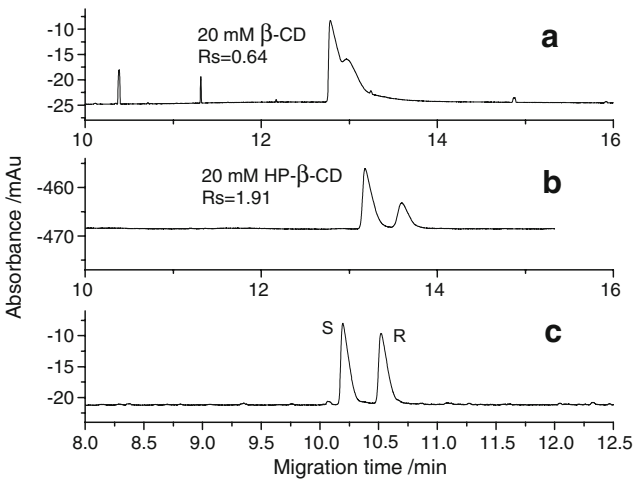


Fig. 2 Enantioseparation comparison of β- and HP-β-CD as the chiral selector. Separation conditions: **a** 50-μm-id×48.5-cm (40.0-cm effective) fused silica capillary, 25 mM SDHP running electrolyte containing 20 mM β-CD, applied voltage 20 kV, capillary temperature 20 °C, UV detection at 220 nm, injection at 50 mbar for 5 s; **b** same as a, except for 20 mM HP-β-CD; **c** same as a, except for 30 mM HP-β-CD, applied voltage 25 kV, capillary temperature 15 °C

Effect of SDHP running electrolyte

Experimental results showed that the chiral resolution and the migration time increased with increasing SDHP running electrolyte concentration from 15 to 50 mM. The baseline at 75 mM SDPH running electrolyte was unstable and there were no peaks eluted within 20 min. In terms of the current, peak shape, and migration time, 25 mM SDHP running electrolyte was considered to be the best (Fig. S1, Electronic supplementary material).

Effects of applied voltage and temperature

The effect of applied voltage on the separation was tested in the range 15–30 kV, indicating that higher applied voltage afforded lower resolution and separation efficiency, and 25 kV resulted in a better separation within reasonable analysis time.

Because the host–guest complexation mechanism is a kinetically driven process and there is potential Joule heating within the capillary under the usual voltage settings, temperature effects are crucial for CD-modified CZE. The optimization of capillary temperature was studied in the range 10–30 °C, showing that the resolution and separation efficiency increased with decreasing temperature. Considering the preferred high Rs and easy manipulation, a capillary temperature of 15 °C was chosen, at which the number of theoretical plates (*N*) was larger than 42,000. The dependence of enantioseparation on temperature is discussed in detail below.

Altogether, the most suitable CZE conditions for separation were 25 mM SDHP running electrolyte containing 30 mM HP-β-CD at pH 2.4, 50-μm-id×48.5-cm (40.0-cm effective) fused silica capillary, applied voltage 25 kV, and capillary temperature 15 °C. A typical electropherogram obtained under these conditions is shown in Fig. 2c.

Quantitation of zolmitriptan enantiomers

Linear regression equations relating the concentration to peak area of zolmitriptan enantiomers using CZE are listed in Table 1, together with their correlation coefficients (R^2), which were higher than 0.99. The limit of detection (LOD) of each analyte is also listed in Table 1: the LODs were both 0.3 μg/ml. The method repeatability was proven by analyzing a mixed solution of 21 μg/ml for zolmitriptan and 19 μg/ml for the (*R*)-enantiomer, and relative standard deviations (RSDs) (n=6) of migration time and peak

