

Complex of a new racemic thiourea substrate: Spectrophotometric study and separation of atropisomers

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Abstract

The activity of a chiral molecule can vary from one atropisomer to the other. In this study, the separation of racemic-N-(2-methylphenyl), N'-(2-chlorophenyl) thiourea (H_2L_2), a hydrophobic heavy metal trap, has been studied using reversed-phase high-performance liquid chromatography (RP-HPLC) with hydroxypropyl-beta-cyclodextrin (HP- β -CD) as a complexing additive to the racemic mixture and hexane-isopropanol as a mobile phase. The stoichiometry and the overall association constant of the complex have been determined using the continuous variation (Job's plot) method and the Scott's method respectively.

Keywords: Thiourea; HP- β -CD; HPLC; atropisomeric separation; inclusion complexes.

1. Introduction

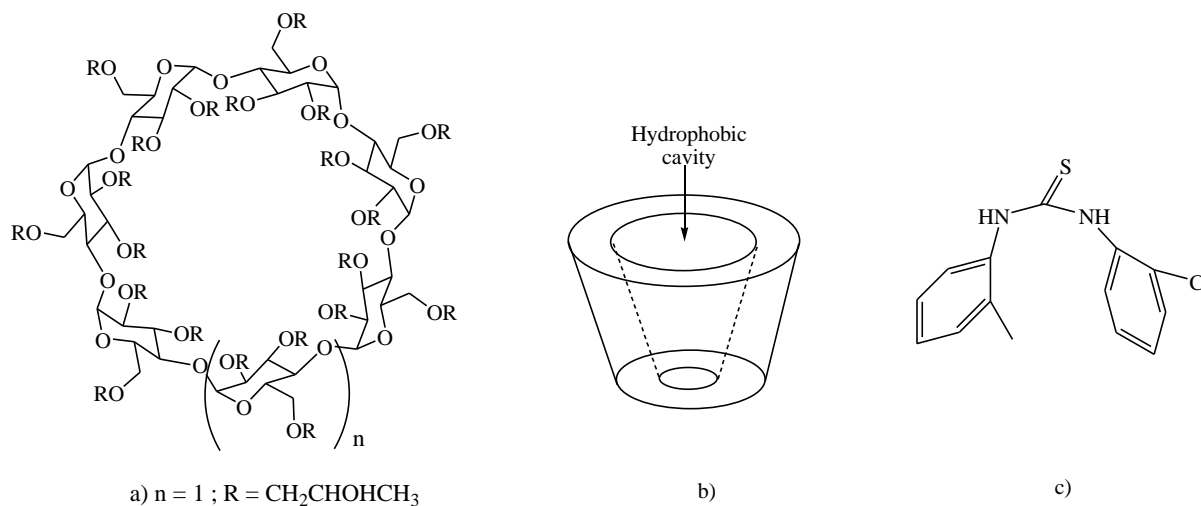
Effects of heavy metals on the human health have been studied for a long time.[1-4] Thiourea substrates, used as traps of the heavy metals play, an important role as organocatalysts for a variety of asymmetric transformations, such as the cyanosilylation of ketones[5], Strecker reactions[6], Michael additions[7] and hydrophosphonylation of imines.[8] In addition, the presence of one or several heteroatoms permits interactions, of electrostatic type (hydrogen-bond, Van der Waals-bond...), with the biologic target, while the aromatic cycles allow other interactions of hydrophobic nature.[9]

The literature is rich with research investigating the separation of isomers with cyclodextrins.[10-13] Natural cyclodextrins (CD) constitute a family of cyclic oligosaccharides comprising repetitive 6, 7, or 8 glucose units (α -, β -, γ -CD, respectively). The inside of the

molecule forms a hydrophobic cavity, enabling it to form molecular inclusion complexes with hydrophobic substrates. Due to the chair conformation of the glucopyranose units, the CD molecules take the shape of a truncated cone rather than a perfect cylinder.[14] A number of the CD derivatives of interest include the hydroxypropyl derivatives (i.e. HP- α -CD, HP- β -CD and HP- γ -CD), the randomly methylated-CD and sulfobutylether-CD.[15-19]

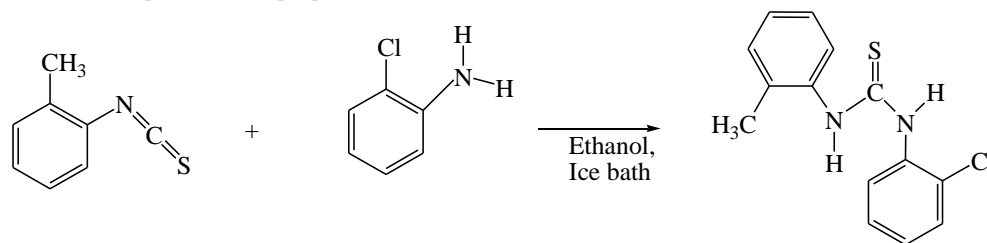
The present paper deals with:

