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Research Article

Separation of racemate hydrophobic thiourea substrates

Inas EL Hassan[1], Fatmé Houmaissi[1], Mohammad Bouchekara[3], Hanna El-Nakat[2], Fawaz El Omar[1] and Ahmad Allouch[*1]

[1]*Laboratory of Applied Chemistry (LAC), Faculty of Science III, Lebanese University, P.O. Box 826, Tripoli, Lebanon*
[2]*Department of Chemistry, Faculty of Science, University of Balamand, P.O. Box 100, Tripoli, Lebanon*
[3]*Department of Chemistry, Faculty of Sciences and Technologies, University Mustapha Stanbouli of Mascara (29000), Algeria*

Abstract

The separation of racemic-N-(2-ethylphenyl)-N'-(2-methylphenyl) thiourea, a hydrophobic substrate trap, to collect its atropisomers, was studied using reversed-phase high-performance liquid chromatography (RP-HPLC). Hydroxypropyl-beta-cyclodextrin (HP-β-CD) was used as a complexing additive to the racemic mixture (to obtain chiral molecules) and hexane-ethanol (50% in volume) as a mobile phase. The effects of mobile phase composition, on separation were systematically investigated to establish the optimum conditions of resolution.

Keywords: Thiourea; HP-β-CD; HPLC; atropisomeric separation

1. Introduction

N-(2-ethylphenyl)-N'-(2-methylphenyl) thiourea compounds have been developed and studied for a long time due to their trapping activity of the heavy metals and their important role in the field of asymmetric organocatalysis. The rigid, semi planar structures of such compounds provide sites for a number of important substitutions. As well, the presence of one or several heteroatoms permits interactions, of electrostatic type (hydrogen-bond, VanderWaals-bond…), with the biologic target, while the aromatic cycles allow other interactions of hydrophobic nature [1].

The separation of isomers with cyclodextrins has been studied intensively in the last decades [2-6]. 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 drugs and components, thus greatly enhancing their solubility in water [7-11]. Due to the chair conformation of the glucopyranose units, the CD molecules take the shape of a truncated cone rather than a perfect cylinder [12]. 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 [13-18].

This study reports the separation of the two atropisomers of racemic-N-(2-ethylphenyl)-N'-(2-methylphenyl) thiourea by reversed phase high-performance liquid chromatography (RP-HPLC) and use of a cyclodextrin derivative, the hydroxypropyl-β-cyclodextrin.

* Correspondence Info
Mr Ahmad Khodr Allouch
Laboratory of Applied Chemistry (LAC),
Faculty of Science III, Lebanese University,
P.O. Box 826, Tripoli, Lebanon
E-mail: aallouch@ul.edu.lb