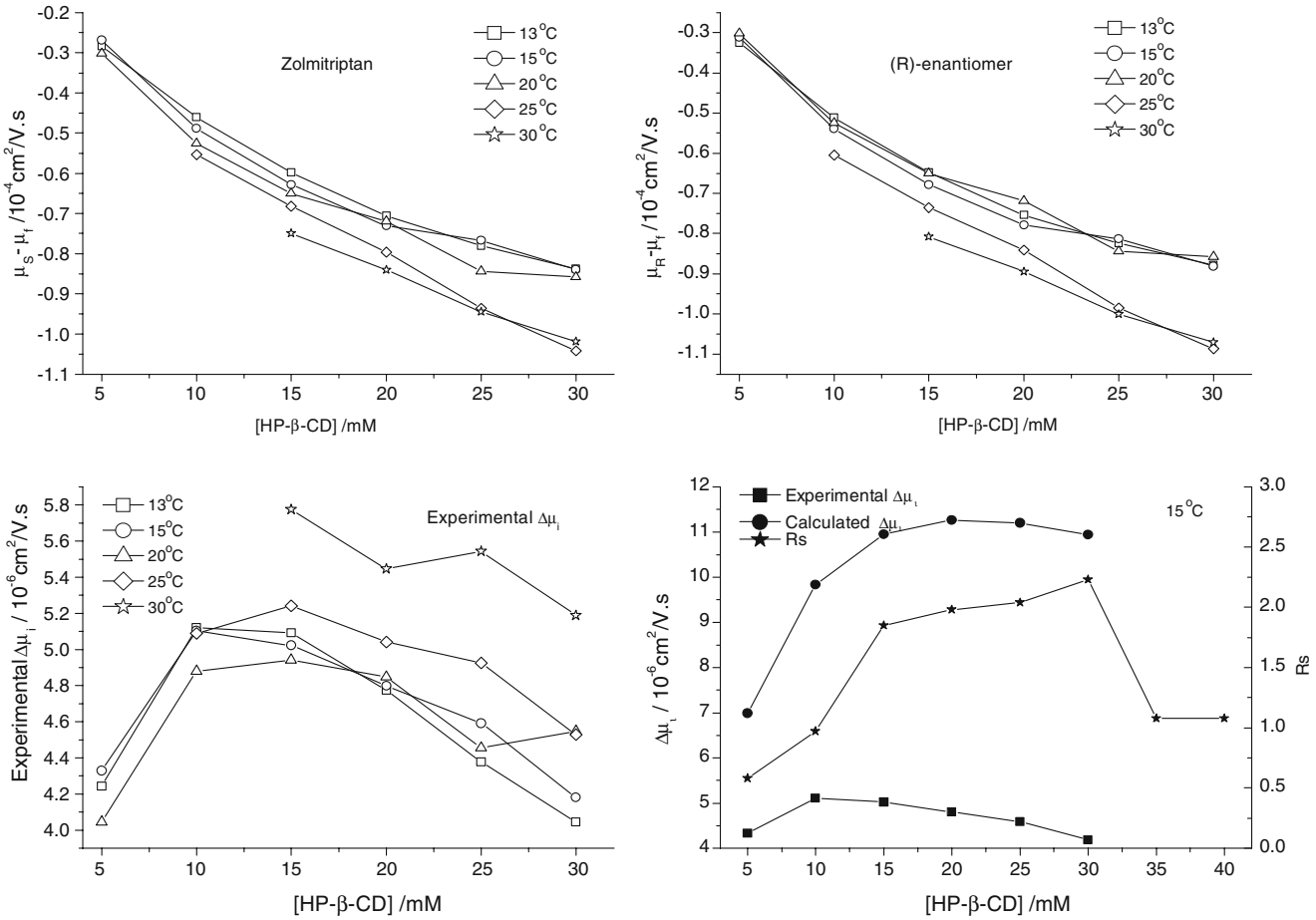


Fig. 3 Plots of the change in solute mobility and differences in electrophoretic mobilities as a function of HP-β-CD concentration for zolmitriptan enantiomers

area are shown in Table 1, illustrating that the method repeatability was acceptable.

Determination of enantiomer binding constants

Knowledge of binding constants of the inclusion complexes can help to elucidate the extent of analyte inclusion into the cavity of a CD. A theoretical model relating mobility to the concentration of the CD selector has been developed by Wren and Rowe [24, 25]. Chiral recognition depends on the difference between the electrophoretic mobilities of each free and complexed enantiomer. Binding constants can be determined by the following expression:

$$K[\mathrm{C}] = \left(\frac{\mu_\mathrm{f} - \mu_\mathrm{i}}{\mu_\mathrm{i} - \mu_\mathrm{c}}\right) \quad (1)$$

where K is the binding constant, [C] is the equilibrium concentration of uncomplexed ligand, μ_f and μ_c are the electrophoretic mobilities of the free and complexed solute, and μ_i is the solute mobility at the ligand concentration [C].

Two procedures should be completed: (1) measuring of the mobilities for enantiomers at different concentrations of

Table 1 LODs, linear relationships, and repeatabilities (n=6) for determination of zolmitriptan enantiomers

Analyte	Concentration range (μg/ml)	Regression equation[a]	R^2	LOD (μg/ml)	RSD% (n=6)	
					MT	Area
Zolmitriptan	0.62–69	Y=3.06X+4.01	0.9999	0.3	0.9	2.2
(*R*)-Enantiomer	0.58–64	Y=3.31X+3.67	0.9998	0.3	0.9	1.7

MT migration time
[a] X = concentration (μg/ml); Y = peak area

chiral selector; (2) using three linear plotting methods listed below to calculate the binding constants.

