



# SPECTROPHOTOMETRIC STUDIES OF THE COMPLEXATION OF 2-ARYL-IMINO-N-(2-ARYL)- THIAZOLINE SUBSTRATES WITH HYDROXYPROPYL- $\gamma$ - CYCLODEXTRIN

INAS EL HASSAN, AHMAD ALLOUCH, ABDEL RAZZAK AL ZEINE,  
MOHAMMAD BOUCHKARA<sup>b</sup>, ADIB ABOU DALLE,  
HANNA EL-NAKAT<sup>a</sup> and FAWAZ EL OMAR<sup>\*</sup>

Laboratory of Applied Chemistry (LAC), Faculty of Science III, Lebanese University,  
P.O. Box 826, TRIPOLI, LEBANON

<sup>a</sup>Department of Chemistry, Faculty of Science, University of Balamand, P.O. Box 100,  
TRIPOLI, LEBANON

<sup>b</sup>Department of Chemistry, University Center of Mascara (29000) ALGERIA

## ABSTRACT

Cyclodextrins molecules can form inclusion complexes with a wide variety of substrates. The relatively hydrophobic cavity of native cyclodextrins and their derivatives induces the ability to complex substrates of appropriate size and shape. Complexation of substrates with cyclodextrins can modify the solubility of the substrate and increase its stability. The most common application of CD in pharmaceutical industry is to enhance drug solubility in aqueous solutions. Inclusion complexes of 2 (2-aryl-imino-N-(2-aryl)-thiazoline) substrates: N-[(2Z)-4-methyl-3-(2-methylphenyl)-1,3-thiazol-2(3H)-ylidene]-N-(2-chlorophenyl) amine (**1**) and N-[(2Z)-4-methyl-3-(2-methylphenyl)-1,3-thiazol-2(3H)-ylidene]-N-(2-chloro-4-nitrophenyl) amine (**2**) with hydroxypropyl- $\gamma$ -cyclodextrin (HP $\gamma$ CD) were investigated by applying a simple and rapid spectrophotometric methods. The 1 : 1 stoichiometry of substrate-CD complexes were determined by continuous variation (Job's plot) method and the overall association constants were determined by using Scott's method. The association constants were determined to be 30866 M<sup>-1</sup> and 5765 M<sup>-1</sup> respectively.

**Key words:** 2-Aryl-imino-N-(2-aryl)-thiazoline, Atropisomerism, Inclusion complexes, Cyclodextrin.

## INTRODUCTION

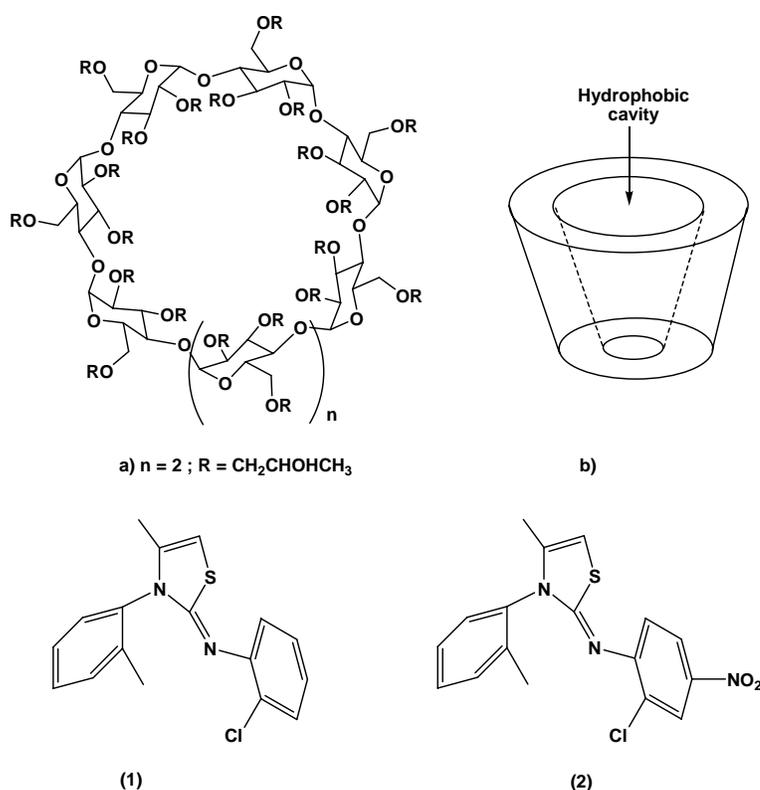
2-Aryl-imino-N-(2-aryl)-thiazoline substrates exist as racemic atropisomers. These heterocyclic substrates play an important role in organic chemistry. They have been

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<sup>\*</sup> Author for correspondence; E-mail: [fomar@ul.edu.lb](mailto:fomar@ul.edu.lb)

developed and studied for a long time due to their biological activities<sup>1-3</sup>. The effect of chirality in bioactive substrates has been recently studied, and stable atropisomers have found applications as ligand for asymmetric catalysis<sup>4,5</sup>.