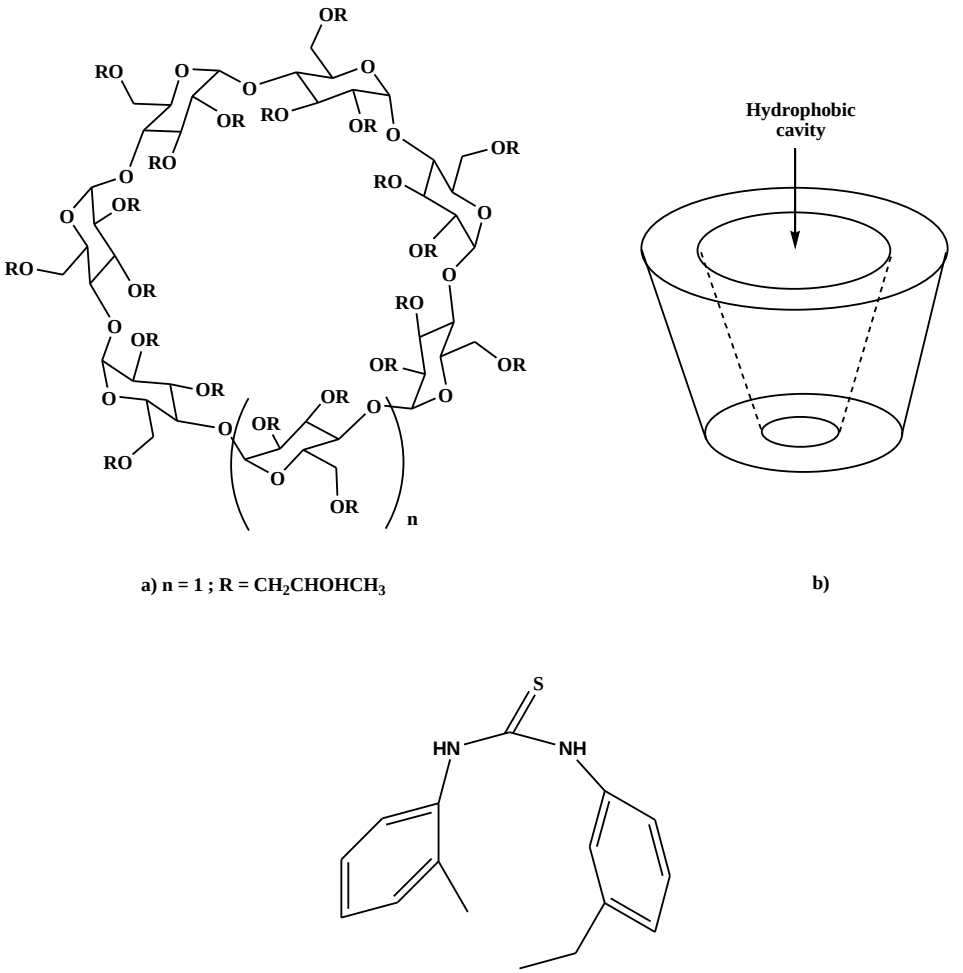

Figure 1: (a) Chemical structure of hydroxypropyl-β-cyclodextrin (HP-β-CD); **(b)** truncated cone shape of (HP-β-CD; **(c)** N-(2-ethylphenyl)-N'-(2-methylphenyl) thiourea.

2. Materials and Methods

2.1. Chemicals and reagents

Racemic-N-(2-ethylphenyl)-N'-(2-methylphenyl) thiourea has been synthesized and purified according to the procedure reported in the literature [19]. HP-β-CD, hexane and ethanol of HPLC grade were purchased from Sigma-Aldrich Co. All the solvents used for column chromatography were of HPLC grade and distilled prior to use. Water was purified by triple distillation.

2.2. Preparation of solutions

6 mg of racemic-N-(2-ethylphenyl)-N'-(2-methylphenyl) thiourea was accurately weighted, transferred to volumetric flasks and dissolved in 10 mL solution of mobile phase 50:50 (v/v) hexane-ethanol to make individual stock solutions of 2.2 µmol/L. The stock solution was stored at 4 °C and was later diluted ten times with mobile phase to the recommended concentration of 0.22 µmol/L.

2.3. Preparation of inclusion complexes

100 µL of 0.22 µmol/L concentration of cyclodextrin and 100 µL of 0.22 µmol/L of solute were mixed, diluted with 500 µL of mobile phase and shaken at the temperature of 25°C to obtain a stable state of solubilization.

2.4. Instrumentation

Chromatographic studies were performed on a Schimadzu 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 × 4.6 mm). The mobile phase was a mixture of hexane and ethanol with a flow rate of

1mL/min. The wavelength of UV detector was set at 220 nm and the column was operated at room temperature. The injection volume was 20 µL.

2.5. Mobile phase optimization

The influence of mobile phase composition was studied, whereby the experiment was carried out in the presence of different volumes of hexane ranging from 20% to 80% (v/v).

3. Results

3.1. Separation of atropisomers

The chromatogram of N-(2-ethylphenyl)-N'-(2-methylphenyl) thiourea **[Figure 2]** showed a single peak indicating the purity of the substrate. In order to achieve the separation of the atropisomers, 100 µL of 0.22 µmol/L of HP-β-CD were added to 100 µL of 0.22 µmol/L of the substrate. The solution was then diluted with 500 µL of a mobile phase composed of 50:50 (v/v) hexane-ethanol. The atropisomeric separation ability was evaluated by resolution. The result, **[Figure 3],** showed the presence of two separated peaks at *Rt 1.79* and 2.06 min. corresponding to two atropisomers.

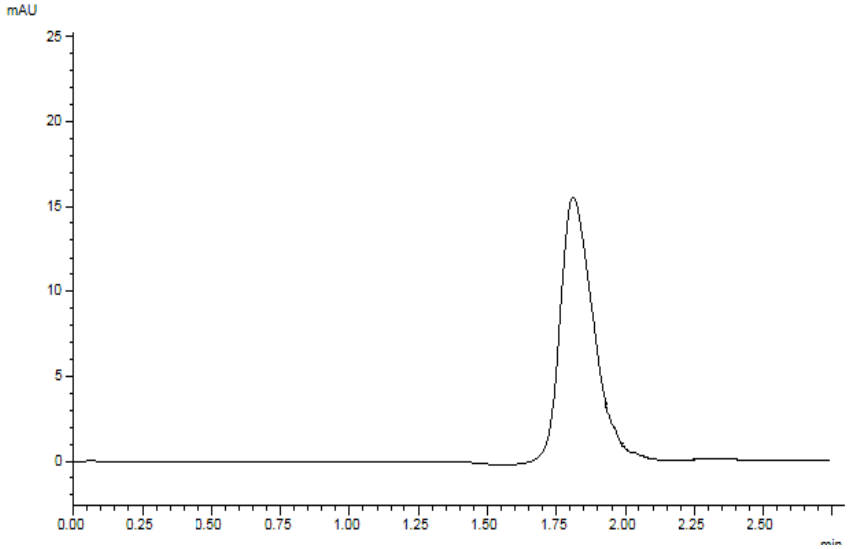

Figure 2: Chromatogram of racemic-N-(2-ethylphenyl)-N'-(2-methylphenyl) thiourea. Flow rate: 1mL/min. Other chromatographic conditions as in Figure 3.

