

# Study of the Inclusion Complexation of Piroxicam - $\beta$ - Cyclodextrin and Determination of the Stability Constant ( $K$ ) by UV-Visible Spectroscopy

N. Gharib Naseri\*, A. Ashnagar<sup>1</sup> and F. Husseini<sup>1</sup>

Over the past four decades, interest in the physical and chemical properties of inclusion complexes has grown considerably. One of the most important reasons for this is the relevance that inclusion complexes have to enzyme-substrate and drug-receptor interactions. Inclusion complexation between the drug piroxicam and  $\beta$ -cyclodextrin was investigated by using the simple and easily accessible UV-visible spectroscopy technique, and the stability constants of the inclusion complexes at two different concentrations of the guest molecule were calculated. The stability constant of the inclusion complex with a diluted solution of the piroxicam drug was  $\bar{K} = 24.75 \pm 5.89 \text{ mol}^{-1} \cdot \text{L}$  at  $\lambda_{\text{max}} = 352 \text{ nm}$  and that of the saturated solution of piroxicam was calculated to be:  $K = 69.35 \pm 5.65 \text{ mol}^{-1} \cdot \text{L}$  at  $\lambda_{\text{max}} = 285 \text{ nm}$  and  $K = 56.34 \pm 8.34 \text{ mol}^{-1} \cdot \text{L}$  at  $\lambda_{\text{max}} = 251 \text{ nm}$ .

## INTRODUCTION

Cyclodextrins are oligosaccharides produced by the action of the amylase of *Bacillus macerans* on starch related compounds. They consist of (+)-D-glucopyranose units joined to each other by  $\alpha$ -(1  $\rightarrow$  4)-linkages. Cyclodextrins are toroidal in shape with all the glucose units in substantially undistorted C<sub>1</sub> (D) chair conformations [1]. The interior of the torus consists only of a ring of C-H groups, a ring of glucosidic oxygens and another ring of C-H groups, rendering the interior of the torus relatively non-polar compared to water. There are three predominant types of cyclodextrins;  $\alpha$ -cyclodextrin ( $\alpha$ -CD), which consists of six,  $\beta$ -cyclodextrin ( $\beta$ -CD), which consists of seven and  $\gamma$ -cyclodextrin ( $\gamma$ -CD), which has eight D-(+)-glucopyranose units, respectively (Figure 1). Cyclodextrins are conveniently depicted by a truncated cone where the narrow end represents the primary hydroxyl groups attached at C-6 positions and the wide end

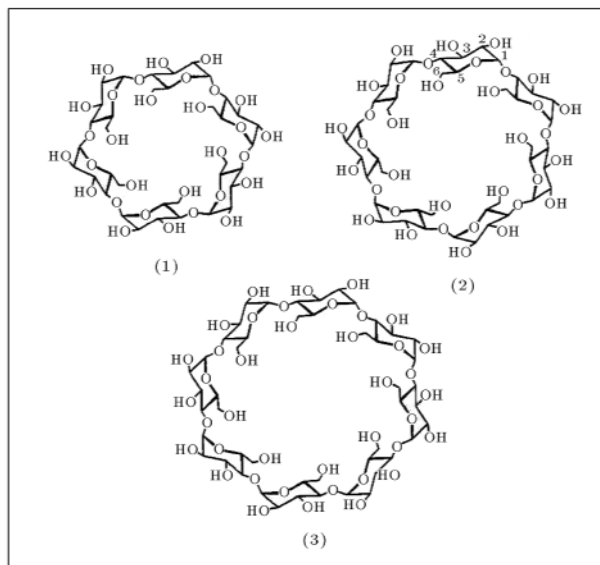


Figure 1. Natural (native) cyclodextrins.

represents the secondary hydroxyl groups attached at C-2 and C-3 positions (Figure 2).

The most interesting property of cyclodextrins is that they contain a hydrophobic cavity, which can encapsulate a guest molecule to form an inclusion complex (Figure 3).

\*. Corresponding Author, Ahwaz Faculty of Petroleum Engineering, Petroleum University of Technology, Ahwas, I.R. Iran.