Cyclodextrins (CD) are cyclic oligosaccharides derived from starch containing 6 ( $\alpha$ ), 7 ( $\beta$ ), 8 ( $\gamma$ ), 9 ( $\delta$ ), 10 ( $\delta$ ) or more glucose residues and characterized by a truncated cone shape<sup>6</sup>. The primary hydroxyls on the narrow side of the cavity can rotate, thus partially blocking the cavity, in contrast to the secondary hydroxyls, which are attached by relatively rigid chains and thus cannot rotate. Substitution of any of the hydrogen bond-forming hydroxyl groups, results in dramatic improvement in their aqueous solubility<sup>7</sup>. CD derivatives of pharmaceutical interest include the hydroxypropyl derivatives (i.e. HP- $\alpha$ -CD, HP- $\beta$ -CD and HP- $\gamma$ -CD), the randomly methylated-CD and sulfobutylether-CD<sup>8-12</sup>.



**Fig. 1: (a) Chemical structure of hydroxypropyl- $\gamma$ -cyclodextrin (HP $\gamma$ CD); (b) Truncated cone shape of HP $\gamma$ CD; (1) N-[(2Z)-4-methyl-3-(2-methylphenyl)-1,3-thiazol-2(3H)-ylidene]-N-(2-chlorophenyl) amine; (2) N-[(2Z)-4-methyl-3-(2-methylphenyl)-1,3-thiazol-2(3H)-ylidene]-N-(2-chloro-4-nitrophenyl) amine**

The inner part of the cavity is hydrophobic<sup>7,13</sup>, as a consequence, CD can encapsulate a variety of poorly water-soluble drugs inside their cavity through non-covalent interactions to form inclusion complexes resulting in an increase of their apparent water solubility<sup>6</sup>. Complexation of pharmaceutical substrates with CD results in altered physicochemical properties of the substrate, such as stability<sup>14</sup>.

There are various methods to study the inclusion complexation like NMR spectroscopy<sup>15</sup>, mass spectrometry and molecular modelling<sup>16</sup>. However, the spectrophotometric method is still the most simple and rapid technique. Accordingly, in this study, the spectrophotometric method was employed to determine the stoichiometries and association constants for the two substrates : N-[(2Z)-4-methyl-3-(2-methylphenyl)-1,3-thiazol-2(3H)-ylidene]-N-(2-chlorophenyl) amine (**1**) and N-[(2Z)-4-methyl-3-(2-methylphenyl)-1,3-thiazol-2(3H)-ylidene]-N-(2-chloro-4-nitrophenyl) amine (**2**).

## EXPERIMENTAL

### Chemicals and reagents

The preparation and purification of N-[(2Z)-4-methyl-3-(2-methylphenyl)-1,3-thiazol-2(3H)-ylidene]-N-(2-chlorophenyl) amine (**1**) and N-[(2Z)-4-methyl-3-(2-methylphenyl)-1,3-thiazol-2(3H)-ylidene]-N-(2-chloro-4-nitrophenyl) amine (**2**) has been described by Bouchkara et-al.,<sup>17</sup> HP- $\gamma$ -CD, hexane and propan-2-ol of analytical grade were purchased from Sigma-Aldrich Co. All solvents and materials used throughout this study were of analytical grade and used as such. All laboratory reagents were freshly prepared. Water was purified by triple distillation.

### Apparatus

Double beam UV-1800 (Schimadzu UV-VIS, Japan) spectrophotometer with matched 1 cm quartz cells was used for all the spectrophotometric measurements. The wavelength of UV detector was set at 254 nm for the two substrates.

### Preparation of solutions

Substrates (**1**) and (**2**) were accurately weighted, transferred to volumetric flasks and dissolved in a solution of 50 : 50 (v/v) hexane-propan-2-ol to make individual stock solutions of 1 mmol/L. The stock solutions were stored at 4°C and were further diluted with the same mixture to obtain working solutions.

