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Research Article

Determination of the stoichiometry and the association constant of a thiourea derivatives substrate - cyclodextrin complex

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Abstract

The determination of stoichiometry and association constant of inclusion complex of N-(2-methylphenyl)-N'-(2-methylphenyl) thiourea with hydroxypropyl-b-cyclodextrin (HP-β-CD) by mean of UV-visible spectrophotometry has been investigated. The stoichiometry of the complex formed was determined by continuous variation (Job's plot) method and the overall association constant and its corresponding uncertainties were determined by using Scott's method.

Key words: Inclusion complex, Cyclodextrin, Chirality, HPLC Chromatography, Partition coefficient, Job's method, Scott's method

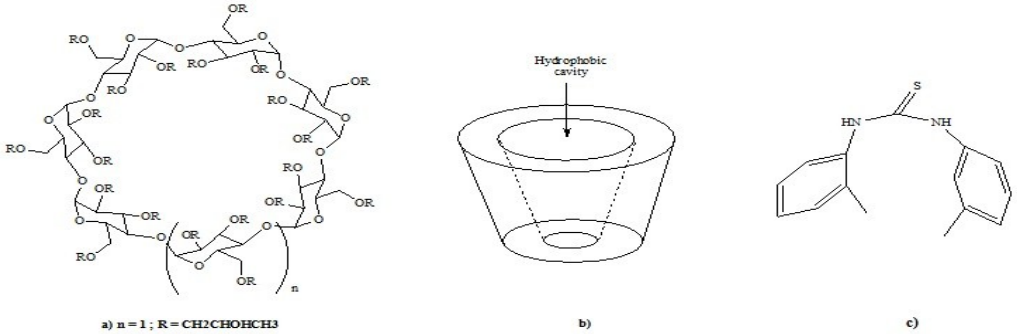
1. Introduction

Cyclodextrins (CD) are cyclic oligosaccharides characterized by a truncated cone shape[1].CD contain a hydrophobic central cavity and a hydrophilic outer surface (Fig. 1). They are well known to be able to interact with poorly-water soluble compounds to form inclusion complexes and to increase their apparent solubilities[2-3].CD derivatives of pharmaceutical interest include, among others, the hydroxypropyl derivatives (HP-α-CD, HP-β-CD and HP-γ-CD), the randomly methylated-CD and sulfobutylether-CD[4-8].

N-(2-methylphenyl)-N'-(2-methylphenyl) thiourea belongs to the thiourea compounds family which are active traps of the heavy metals and play an important role in the field of asymmetric organocatalysis[9].

The present paper deals with the application of spectrophotometric Job's and Scott's methods to study the inclusion complexation of N-(2-methylphenyl)-N'-(2-methylphenyl) thiourea with hydroxypropyl-β-cyclodextrin (HP-β-CD).

Figure1. (a) Chemical structure of hydroxypropyl-β-cyclodextrin (HP-β-CD); (b) Truncated cone shape of HP-β-CD; (c) Structure of N-(2-methylphenyl)-N'-(2-methylphenyl) thiourea.



a) n = 1 ; R = CH2CHOHCH3 b) c)

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2. Experimental

2.1. Chemicals and Reagents

The preparation and purification of N-(2-methylphenyl)-N'-(2-methylphenyl) thiourea substrate has been prepared according to the procedure reported in the literature[10]. HP-β-CD has been purchased from Sigma-Aldrich Company. All solvents and materials used throughout this study areof analytical grade and used as such. All laboratory reagents have been freshly prepared. Water has been purified by triple distillation.

2.2. Apparatus

Double beam UV-1800 (Schimadzu UV-VIS, Japan) spectrophotometer with matched 1 cm quartz cells has been used for all the spectrophotometric measurements. The wavelength of UV detector has been set at 215 nm; the wavelength of maximum absorption for the substrate in solvent.

2.3. Preparation of solutions

N-(2-methylphenyl)-N'-(2-methylphenyl) thiourea standard solution (substrate): An accurately weighed amount (25.6 mg) of the substrate was quantitatively transferred into a 10 mL calibrated flask and dissolved in 5 mL mixture of solvents (water, propan-2-ol, acetonitrile; 1/3 % volume each). The contents of the flask were swirled, sonicated for 5 minutes, and then diluted to the mark with the same solvent to obtain a stock solution of 10 mmol.L^{-1}. This stock solution was stored at 4°C. Later, different dilutions were employed to obtain working solutions in the range of 0.1-10 mmol.L^{-1}.

Hydroxypropyl-β-cyclodextrin (HP-β-CD) standard solution:An accurately weighed amount (146 mg) of HP-β-CD was quantitatively transferred into a 10 mL calibrated flask. The same procedure described above was implemented as well.

2.4. Preparation of inclusion complexes

For each measurement, cyclodextrin and substrate have been mixed and shaken for 10 min to obtain a stable state of solubilisation.