Double reciprocal plot of $\frac{1}{\mu_i-\mu_f}$ versus $\frac{1}{[C]}$:

$$\frac{1}{\mu_i-\mu_f}=\frac{1}{(\mu_c-\mu_f)K}\frac{1}{[C]}+\frac{1}{\mu_c-\mu_f} \quad K=\frac{\text{intercept}}{\text{slope}}$$

Y-reciprocal plot of $\frac{[C]}{\mu_i-\mu_f}$ versus [C]:

$$\frac{[C]}{\mu_i-\mu_f}=\frac{1}{\mu_c-\mu_f}[C]+\frac{1}{(\mu_c-\mu_f)K} \quad K=\frac{\text{slope}}{\text{intercept}}$$

X-reciprocal plot of $\frac{\mu_i-\mu_f}{[C]}$ versus $(\mu_i-\mu_f)$:

$$\frac{\mu_i-\mu_f}{[C]}=-K(\mu_i-\mu_f)+K(\mu_c-\mu_f) \quad K=-\text{slope}$$

The mobilities of zolmitriptan enantiomers were measured at different temperatures. Formamide was used as a neutral marker to correct EOF changes.

Under these plotting forms, the direct measurement of μ_c, which is difficult or impossible due to the difficulty of finding suitable CD markers and reaching saturated conditions, is not required. This approach also enables the calculation of μ_c from the regression equations. As shown in Table 2, μ_c increased with temperature, because the viscosity of the medium decreases and the frictional forces of the complexed species reduce.

Plots showing the effect of HP-β-CD concentration on solute mobilities at several temperatures are shown in Fig. 3. The lowest CD concentration needed for the measurement of solute mobilities at higher temperatures had to be enhanced for sufficient resolution. At the five temperatures checked for zolmitriptan enantiomers, electrophoretic mobilities decreased with increasing CD concentrations in all ranges tested. On the other hand, differences in electrophoretic mobilities of enantiomers, $\Delta\mu_i$, initially increased with increasing CD concentrations and decreased after a maximum was reached. These differences at 25 °C and 30 °C were larger than those at lower temperatures.

From the rearrangement of Eq. (1), μ_i for each analyte can be calculated using the following expression:

$$\mu_i=\frac{\mu_f-\mu_c}{1+K[C]}+\mu_c \quad (2)$$

As a result, theoretical $\Delta\mu_i$ can be obtained. Maximum $\Delta\mu_i$ between enantiomers occurs at the optimal CD concentration $[C]_{opt}$ [27, 39]. It is calculated by:

$$[C]_{opt}=\frac{1}{\sqrt{K_S K_R}} \quad (3)$$

where K_S and K_R are the binding constants for both enantiomers. The effects of HP-β-CD concentration on experimental $\Delta\mu_i$, calculated $\Delta\mu_i$, and experimental reso-

Table 2 Binding constants and enantioselectivities of complexation for zolmitriptan enantiomers with HP-β-CD in 25 mM SDHP buffer (pH 2.4) at several temperatures

Plotting method	K_S[a] (M^{-1})	K_R[a] (M^{-1})	α[b]	$\mu_c(S)$[c] (10^{-5} cm^2/V s)	$\mu_c(R)$[c] (10^{-5} cm^2/V s)	$R^2(S)$	$R^2(R)$	$[C]_{opt}$ (mM)
13 °C								
Double reciprocal	51.8	65.3	1.26	4.11	4.65	0.999	0.999	17
Y-reciprocal	50.0	62.5	1.25	3.82	4.34	0.999	0.999	18
X-reciprocal	50.3	63.3	1.26	3.85	4.42	0.996	0.996	18
15 °C								
Double reciprocal	41.0	54.4	1.33	2.42	3.67	0.995	0.996	21
Y-reciprocal	51.4	64.5	1.26	4.42	4.95	0.98	0.991	17
X-reciprocal	46.2	58.9	1.27	3.55	4.31	0.94	0.96	19
20 °C								
Double reciprocal	53.3	66.0	1.24	5.73	6.32	0.996	0.996	17
Y-reciprocal	59.4	69.2	1.16	6.57	6.65	0.99	0.991	16
X-reciprocal	56.1	67.2	1.20	6.16	6.51	0.959	0.970	16
25 °C								
Double reciprocal	46.5	55.1	1.19	6.01	6.44	0.98	0.98	20
Y-reciprocal	38.4	45.5	1.19	4.08	4.69	0.97	0.97	24
X-reciprocal	40.2	47.7	1.19	4.55	5.10	0.91	0.91	23
30 °C								
Double reciprocal	59.8	68.9	1.15	9.59	9.57	0.990	0.99	16
Y-reciprocal	56.2	64.7	1.15	9.16	9.16	0.995	0.995	17
X-reciprocal	57.2	65.8	1.15	9.28	9.27	0.97	0.97	16