1. School of Pharmacy, Ahwaz Jundi Shapour University of Medical Sciences, Ahwas, I.R. Iran.

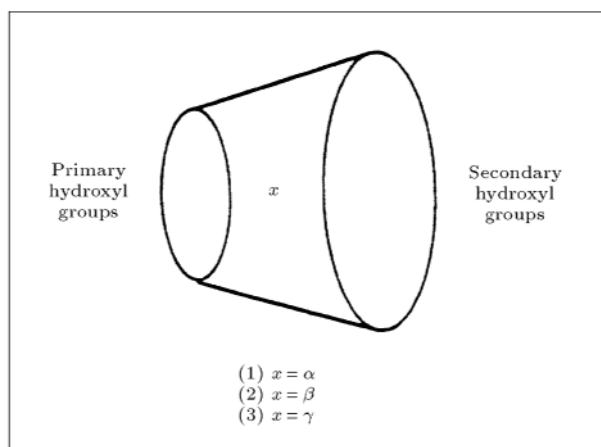


Figure 2. Truncated cone representing cyclodextrins.

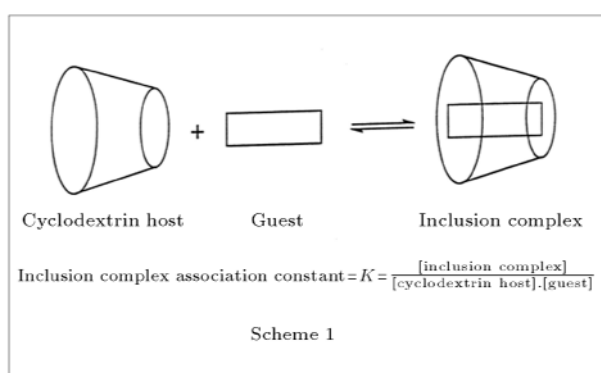


Figure 3. Schematic of inclusion complex formation.

Cyclodextrins are known to form inclusion complexes with a variety of guest molecules in solution and in a solid state, since their inherent annular structure exists stably in both phases. Inclusion complexes are chemical species consisting of two or more associated molecules, in which one of the molecules, the “host”, forms or possesses a cavity into which it can admit a “guest” molecule, resulting in a stable association without formation of any covalent bonds. Secondary forces are alone responsible for maintenance of the integrity of all inclusion complexes. The molecular ratio of guest to cyclodextrin is usually found to be 1:1 [2,3], however, this can change, depending on the shape and geometry of the guest and the cyclodextrin. The minimum requirement for an inclusion complex formation is a size compatibility between host and guest molecules, i.e., guest molecules must fit, entirely or at least partially, into the cyclodextrin cavity. In general, hydrophobic molecules or residues, rather than hydrophilic ones, have a higher affinity to the cyclodextrin cavity in aqueous solution, because the cavity provides a microheterogeneous matrix in such a polar solvent. Inclusion complex formation in aqueous media usually involves the displacement of water molecules

from the cavity of the cyclodextrin and subsequent replacement with the guest molecule. A vast array of guest molecules has been seen to be included in the cavities of cyclodextrins, ranging from polar amines, acids and ions to hydrophobic non-polar aliphatic and aromatic hydrocarbons [4,5]. An inclusion complex can be thermodynamically quite stable and each is characterized by a thermodynamic stability constant or association constant,  $K$ , defined by the position of equilibrium between the host, the guest and the complex (equation in Figure 3).

In the pharmaceutical field, the number of papers and patents dealing with the practical applications of cyclodextrins has also shown an explosive increase during the past three decades [6-12]. This is probably due to the following facts:

1. Fundamental research furnishes information about host-guest interactions;
2. Pure cyclodextrins can be obtained on a large scale;
3. Cyclodextrins can scarcely be considered as having toxic action.

Although the natural cyclodextrins have been extensively used in pharmaceutical formulation, they have some undesirable properties as drug carriers [13,14]. The limited application of natural cyclodextrins in the pharmaceutical formulation seems to be related to their relatively low aqueous solubility, particularly in the case of  $\beta$ -cyclodextrin (1.8 w/v% at 25°C).

The narrow end represents the primary hydroxyl groups attached at C-6 positions.

The wide end represents the secondary hydroxyl groups attached at C-2 and C-3 positions.

## EXPERIMENTS

The experimental part of this research work in comprised of three different steps:

- (i) Determination of the proper pH,
- (ii) Determination of the maximum absorbance wavelength ( $\lambda_{\max}$ ),
- (iii) Determination of the stability constant of the inclusion complex.