1. The separation of the two atropisomers of racemic-N-(2-methylphenyl),N'-(2-chlorophenyl) thiourea, (H_2L_2), [Scheme 1] by reversed phase high-performance liquid chromatography (RP-HPLC) and the use of a cyclodextrin derivative and the hydroxypropyl- β -cyclodextrin as a complexing additive
2. The application of spectrophotometric Job's and Scott's methods to study the inclusion complexation



Scheme 1. (a) Chemical structure of hydroxypropyl- β -cyclodextrin (HP- β -CD); (b) truncated cone shape of HP- β -CD; (c) N-(2-methylphenyl),N'-(2-chlorophenyl) thiourea (H_2L_2).

Racemic-N-(2-methylphenyl),N'-(2-chlorophenyl) thiourea, (H_2L_2), has been prepared according to the procedure reported in the literature as shown in [Scheme 2].[20]



Scheme 2: Synthesis of racemic-N-(2-methylphenyl),N'-(2-chlorophenyl) thiourea, (H_2L_2).

2. Materials and Methods

2.1. Chemicals and reagents

Racemic-N-(2-methylphenyl),N'-(2-chlorophenyl) thiourea, (H_2L_2), was synthesized and purified according to the procedure reported in the literature.[20] HP- β -CD, hexane and isopropanol of HPLC grade were purchased from Sigma-Aldrich Company. All the solvents used for column chromatography were of HPLC grade and distilled prior to use. Water was purified by triple distillation to obtain ultrapure water.

2.2. Preparation of solutions

3 mg of racemic-N-(2-methylphenyl),N'-(2-chlorophenyl) thiourea, (H_2L_2), was accurately weighted, transferred to volumetric flasks and dissolved in 10 mL solution of mobile phase 50:50 (v/v) hexane-isopropanol to make individual stock solutions of 1 mmol/L. The stock solution was stored at 4 °C and was later diluted with mobile phase to the recommended concentration of 10^{-5} mmol/L.

2.3. Preparation of inclusion complexes

100 μL of 10^{-5} mmol/L concentration of cyclodextrin and 100 μL of 10^{-5} mmol/L of H_2L_2 were mixed and shaken at the temperature of 25°C to obtain a stable state of solubilization.

2.4. Instrumentation

Chromatographic studies were performed on a Shimadzu HPLC system (UFLC) equipped with a thermostated-column device, a degasser and a variable-wavelength UV detector. The column used for analytical HPLC was C-18 (150 mm \times 4.6 mm). The mobile phase was a mixture of hexane-isopropanol with a flow rate of 1mL/min. The wavelength of UV detector was set at 206 nm (λ_{max} of the substrate) and the column was operated at room temperature. The injection volume was 20 μL .

2.5. Partition coefficient determination

The lipophilicity of racemic-N-(2-methylphenyl),N'-(2-chlorophenyl) thiourea, (H_2L_2), was evaluated through the calculation of their *n*-hexane-water partition coefficient, K_p , as follows: 3 mg of H_2L_2 , dissolved in equal volumes of hexane and triple distilled water, were vigorously stirred at 25 °C for 1 h. The two phases were separated by brief centrifugation (1000 g for 20 s). The substrate concentration in either the organic or the aqueous phase was determined by a Shimadzu UV-VIS spectrophotometer (UV-1800). K_p was calculated as the ratio of the substrate concentration in *n*-hexane to that in water.

2.6. UV-Vis spectra

Schimidzu UV-Vis Absorption spectrophotometer (UV-1800), was employed to determine the wavelength of maximum absorption (206 nm) for the substrate in water.

2.7. Standard curves for calibration

A 10^{-5} mmol/L mother solution of substrate in water was used. A series of 5 mL solutions of concentrations between 10^{-6} and 10^{-5} mmol/L were prepared and left at room temperature for 10 min. Absorbance was measured at 206 nm for each solution against a blank solution. The molar extinction coefficient was determined by measuring the absorbance as a function of concentration.