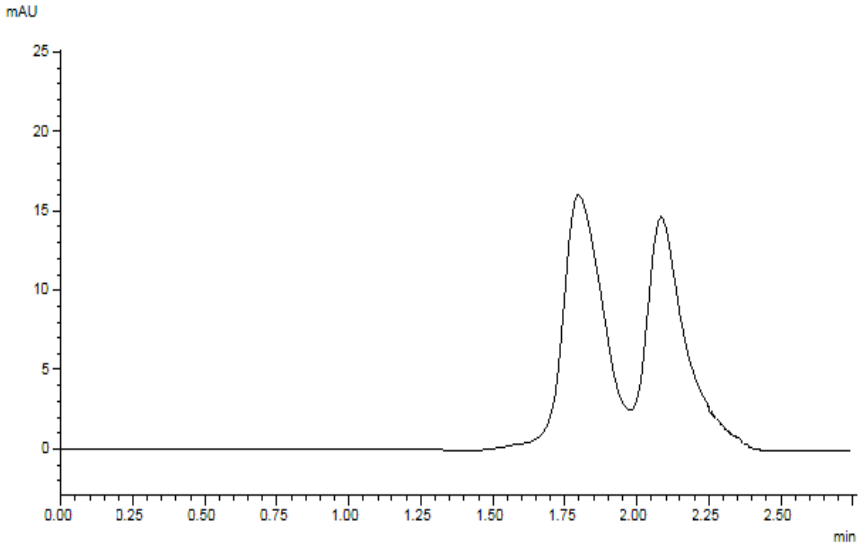

Figure 3: Separation of atropisomers: conditions: Schimadzu HPLC system, (150mm×4.6mm) column. Mobile phase: 50:50 (v/v) hexane-ethanol. Flow rate: 1mL/min. Injection volume: 20µL. Wavelength used for UV detection: 220 nm. at room column temperature.

3.2. Effect of mobile phase parameter

The variation of the composition of the hexane-ethanol mobile phased was found to be important for the improvement of enantioselectivity. Optimization of the mobile phase composition was achieved by testing the different percentages (a range of 20-80%) of hexane-ethanol. The results are described in **Figure 4**, where the best resolution was obtained for a 50% hexane by volume. Resolutions less than 1 were observed for hexane percentages higher than 60%.

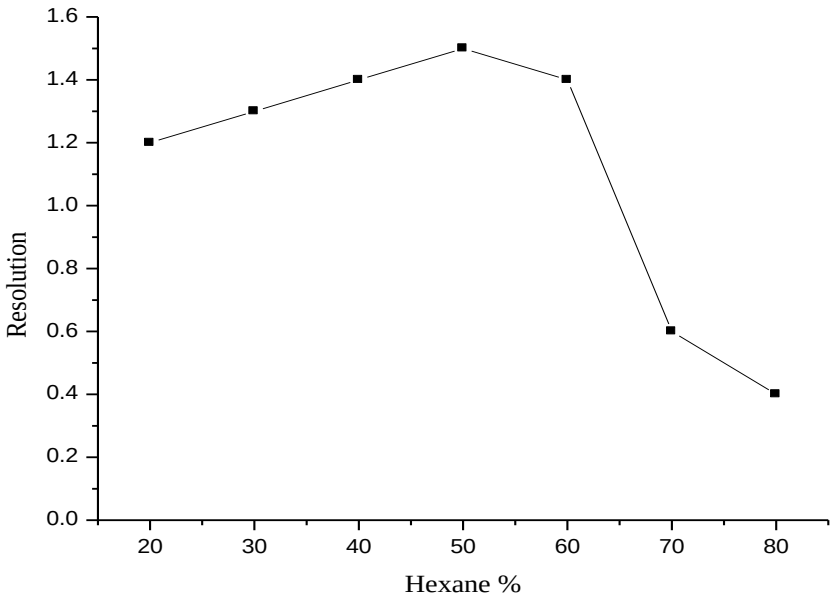

Figure 4: Effect of hexane proportion on the atropisomers resolution. 0.03µmol/L concentration of HP-beta-CD, 0.03µmol/L concentration of substrate are used. Flow rate: 1mL/min. Other operating conditions as in Figure 3.

4. Discussion

In this study, the substrate showed a stereoselective interaction with HP-β-CD. The reason could be related to the capacity and the polarity of HP-β-CD cavity which allow the substrate inclusion phenomenon. These results suggest that the predominating separation mechanism of CD for N-(2-ethylphenyl)-N'-(2-methylphenyl) thiourea compounds is based on the phenomenon of CD-substrate inclusion, where a transient diastereomeric complex is formed between the CD and the substrate [20-21].

5. Conclusion

The objective of this study was the atropisomers separation of N-(2-ethylphenyl)-N'-(2-methylphenyl) thiourea with HPLC using HP-β-CD as chiral additive.

The method used is based on the addition of the cyclodextrin with the substrate. A complex cyclodextrin-substrate is formed, and passed on a stationary phase of a RP-HPLC. The high solubility of HP-β-CD and the substrate in the mobile phase of 50:50 (v/v) hexane-ethanol facilitates the optimization of the chromatographic conditions. 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. Supplementary studies in NMR or molecular modelling must be made to confirm the inclusion of the analyte inside the cavity of cyclodextrin.

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