### Preparation of Phosphate Buffer Solution with pH 7.4

Potassium dihydrogen phosphate (1.2 g, 8.8 mmol) and disodium hydrogen phosphate dodecahydrate (10.89 g, 30.4 mmol) were dissolved in deionized water in a 2000 mL volumetric flask and the volume was made up to 2000 mL by adding more deionized water. The pH was measured and confirmed to be 7.4

### Preparation of Phosphate Buffer Solution with pH 7

#### Solution A

Potassium dihydrogen phosphate (0.908 g, 6.67 mmol) was dissolved in deionized water in a 100 mL volumetric flask and the volume was made up to 100 mL by adding more deionized water.

#### Solution B

Disodium hydrogen phosphate dodecahydrate (2.38 g, 6.65 mmol) was dissolved in deionized water in a 100 mL volumetric flask and the volume was made up to 100 mL by adding more deionized water.

Then, 38.9 mL of Solution A was added to 61.1 mL of Solution B and stirred well. The pH was measured and confirmed to be 7.

### Preparation of Citrate Buffer Solution with pH 6.5

Disodium hydrogen phosphate dodecahydrate (7.16 g, 19.98 mmol) and sodium dihydrogen phosphate (2.43 g, 20.25 mmol) were dissolved in deionized water in a 1000 mL volumetric flask and the volume was made up to 1000 mL by adding more deionized water. The pH was measured and confirmed to be 6.5.

### Preparation of Diluted Piroxicam Solution (I) [ $1.024 \times 10^{-3}$ mol.L<sup>-1</sup>]

Piroxicam (17 mg, 51.2  $\mu$ L) was dissolved in the desired buffer solution in a 50 mL volumetric flask and the volume was made up to 50 mL by adding more of the buffer solution used. This procedure was carried out for each of the three buffer solutions (pH 7.4, 7, 6.5).

### Preparation of Piroxicam Solution (II) [ $3.62 \times 10^{-3}$ mol.L<sup>-1</sup>] (Almost Saturated Solution)

Piroxicam (60 mg, 181  $\mu$ L) was dissolved in the desired buffer solution in a 50 mL volumetric flask and the volume was made up to 50 mL by adding more of the buffer solution used. This procedure was carried out for each of the three buffer solutions (pH 7.4, 7, 6.5).

### Preparation of $\beta$ -Cyclodextrin Stock Solution

$\beta$ -cyclodextrin (0.4494 g, 0.396 mmol) was dissolved in the desired buffer solution in a 25 mL volumetric flask and the volume was made up to 25 mL by adding more of the buffer solution used. This procedure was carried out for each of the three buffer solutions (pH 7.4, 7, 6.5).

### Determination of the Proper pH

#### (i) pH 7.4

5 mL of piroxicam solution (II) [ $3.62 \times 10^{-3}$  mol.L<sup>-1</sup>] was added to each of the five various concentrations of  $\beta$ -cyclodextrin (Table 1) in 5 mL volumetric flasks and the final volume was made up to 10 mL by adding more of the buffer solution (pH 7.4). The solutions were kept at room temperature for 2 hours and occasionally were shaken, then filtered, through filter paper. Finally, 2.95 mL of the buffer solution with pH 7.4 was added to 50  $\mu$ L of each filtrate. The UV visible spectrum of each of the solutions was taken at wavelengths 200-400 nm. This procedure was repeated 3 times at 15 minute intervals.

#### (ii) pH 7 and (iii) pH 6.5

Exactly the same amounts were used and the same procedure was carried out as described in (i).

### Determination of $\lambda_{\max}$

The measurements were made with pH 7.4 buffer solution. 166  $\mu$ L of the piroxicam solution (I) [ $1.024 \times 10^{-3}$  mol.L<sup>-1</sup>] was added to each of the  $\beta$ -cyclodextrin concentrations (Table 2) in a 5 mL volumetric flask and the final volume was made up to 5 mL by adding more of the buffer solution (pH 7.4). The UV-visible spectrum of each of the solutions was taken at wavelength 200 - 400 nm) at 25°C. From the recorded spectra, it was found that  $\lambda_{\max}$  is 352 nm.