### Preparation of inclusion complexes

For each measurement, cyclodextrin and substrate were mixed and shaken at the

temperature of 25°C to obtain a stable state of solubilization.

### Partition coefficient determination

The lipophilicity of the two substrates was evaluated from their *n*-hexane–water partition coefficient,  $K_P$ , as follows: Equal volumes of freshly prepared substrate/hexane solution (0.25 mol/L) and of triple distilled water were mixed and vigorously stirred at 37°C for 1 h. The two phases were separated by brief centrifugation (1000 *g* for 20 s). The substrate concentration in either the hexanic or the aqueous phase was determined by a Shimadzu UV-VIS spectrophotometer (UV-1800).  $K_P$  was evaluated as the ratio of the substrate concentration in *n*-hexane to that in water.

### Absorption spectra

Schimidzu UV-VIS spectrophotometer (UV-1800) was employed to determine the wavelengths of maximum absorption for each of the two solutions of substrate (1) and (2) in a 50 : 50 (v/v) hexane-propan-2-ol mixture.

### Standard curves

For calibration, a 0.1 mmol.L<sup>-1</sup> mother solution of substrate (1) in 50 : 50 (v/v) hexane-propan-2-ol was used. A series of 5 mL solutions of concentrations between 0.01 and 0.1 mmol/L were prepared and left at room temperature for 10 min. Absorbance was measured at 254 nm for each solution against a blank which was prepared simultaneously without substrate. The molar extinction coefficient was determined by measuring the absorbance as a function of concentration. This procedure was repeated for substrate (2) whereas a mother solution of 0.01 mmol/L was prepared and a series of concentrations ranging between 0.001 to 0.01 mmol/L was employed. The extinction coefficients  $\epsilon_1$  and  $\epsilon_2$  for substrates (1) and (2) were found to be 15916 L/mol.cm and 73888 L/mol.cm respectively.

### Stoichiometries ratio of the complexes

**Job's Method:** Job's method of continuous variation was employed<sup>18</sup>. Initial (10<sup>-4</sup> M) concentrations of each HP- $\gamma$ -CD and substrate (1) were prepared. Series of 5 mL quantities of HP- $\gamma$ -CD and (1) were 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 was allowed to stand for 10 min before analysis at 254 nm. This procedure was repeated for substrate (2) with initial (10<sup>-5</sup> M) solutions. 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.

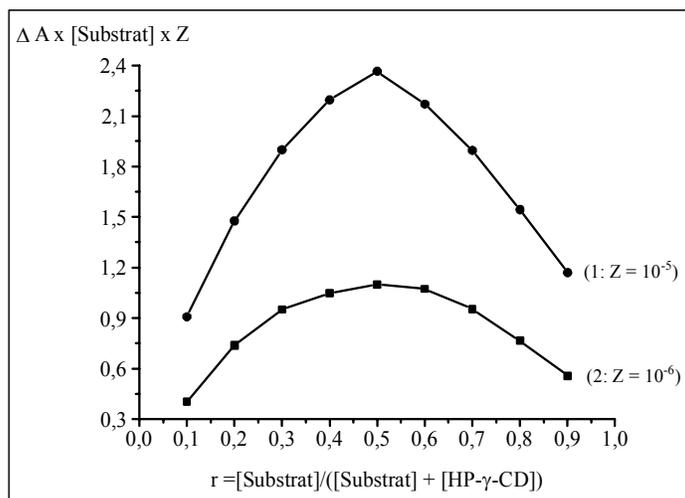
## Association constants $K_a$

Scott's plot method was employed<sup>19</sup>. From the same master equimolar ( $10^{-4}$  M) aqueous solutions of HP- $\gamma$ -CD and substrate (1), serial volumes of 0 to 4.5 mL of HP- $\gamma$ -CD solution were transferred to different test tubes. 0.25 mL of (1) were added to each test tube and completed to 5 mL by addition of the necessary volumes of propan-2-ol. The procedure was continued as described in section 2.8.1. The same procedure was followed for substrate (2) with master equimolar ( $10^{-5}$  M) solutions.

## RESULTS AND DISCUSSION

### Stoichiometries of the complexes

Under the optimum conditions, the stoichiometries of the complexes formation between substrates (1) and (2) with HP- $\gamma$ -CD were investigated. Job's method of the continuous variation was employed<sup>18</sup>. Keeping the sum of the molar concentrations of substrate and CD fixed, the ratio of the concentrations of the two substances in the mixture was varied and the absorbance of the mixtures was recorded at 254 nm against a convenient blank solution prepared for each point of the experiment. As shown in Fig. 2, the substrates (1) and (2) molar ratios which gave maximum absorbance were 0.5, indicating that they react with HP- $\gamma$ -CD in a proportion of 1 : 1.