2.8. Stoichiometry ratio of the complex

Job's method of continuous variation has been employed.²¹ Initial (10^{-5} mmol/L) concentrations of each HP- β -CD and racemic-N-(2-methylphenyl), N'-(2-chlorophenyl) thiourea (H_2L_2) have been prepared. Series of 5ml quantities of HP- β -CD and H_2L_2 have been made up comprising different complementary proportions (0: 5; 0.5: 4.5;4.5: 0.5; 5: 0). The complex formed for each reaction mixture has been allowed to stand for 10 min before analysis at 206 nm. The method is based on the graphical representation of curves, obtained by means of the experimental measurements from a chemical system in equilibrium using Origin 6.0 professional program.

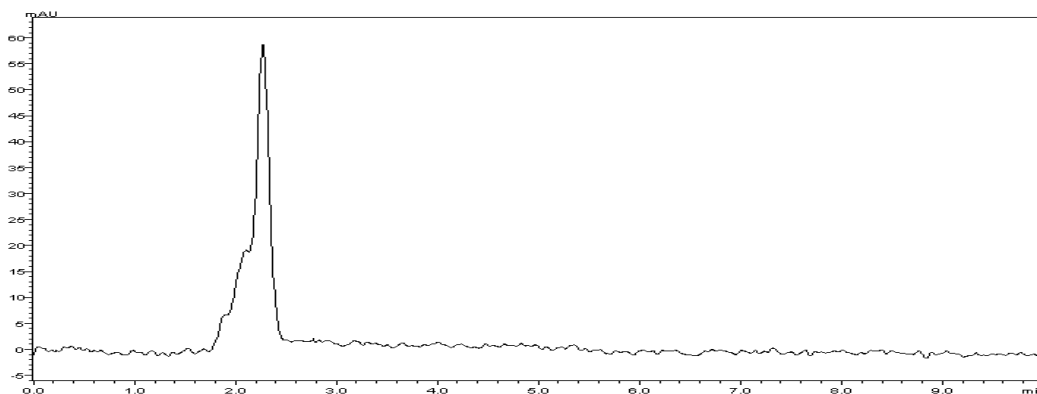
2.9. Association Constants K_a

Scott's plot method was employed.[22] Starting from the same master equimolar (10^{-5} mmol/L) aqueous solutions of HP- β -CD and racemic-N-(2-methylphenyl),N'-(2-chlorophenyl) thiourea (H_2L_2), serial volumes of 0 to 4.5 ml of HP- β -CD solution were transferred to different test tubes. 0.25 ml of racemic-N-(2-methylphenyl), N'-(2-chlorophenyl) thiourea (H_2L_2) were added to each test tube and completed to 5 ml by the addition of the necessary volumes of hexane-isopropanol 50:50 (v/v). The procedure was continued as previously described in section 2.8.

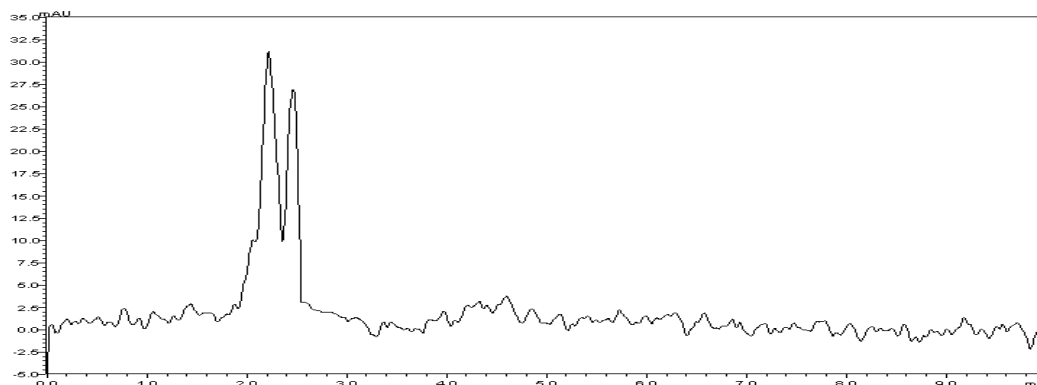
3. Results

3.1. Separation of atropisomers

The chromatogram of racemic-N-(2-methylphenyl),N'-(2-chlorophenyl) thiourea (H_2L_2) without cyclodextrin [Scheme 3] showed a single peak indicating the purity of the substrate. In order to achieve the separation of the two atropisomers, 100 μ L of 10^{-5} mmol/L of HP- β -CD were added to 100 μ L of 10^{-5} mmol/L of the substrate. The result, [Scheme 4], showed the presence of two separated peaks at R_t 2.21 and 2.46 min. corresponding to two atropisomers.