### Determination of the Stability Constant (K) by Using Diluted Piroxicam Solution (I).

Piroxicam solution (I) [166  $\mu$ L,  $1.024 \times 10^{-3}$  mol.L<sup>-1</sup>] was added to each of the diluted solutions of  $\beta$ -cyclodextrin given in Table 2 in 5 mL volumetric flasks and the volume was made up to 5 mL by adding more of the buffer solution with pH 7.4. The flasks were kept at room temperature for 2 hours, then filtered on filter paper. Each time, the filtrate was placed

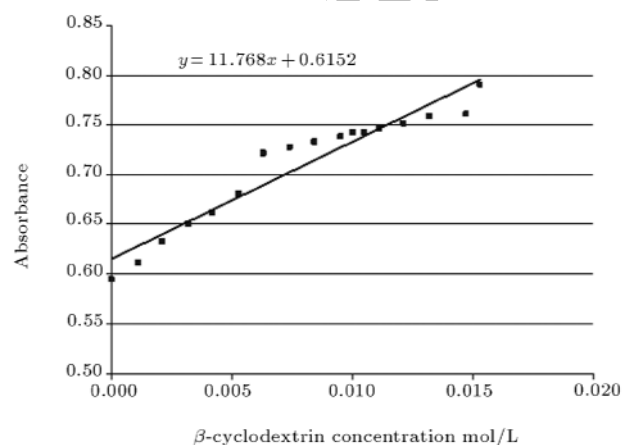
**Table 1.** Solutions of the host ( $\beta$ -cyclodextrin) for complexation with almost saturated piroxicam solution (II).

| Volume of Stock Solution Taken to Make 10 mL Final Solution (mL) | Molar Concentration of Diluted $\beta$ -Cyclodextrin (mol.L <sup>-1</sup> ) |
|--|---|
| 0.632  | 0.0010  |
| 1.264  | 0.0020  |
| 1.582  | 0.0025  |
| 3.164  | 0.0050  |
| 4.746  | 0.0075  |

**Table 2.** Solutions of the host ( $\beta$ -cyclodextrin) for complexation with diluted piroxicam solution (I).

| Volume of Stock Solution Taken to Make 5 mL Final Solution (mL) | Molar Concentration of Diluted $\beta$ -cyclodextrin (mol.L <sup>-1</sup> ) |
|---|---|
| 0.347   | 0.0011  |
| 0.662   | 0.0021  |
| 1.010   | 0.0032  |
| 1.325   | 0.0042  |
| 1.672   | 0.0053  |
| 1.980   | 0.0063  |
| 2.330   | 0.0074  |
| 2.650   | 0.0084  |
| 2.990   | 0.0095  |
| 3.314   | 0.0105  |
| 3.500   | 0.0111  |
| 3.810   | 0.0121  |
| 4.160   | 0.0132  |
| 4.640   | 0.0147  |
| 4.820   | 0.0153  |

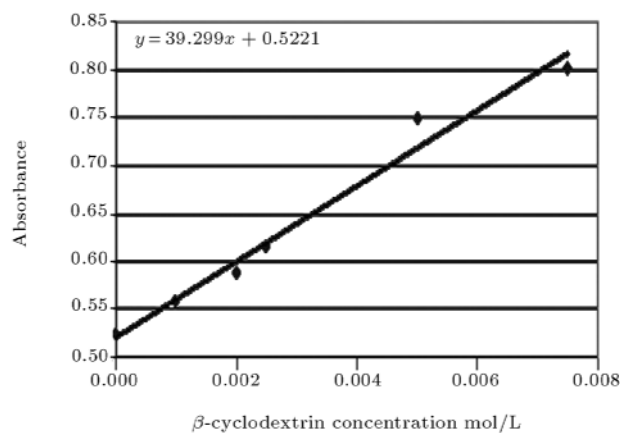
in a UV-visible cell at 25°C and, finally, the UV-visible spectrum was taken at wavelengths 200-400 nm. The absorbance at  $\lambda_{\max} = 352$  nm of each of the various concentrations of  $\beta$ -cyclodextrin added to the piroxicam solution was recorded (Table 3) and then plotted versus  $\beta$ -cyclodextrin (Figure 4). For each entry of Table 2, the measurements were repeated 3 times at 15 minute intervals. On the basis of  $\lambda_{\max} = 352$  nm of piroxicam solution and various concentrations of  $\beta$ -cyclodextrin, the stability constant was calculated, as given in the results and discussion section of this article (Table 4).