2.5. Standard Curves

For calibration, a 10 mmol.L^{-1}mother solution of substrate in solvent has been used. A series of 5 mL solutions of concentrations between 0.1 and 10 mmol.L^{-1}have been prepared and left at room temperature (25 °C) for 10 min. Absorbance has been measured at 215 nm for each solution against a blank solution.

2.6. Stoichiometry ratio of the complex

Job's method of continuous variation has been employed[11]. Initial (10^{-3}M) concentrations of each HP-β-CD and N-(2-methylphenyl)-N'-(2-methylphenyl) thiourea were prepared. Series of 5mL quantities of HP-β-CD and N-(2-methylphenyl)-N'-(2-methylphenyl) thiourea 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 215 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.

2.7. Association Constant *Ka*

Scott's plot method has been employed[12]. From the same master equimolar (10^{-3} M) aqueoussolutions of HP-β-CD and N-(2-methylphenyl)-N'-(2-methylphenyl) thiourea, serial volumes of 0 to 4.5 mL of HP-β-CD solution have been transferred to different test tubes. 0.25 mL of N-(2-methylphenyl)-N'-(2-methylphenyl) thiourea have been added to each test tube and completed to 5 mL by addition of the necessary volumes of solvent. The procedure has been continued as described in section 2.6.

2.8. Partition coefficient determination

The lipophilicity of N-(2-methylphenyl)-N'-(2-methylphenyl) thiourea has been evaluated from their *n*-hexane–water partition coefficient, K_P, as follows: Equal volumes of freshly prepared substrate/hexane solutions (0.25 mol/L) and of substrate/water solutions have been mixed and vigorously stirred at 37°C for 1 h. The two phases have been separated by brief centrifugation (1000 *g* for 20 s). The substrate concentration in either the hexanic or the aqueous phase has been

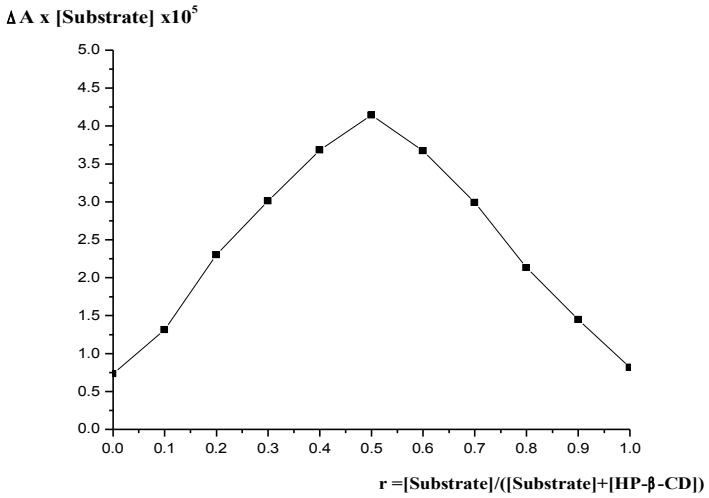
determined by a Schimadzu UV-VIS spectrophotometer (UV-1800). $K_P$has been evaluated as the ratio of the substrate concentration in *n*-hexane to that in water.

3. Results and Discussion

3.1. Determination of the N-(2-methylphenyl)-N'-(2-methylphenyl) thiourea-HP-β-CD inclusion complex stoichiometry by continuous variation method (Job's Plot).

The stoichiometry of N-(2-methylphenyl)-N'-(2-methylphenyl) thiourea-HP-β-CD complex has been determinedby the continuous variation (Job's plot) method.[11]It involves preparing series of solutions containing both the N-(2-methylphenyl)-N'-(2-methylphenyl) thiourea (Substrate) and the HP-β-CD in varying proportions so that a complete range of mole ratios is sampled (0 < r = [Substrate]/[HP-β-CD] + [Substrate] < 1). As well, the total concentration [HP-β-CD] + [Substrate] is kept constant for each solution. The absorbance of the mixtures has been recorded at 215 nm against a convenient blank solution. The plot of ΔA× [Substrate]against the mole fraction of substrate is presented infigure 2. The plot shows a maximumvalue at r = 0.5. The highly symmetrical shape demonstrates the existence of aN-(2-methylphenyl)-N'-(2-methylphenyl) thiourea-HP-β-CD complexwith a 1:1 stoichiometry.

Figure 2. Job's plot (continuous variation method) of N-(2-methylphenyl)-N'-(2-methylphenyl) thiourea with HP-b-CD inclusion complex showing 1:1 stoichiometry. Initial concentration of N-(2-methylphenyl)-N'-(2-methylphenyl) thiourea is 1mM. Absorbance measurements were carried out at 215 nm.