Scheme 3: Chromatogram of racemic-N-(2-methylphenyl), N'-(2-chlorophenyl) thiourea. Flow rate: 1mL/min. Other chromatographic conditions as in Scheme 4



Scheme 4: Separation of atropisomers: conditions: Shimadzu HPLC system, (150mm \times 4.6mm) column. Mobile phase: 50:50 (v/v) hexane-isopropanol. Flow rate: 1mL/min. Injection volume: 20 μ L. Wavelength used for UV detection: 206 nm at room column temperature.

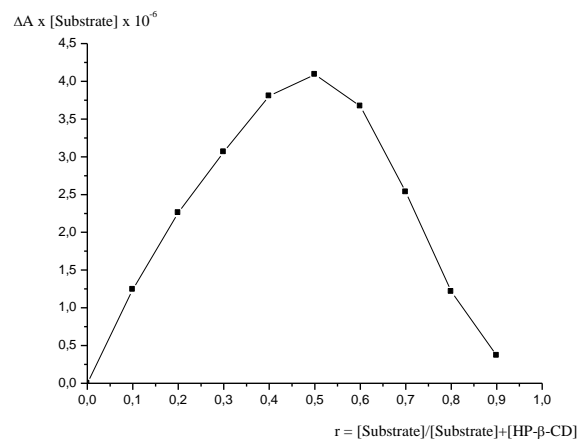
The method involves direct separation of the atropisomers of racemic-N-(2-methylphenyl),N'-(2-chlorophenyl) thiourea (H_2L_2) on a non-chiral stationary phase with cyclodextrin as the chiral selector in the mobile phase. The selectivity and the migration behavior of the substrate are greatly influenced by the interaction of H_2L_2 with the cyclodextrin. In this respect, the inclusion complex formation and the hydrogen bonding play a significant role; where a transient diastereomeric complex is formed between the CD and the compound.[23,24]

3.2. Partition coefficient determination

The success of atropisomers separation greatly depends on the polarity of the substrate. In fact, the use of a lipophilic substrate gives the best results due to the lipophilic cavity of the cyclodextrin. Therefore, the lipophilic characteristic will enhance the inclusion of the substrate in the lipophilic cyclodextrin cavity.[25,26] The partition coefficient K_p is the ratio of the concentrations of the substrate in an organic solvent (hexane) versus that in water. Since the capacity of inclusion of the substrate in the cavity of the cyclodextrin is directly related to lipophilicity, the determination of this coefficient is essential to determine whether the inclusion process is possible. Using spectrophotometry[27], K_p has been found to be 2.92, which indicates a preference for racemic-N-(2-methylphenyl),N'-(2-chlorophenyl) thiourea (H_2L_2) towards the organic phase thus proving the possibility of penetrating the cyclodextrin cavity.

3.3. Determination of stoichiometry

The stoichiometry of H_2L_2 -HP- β -CD inclusion complex has been determined by the continuous variation (Job's plot) method.[21] A series of solutions containing both the H_2L_2 and the HP- β -CD in varying proportions have been prepared in such a way that a complete range of mole ratios is sampled ($0 \leq r = [H_2L_2]/[HP-\beta-CD] + [H_2L_2] \leq 1$). The total concentration $[HP-\beta-CD] + [H_2L_2]$ has been kept constant for each solution. The absorbances of the mixtures have been recorded at 206 nm against a convenient blank solution. The plot of $\Delta A \times [H_2L_2]$ against the mole fraction of H_2L_2 is presented in [Scheme 5]. The plot shows a highly symmetrical shape with a maximum value at $r = 0.5$, which demonstrates the existence of a H_2L_2 -HP- β -CD complex with a 1:1 stoichiometry.



Scheme 5: Job's plot (continuous variation method) of H_2L_2 with HP- β -CD inclusion complex showing 1:1 stoichiometry. Initial concentration of H_2L_2 is 10^{-5} mmol/L. Absorbance measurements were carried out at 206 nm.

3.4. Determination of the association constant K_a

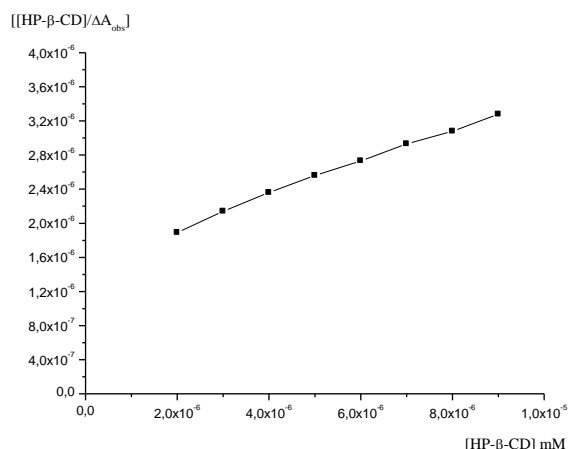
The association constant, K_a , of the racemic-N-(2-methylphenyl),N'-(2-chlorophenyl) thiourea-HP- β -CD complex has been determined by using Scott's method:[22]

$$[HP-\beta-CD]_0/\Delta A_{obs} = [HP-\beta-CD]_0/\Delta A_{max} + 1/K_a \cdot \Delta A_{max}$$