**Figure 4.** Observed linear absorption diagram of inclusion complexation of beta cyclopextrin and diluted solution of Piroxicam (I).**Table 3.** Absorbance of the inclusion complex formed by addition of various concentrations of  $\beta$ -cyclodextrin to diluted piroxicam solution (I) with pH 7.4.

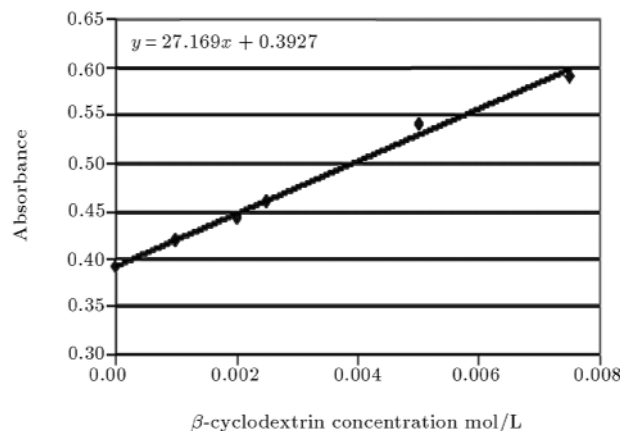
| $\beta$ -Cyclodextrin (mol.L <sup>-1</sup> ) | Absorbance (A)            |                           |                           |
|--|---------------------------|---------------------------|---------------------------|
|  | $\lambda_{\max} = 352$ nm | $\lambda_{\max} = 285$ nm | $\lambda_{\max} = 251$ nm |
| 0.0000                                       | 0.595                     | 0.465                     | 0.570                     |
| 0.0011                                       | 0.611                     | 0.471                     | 0.582                     |
| 0.0021                                       | 0.633                     | 0.483                     | 0.586                     |
| 0.0032                                       | 0.650                     | 0.498                     | 0.605                     |
| 0.0042                                       | 0.662                     | 0.525                     | 0.612                     |
| 0.0053                                       | 0.680                     | 0.542                     | 0.617                     |
| 0.0063                                       | 0.721                     | 0.548                     | 0.618                     |
| 0.0074                                       | 0.728                     | 0.552                     | 0.622                     |
| 0.0084                                       | 0.733                     | 0.568                     | 0.626                     |
| 0.0095                                       | 0.738                     | 0.569                     | 0.637                     |
| 0.0100                                       | 0.742                     | 0.578                     | 0.645                     |
| 0.0105                                       | 0.742                     | 0.589                     | 0.652                     |
| 0.0111                                       | 0.746                     | 0.579                     | 0.660                     |
| 0.0121                                       | 0.751                     | 0.626                     | 0.663                     |
| 0.0132                                       | 0.758                     | 0.629                     | 0.676                     |
| 0.0147                                       | 0.760                     | 0.629                     | 0.676                     |
| 0.0153                                       | 0.790                     | 0.635                     | 0.710                     |