[a] Binding constants for zolmitriptan (*S*) and (*R*)-enantiomer (*R*)
[b] Enantioselectivities of complexation (α) calculated as $\alpha = K_R/K_S$
[c] Electrophoretic mobilities of the enantiomer–CD complexes for zolmitriptan (*S*) and (*R*)-enantiomer (*R*)

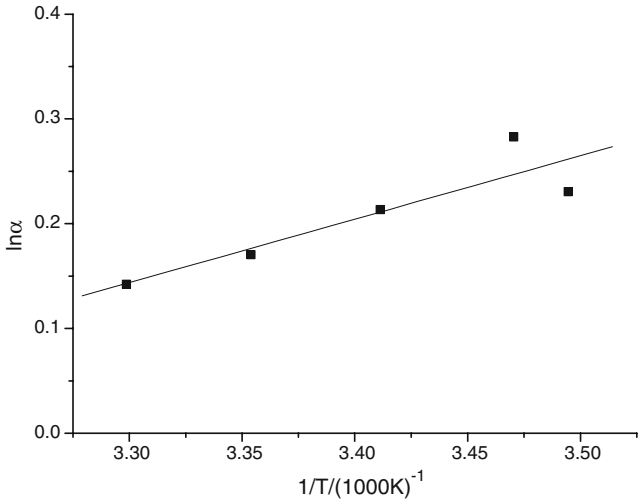


Fig. 4 Plot of lnα versus $1/T$ for zolmitriptan enantiomer–HP-β-CD complexes

lution could be studied at different temperatures as shown in Fig. 3. Considering the isoelectric point of silica, all of experimental $\Delta\mu_i$ values were smaller than the calculated values and the maximum of experimental $\Delta\mu_i$ occurred at lower HP-β-CD concentration than $[C]_{opt}$ at 15 °C in agreement with the viscosity change caused by the variations in CD concentration [46]. On the other hand, maximal resolution was observed at higher CD concentration than maximal experimental and calculated $\Delta\mu_i$. Resolution is more complex because it must also consider EOF, band broadening due to diffusion, and other factors such as injection concentration and detector path length. So maximal resolution will not occur at the same concentration as those where maximal $\Delta\mu_i$ values occur. Besides, a part of the observed variations must come from the inaccuracy of the measurements.

Table 2 lists the binding constants, enantioselectivities of complexation α (calculated as $\alpha=K_R/K_S$) at different temperatures using these linear plotting forms. Acceptable enantioselectivities of complexation were achieved ($\alpha\geq1.15$). The double reciprocal fit for each complexation showed better linearity than those of *Y*- and *X*-reciprocal ones, because it masked deviations from linearity at low ligand concentrations [47]. So the double reciprocal fit was more reliable to assess analyte–ligand interaction. The thermodynamic parameters in this work were deduced from it.

Thermodynamic study of the complexation

Temperature dependence of the binding constants can be described as:

$$\ln K = -\frac{\Delta H^\circ}{RT} + \frac{\Delta S^\circ}{R} \quad (4)$$

where ΔH° is the enthalpy change associated with inclusion complex formation, ΔS° is the corresponding entropy change, T is the absolute temperature, and R is the gas constant.

Usually, the Gibbs energy is known to be mainly dependent on the enthalpy of the complex formation, showing a linear relationship in the van't Hoff plot. Nonlinearity indicates temperature dependence of the partitioning of analyte from the aqueous phase to the pseudostationary phase and entropy-controlled factors involved in complexation [36, 37].

At temperatures between 13 and 30 °C, distinct deviation from linearity in the van't Hoff plot was observed for zolmitriptan enantiomers (data not shown). So the migration behavior of the complex was influenced significantly by the contribution of entropy to the CD complex formation. The entropy-controlled factors including the disarrangement of the solvation status of the CD and the loss of translational and rotational degrees of freedom during complex formation are attributed to temperature-controlled changes in the bulk liquid structure and viscosity [36, 40].