Where:

- ΔA_{obs} is the observed absorbance variation of the racemic-N-(2-methylphenyl),N'-(2-chlorophenyl) thiourea (H_2L_2) in the solution for a given racemic-N-(2-methylphenyl),N'-(2-chlorophenyl) thiourea concentration
- ΔA_{max} is the absorbance variation of the racemic-N-(2-methylphenyl),N'-(2-chlorophenyl) thiourea (H_2L_2) between a pure sample of complex and the free racemic-N-(2-methylphenyl),N'-(2-chlorophenyl) thiourea (H_2L_2) at saturation.

In this procedure, the plot of $[HP-\beta-CD]_0/\Delta A_{obs}$ against $[HP-\beta-CD]$ should be linear for 1:1 inclusion complex. The slope of the plot, $(1/\Delta A_{max})$, and the intercept with the vertical axis, $(1/K_a \cdot \Delta A_{max})$, allow the estimation of the association constant K_a . The obtained Scott's plot for the H_2L_2 -HP- β -CD inclusion complex is shown in [Scheme 6] and the association constant (K_a) has been calculated to be 125 M^{-1} .

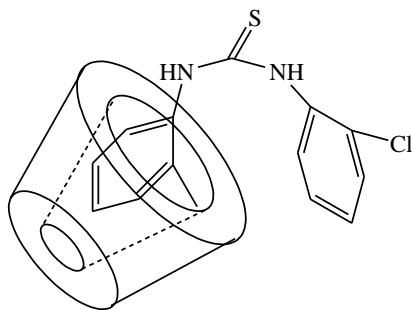


Scheme 6: Scott's plot for racemic-N-(2-methylphenyl),N'-(2-chlorophenyl) thiourea (H_2L_2) with HP- β -CD inclusion complex, showing overall association constant $K_a = 125 \text{ M}^{-1}$, correlation $R = 0.99797$.

4. Discussion

In this study, the substrate showed a stereoselective interaction with HP- β -CD due to the capacity and the polarity of HP- β -CD cavity that allow the substrate inclusion phenomenon. These results confirm that the predominating separation mechanism of CD for racemic-N-(2-methylphenyl),N'-(2-chlorophenyl) thiourea (H_2L_2) was based on the phenomenon of CD-substrate inclusion, in which a transient diastereomeric complex was formed between the CD and the substrate.

Taking into consideration the 1:1 ratio obtained and the presence of only one cavity in the HP- β -CD molecule, it may be postulated that the size of the H_2L_2 matches the size of the cavity. Studies suggest that the penetration of the hydrophobic ring (2-methylphenyl in our study) may take place either from the wider or the narrower rim of the HP- β -CD cavity.[28] The structure of the 1:1 H_2L_2 -HP- β -CD inclusion complex may be represented as in [Scheme 7].



Scheme 7: Possible structure of the H_2L_2 -HP- β -CD inclusion complex. The penetration of the 2-methylphenyl ring may take place either from the wider or the narrower rim of the HP- β -CD cavity.

5. Conclusion

The first objective was the separation of the atropisomers of racemic-N-(2-methylphenyl),N'-(2-chlorophenyl) thiourea (H_2L_2) with HPLC using HP- β -CD as chiral additive. The method used was based on the addition of the cyclodextrin with the racemic substrate. A complex cyclodextrin-compound was formed, and passed on a stationary phase of a RP-HPLC. The separation was easily achieved and pure atropisomers were obtained. The chromatographic conditions described herein provide a novel, rapid and reliable approach for the separation and the analysis of atropisomers from synthesized sample.

The determination of the stoichiometry and the association constant of the complex had been an important subject in analytical chemistry and other branches of chemistry. UV-VIS Spectrophotometry was used for the study of interaction between H_2L_2 and HP- β -CD. The inclusion complex exhibited a high value of the inclusion complex association constant, reflecting the good stability. Our experiment confirmed that our substrate formed 1: 1 complex with HP- β -CD indicating a good interaction.

The method described herein has many advantages: it does not need expensive or sophisticated apparatus; it is simple, rapid and with high sensitivity. These advantages encourage its application.

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