#### Determination of the Stability Constant ( $K$ ) by Using an Almost Saturated Solution of Piroxicam (II)

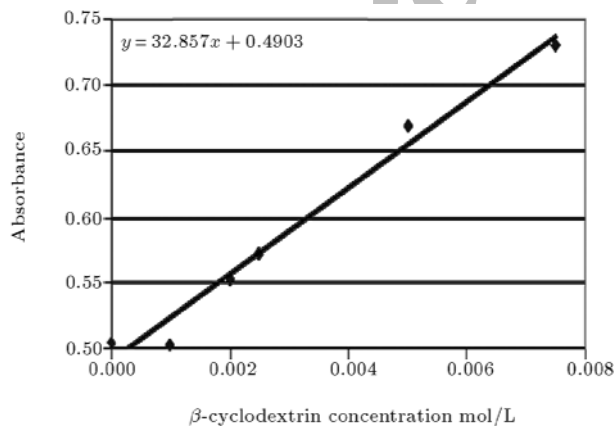
Piroxicam solution (II) [5 mL, with a concentration of  $3.62 \times 10^{-3}$  mol.L<sup>-1</sup>] (almost saturated solution) was added to each of the diluted solutions of  $\beta$ -cyclodextrin given in Table 1 in 10 mL volumetric flasks and the volume was made up to 10 mL by adding more of the buffer solution with pH 7.4. The concentration of diluted piroxicam solution (II) became [ $1.81 \times 10^{-3}$  mol.L<sup>-1</sup>]. The flasks were kept at room temperature for 2 hours, then, filtered on filter paper. 50  $\mu$ L of the inclusion complex solution was added to 2.95 mL of the buffer solution with pH 7.4 (concentration of more diluted piroxicam solution (II) became [ $3.02 \times 10^{-5}$  mol.L<sup>-1</sup>]). Each time, the filtrate was placed in a UV-visible cell at 25°C and, finally, the UV-visible spectrum was taken at wavelength 200-400 nm. The absorbances at  $\lambda_{\max} = 352, 285$  and 251 nm, of each of the various concentrations of  $\beta$ -cyclodextrin added to the piroxicam solution, were recorded (Tables 5 to 7) and then plotted versus  $\beta$ -cyclodextrin concentration (Figures 5 to 7). For each entry of Table 1, the measurements were repeated 3 times at 15-minute intervals. On the basis of  $\lambda_{\max} = 352, 285$  and 251 nm of piroxicam solution and various concentrations of  $\beta$ -cyclodextrin, the stability constants were calculated, as



**Figure 5.** Observed linear absorption diagram of inclusion complexation of  $\beta$ -cyclodextrin and saturated solution of piroxicam (II) at pH 7.4 and maximum absorption wavelength 352 nm.



**Figure 6.** Observed linear absorption diagram of inclusion complexation of  $\beta$ -cyclodextrin and saturated solution of piroxicam (II) at pH 7.4 and maximum absorbance wavelength 285 nm.



**Figure 7.** Observed linear absorption diagram of inclusion complexation of  $\beta$ -cyclodextrin and saturated solution of piroxicam (II) at pH 7.4 and maximum absorption wavelength 251 nm.

**Table 4.** Calculated stability constant ( $K$ ) of the inclusion complex between  $\beta$ -cyclodextrin and diluted piroxicam solution (I) [ $3.39 \times 10^{-5}$  mol.L $^{-1}$ ] at pH 7.4 and  $\lambda_{\max} = 352$  nm.

| Molar Concentration of Diluted $\beta$ -cyclodextrin (mol.L $^{-1}$ ) | Calculated Stability Constant ( $K$ ) mol $^{-1}$ .L |
|---|--|
| 0.0011  | 24.5   |
| 0.0021  | 30.64  |
| 0.0032  | 28.94  |
| 0.0042  | 26.90  |
| 0.0053  | 26.98  |
| 0.0063  | 33.60  |
| 0.0074  | 30.20  |
| 0.0084  | 27.65  |
| 0.0095  | 25.35  |
| 0.0105  | 23.50  |
| 0.0111  | 22.90  |
| 0.0121  | 21.69  |
| 0.0132  | 20.79  |
| 0.0147  | 18.87  |
| 0.0153  | 21.40  |

**Table 5.** Absorbance of the inclusion complex formed by addition of various concentrations of  $\beta$ -cyclodextrin to saturated piroxicam solution (II) with pH 7.4.

| $\beta$ -Cyclodextrin (mol.L $^{-1}$ ) | Absorbance ( $A$ )        |                           |                           |
|--|---------------------------|---------------------------|---------------------------|
|  | $\lambda_{\max} = 352$ nm | $\lambda_{\max} = 285$ nm | $\lambda_{\max} = 251$ nm |
| 0.0000                                 | 0.525                     | 0.392                     | 0.505                     |
| 0.0010                                 | 0.560                     | 0.420                     | 0.503                     |
| 0.0020                                 | 0.589                     | 0.443                     | 0.553                     |
| 0.0025                                 | 0.616                     | 0.460                     | 0.573                     |
| 0.0050                                 | 0.748                     | 0.540                     | 0.668                     |
| 0.0075                                 | 0.802                     | 0.590                     | 0.731                     |

given in the results and discussion section of this article (Table 8).