The Gibbs free energy ΔG° at different temperatures can be calculated by:

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

The negative ΔG° indicated that the complexation was thermodynamically favored. Besides, the inclusion into the cavity of HP-β-CD was favored for the (*R*)-enantiomer with more negative ΔG° in agreement with the migration order (data not shown).

Enantioselectivity (α) is dependent on the enthalpy difference ($\Delta\Delta H^\circ$) and the entropy difference ($\Delta\Delta S^\circ$) of the inclusion interaction following Eq. (6):

$$\ln \alpha = -\frac{\Delta\Delta H^\circ}{RT} + \frac{\Delta\Delta S^\circ}{R} \quad (6)$$

$$\Delta\Delta G^\circ = \Delta\Delta H^\circ - T\Delta\Delta S^\circ \quad (7)$$

where $\Delta\Delta G^\circ$ is the difference in the molar Gibbs energy of the two enantiomers.

A van't Hoff plot of lnα versus $1/T$ (Fig. 4) showed an approximately linear relationship in the temperature range from 13 to 30 °C (R^2=0.8). An increase of temperature caused a decrease of separation factor. The aberrant values obtained at 15 °C for both K and μ_c (S and R) explain this low value of the correlation coefficient. Table 3 summarizes the thermodynamic data from Fig. 3 according to Eqs. (6) and (7).

Table 3 Key thermodynamic parameters for the association between zolmitriptan enantiomers and HP-β-CD

$\Delta\Delta H^\circ$ (kJ/mol)	$\Delta\Delta S^\circ$ (J/mol K)	$\Delta\Delta G^\circ_{298}$ (kJ/mol)	T_{iso} (°C)
−5.03	−15.4	−0.437	53

Usually, the Gibbs free energy of the inclusion complex is associated with a favorable proportion of the enthalpy and an unfavorable entropy proportion, as was applicable to the complex formation of HP-β-CD with zolmitriptan enantiomers. Its large negative $T\Delta\Delta S°$ term represented an unfavorable influence of the entropy on $\Delta\Delta G°$ and thus on the separation. The entropy value is influenced by two main counteracting effects: (1) hydrate water surrounding host and guest becomes disordered in particular if lateral hydrogen bonds are formed during complexation; (2) there are losses in the translational and rotational degrees of freedom because of the association of host and guest [40]. For the complexation of HP-β-CD and zolmitriptan enantiomers, the loss of a degree of freedom dominated over the release of hydrate water. It also indicated that enantiomers formed only weak lateral hydrogen bonds with the host.

For enantiorecognition based on two mechanisms, namely, steric barrier and coulometric interactions, thermodynamic parameters of complexation should differ significantly [40]. Because negative $\Delta\Delta G°$ was mainly controlled by the contribution of $\Delta\Delta H°$, the chiral separation of zolmitriptan enantiomers should be based on a steric barrier mechanism, not the electrostatic interaction characterized by the positive $T\Delta\Delta S°$ value [40].

$\Delta\Delta H°$ and $\Delta\Delta S°$ are both negative which means that an isoenantioselective temperature (T_{iso}) exits [42, 48]. T_{iso} is the particular temperature at which $\Delta\Delta G°$ is nil with no enantiomer resolution. T_{iso} is expressed by:

$$T_{iso} = \frac{\Delta\Delta H^o}{\Delta\Delta S^o} \quad (8)$$

At temperatures higher than T_{iso}, the enantiomer elution order should be reversed. Although the elution reversal was not observed, enantiometric resolution at temperatures close to the calculated T_{iso} listed in Table 3 disappeared.

Conclusions

In this work, a CD-modified CZE method using relatively cheap HP-β-CD was established for the separation of zolmitriptan enantiomers. The developed method is simple, environmentally friendly, and involves low reagent consumption. Moreover, because all measurements are performed under defined conditions and in pure aqueous solutions, the method could be used to estimate the thermodynamic values with more precision. The binding constants of enantiomers were obtained using three linear plotting methods. The double reciprocal fit provided better reliability. The nonlinear plot of ln$K$ versus $1/T$ suggested that the entropy-controlled factors dependent on temperature were involved in the complexation between zolmitriptan enantiomers and HP-β-CD. Because of the overwhelming contribution of $\Delta\Delta H°$ to $\Delta\Delta G°$, the chiral recognition of zolmiotriptan enantiomers was based on a barrier mechanism. The calculations of related parameters such as μ_c, $[C]_{opt}$, and T_{iso} have proven particularly useful to permit an additional insight into the enantioseparation.

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