**Fig. 2: Job's plot (continuous variation method) of substrates: (1 : ●) and (2 : ■) with HP- $\gamma$ -CD inclusion complex showing 1 : 1 stoichiometries. Initial concentrations of substrates 1 and 2 are 0.1 mM and 0.01 mM respectively. Absorbance measurements were carried out at 254 nm**

Taking into consideration the presence of only one cavity in the HP- $\gamma$ -CD molecule and the size of the substrate, it can be postulated that only phenyl ring penetrates in the HP- $\gamma$ -CD cavity resulting in the formation of 1 : 1 substrate-HP- $\gamma$ -CD complexes. This is confirmed by the 1 : 1 ratio obtained. Many studies suggest that the penetration of phenyl ring may take place either from the wider or the narrower rim of the HP- $\gamma$ -CD cavity<sup>15</sup>. As well, the involvement of other parts of the substrate molecule in complexation was ruled out on the basis of the presence of polar groups. The structures of the two 1 : 1 substrate-HP- $\gamma$ -CD inclusion complexes were characterized as shown in Fig 4.

### Determination of association constants $K_a$

The association constants ( $K_a$ ) of the substrate-HP- $\gamma$ -CD complexes were determined by using Scott's method. Eq. 1 refers the Scott's equation: where  $[\text{HP-}\gamma\text{-CD}]_0$  is the molar concentration of the HP- $\gamma$ -CD,  $\Delta A_{\text{obs}}$  the observed absorbance variation of the substrate in the mixture for a given substrate concentration,  $\Delta A_{\text{max}}$  the absorbance variation of the substrate between a pure sample of complex and the free substrate at saturation.

$$[\text{HP-}\gamma\text{-CD}]_0/\Delta A_{\text{obs}} = [\text{HP-}\gamma\text{-CD}]_0/\Delta A_{\text{max}} + 1/K_a \cdot \Delta A_{\text{max}} \quad \dots(1)$$

In this procedure, the plot of  $[\text{HP-}\gamma\text{-CD}]_0/\Delta A_{\text{obs}}$  against  $[\text{HP-}\gamma\text{-CD}]$  should be linear for 1 : 1 inclusion complexes. The slope of the plot ( $1/\Delta A_{\text{max}}$ ) and the intercept with the vertical axis ( $1/K_a \cdot \Delta A_{\text{max}}$ ) allow the estimation of association constant ( $K_a$ ). A typical Scott's plot for the two substrate-HP- $\gamma$ -CD inclusion complexes is shown in Fig. 3 and the association constants ( $K_a$ ) were calculated to be 30866 M<sup>-1</sup> and 5765 M<sup>-1</sup> for substrates (1) and (2) respectively.

### Partition coefficient determination

The inclusion of a substrate in cyclodextrin molecule greatly depends on its polarity. In fact, the hydrophobic characteristic will enhance the inclusion of the substrate in the hydrophobic cyclodextrin cavity<sup>20,21</sup>.

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 if the inclusion process is possible.

Using Spectrophotometry<sup>22</sup>,  $K_p$  were found to be 29 and 26 for substrate (1) and (2) respectively, which indicate a preference for N-[(2Z)-4-methyl-3-(2-methylphenyl)-1,3-thiazol-2(3H)-ylidene]-N-(2-chlorophenyl)amine and N-[(2Z)-4-methyl-3-(2-methylphenyl)-1,3-thiazol-2(3H)-ylidene]-N-(2-chloro-4-nitrophenyl)amine towards the lipid phase thus



## CONCLUSION

The determination of the stoichiometries and the association constants of complexes is an important subjects in analytical chemistry and other branches of chemistry. UV-VIS Spectrophotometry was used for the study of interactions between substrates (1) and (2) and HP- $\gamma$ -CD.

The substrate-HP- $\gamma$ -CD inclusion complexes exhibit high values of the inclusion complex association constants, reflecting the good stability. Our experiments confirm that our two substrates form 1 : 1 complexes with HP- $\gamma$ -CD indicating a good interaction. However poor solubility continues to impact the development of a large number of potential non-water soluble drug candidates<sup>23</sup>. This factor has a significant impact on what is required to use CD molecules as a true added value in this context. These starch derivatives are useful solubilizers, increasing the bioavailability of substrate through an increase in its apparent solubility.

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