3.2. Determination of the association constant *Ka*

The association constant (*Ka*) of the N-(2-methylphenyl)-N'-(2-methylphenyl) thiourea-HP-β-CD complex has been determined by using Scott's method which is a modification of Benesi-Hildebrand equation[12,14]. Eq. 1 refers the Scott's equation:

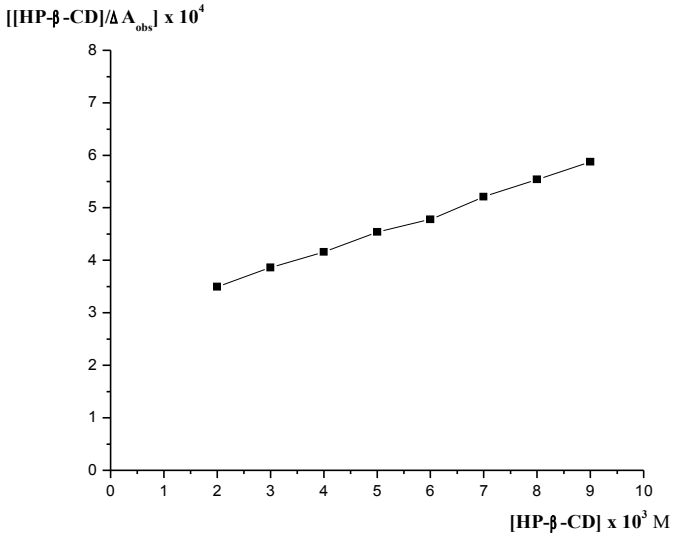
$$[\text{HP-}\beta\text{ CD}]_0/\Delta A_{obs} = [\text{HP-}\beta\text{ -CD}]_0/\Delta A_{max} + 1/Ka.\Delta A_{max} \quad \text{(Eq. 1)}$$

where:

- $[\text{HP-}\beta\text{ -CD}]_0$ is the molar concentration of the HP-β -CD
- ΔA_{obs} is the observed absorbance variation of the N-(2-methylphenyl)-N'-(2-methylphenyl) thiourea in the solution for a given N-(2-methylphenyl)-N'-(2-methylphenyl) thiourea concentration
- $\Delta A_{max}$is the absorbance variation of the N-(2-methylphenyl)-N'-(2-methylphenyl) thiourea between a pure sample of complex and the free N-(2-methylphenyl)-N'-(2-methylphenyl) thiourea at saturation.

In this procedure, the plot of $[HP\text{-}\beta\text{-}CD]_0/\Delta A_{obs}$ against [HP-β-CD] shouldbe linear for 1:1 inclusion complex. The slope of the plot,$(1/\Delta A_{max})$, and the intercept with the vertical axis,$(1/Ka.\Delta A_{max})$, allow the estimation of associationconstant (*Ka*). A typical Scott's plot for the N-(2-methylphenyl)-N'-(2-methylphenyl) thiourea-HP-β-CD inclusioncomplex is shown in figure 3 and the association constant (*Ka*) was calculated to be 120 M^{-1}.

Figure 3. Scott's plot for N-(2-methylphenyl)-N'-(2-methylphenyl) thiourea with HP-β-CD inclusion complex, showing overall association constant *Ka* = 120 M^{-1}, correlation R = 0.9992, standard deviation SD= 3.584.10^{-6}.



3.3. Partition coefficient determination

The use of a lipophylic compound gives the best results due to the lipophylic cavity of the cyclodextrin. Therefore, the lipophylic characteristic will enhance the inclusion of the substrate in thelipophylic cyclodextrin cavity[15,16].

The partition coefficient *Kp* 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[17], *Kp*was found to be 1.6, which indicates a slight preference for N-(2-methylphenyl)-N'-(2-methylphenyl) thiourea towards the lipid phase thus proving the possibility of penetrating the cyclodextrin cavity.

3.4. Reproducibility

The reproducibility of the methods has been determined by replicate analysis of three separate solutions of the working standard. The method gave satisfactory results with relative standard deviations not exceeding 2 %.

4. Conclusion

Cyclodextrins (CDs) act as host molecules to form inclusion complexes with a wide variety of substrate. The relatively hydrophobic cavity of native cyclodextrins and their derivatives enhances the ability to complex substrate molecules of appropriate size and shape. Due to its molecular structure, N-(2-methylphenyl)-N'-(2-methylphenyl) thiourea is a suitable substrate for the macrocycle of β-cyclodextrins.

In this paper, UV-VIS Spectrophotometry was used for the study of interactions betweenN-(2-methylphenyl)-N'-(2-methylphenyl) thiourea and HP-β-CD. Our spectrophotometric experiments confirm that N-(2-methylphenyl)-N'-(2-methylphenyl) thiourea forms 1:1 complex with HP-β-CD.This obtained result has been confirmed for many other similar compounds using NMR spectroscopy[13,14].N-(2-methylphenyl)-N'-(2-methylphenyl) thiourea-HP-β-CD inclusion complex

exhibit high value of the inclusion complex association constant, reflecting the good stability.

The method described herein has many advantages: it does not need expensive sophisticated apparatus, it is simple, rapid, reproducible and it has high sensitivity. Furthermore, all the analytical reagents are inexpensive, and are available in anyanalytical laboratory. These advantages encourage its application. The precision of the method was satisfactory; the values of relative standard deviation did not exceed 2%.

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