## RESULTS AND DISCUSSION

Because of the therapeutic importance of the piroxicam drug (IV), [4-hydroxy-2-methyl-N-(2-pyridyl)-2H-1,2-benzothiazine-3-carboxamide 1,1,-dioxide] (Scheme 1) and its widespread use, it was decided to study its inclusion complexation with  $\beta$ -cyclodextrin and determine the stability constant ( $K$ ) of the complex by UV-visible spectroscopy at two different concentrations; i.e. diluted and almost saturated solutions of piroxicam.

**Table 6.** Absorbance of the inclusion complex formed by addition of various concentrations of  $\beta$ -cyclodextrin to saturated piroxicam solution (II) with pH 7.

| $\beta$ -Cyclodextrin<br>(mol.L <sup>-1</sup> ) | Absorbance (A)               |                              |                              |
|---|------------------------------|------------------------------|------------------------------|
|   | $\lambda_{\max} = 352$<br>nm | $\lambda_{\max} = 285$<br>nm | $\lambda_{\max} = 251$<br>nm |
| 0.0000  | 0.232                        | 0.169                        | 0.225                        |
| 0.0010  | 0.235                        | 0.172                        | 0.228                        |
| 0.0020  | 0.238                        | 0.174                        | 0.230                        |
| 0.0025  | 0.245                        | 0.180                        | 0.238                        |
| 0.0050  | 0.279                        | 0.215                        | 0.271                        |
| 0.0075  | 0.295                        | 0.237                        | 0.293                        |

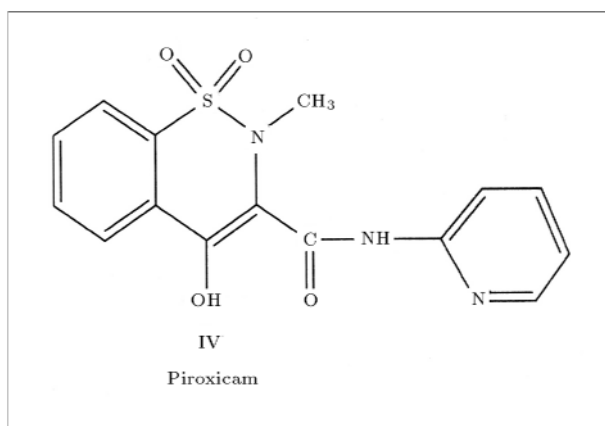
**Table 7.** Absorbance of the inclusion complex formed by addition of various concentrations of  $\beta$ -cyclodextrin to saturated piroxicam solution (II) with pH 6.5.

| $\beta$ -Cyclodextrin<br>(mol.L <sup>-1</sup> ) | Absorbance (A)               |                              |                              |
|---|------------------------------|------------------------------|------------------------------|
|   | $\lambda_{\max} = 352$<br>nm | $\lambda_{\max} = 285$<br>nm | $\lambda_{\max} = 251$<br>nm |
| 0.0000  | 0.116                        | 0.124                        | 0.142                        |
| 0.0010  | 0.118                        | 0.127                        | 0.156                        |
| 0.0020  | 0.119                        | 0.129                        | 0.162                        |
| 0.0025  | 0.148                        | 0.137                        | 0.178                        |
| 0.0050  | 0.166                        | 0.165                        | 0.208                        |
| 0.0075  | 0.175                        | 0.169                        | 0.211                        |

**Table 8.** Calculated stability constant ( $K$ ) of the inclusion complex between  $\beta$ -cyclodextrin and saturated piroxicam solution (II) [ $3.02 \times 10^{-5}$  mol.L<sup>-1</sup>] at pH 7.4 and  $\lambda_{\max} = 352, 285$  and  $251$  nm.

| $\beta$ -Cyclodextrin<br>(mol.L <sup>-1</sup> ) | $K$ (mol <sup>-1</sup> .L)   |                              |                              |
|---|------------------------------|------------------------------|------------------------------|
|   | $\lambda_{\max} = 352$<br>nm | $\lambda_{\max} = 285$<br>nm | $\lambda_{\max} = 251$<br>nm |
| 0.0010  | 66.9                         | 68.8                         | 49.6                         |
| 0.0020  | 61.5                         | 63.7                         | 48.0                         |
| 0.0025  | 70                           | 68.36                        | 53.96                        |
| 0.0050  | 85.76                        | 75.0                         | 65.2                         |
| 0.0075  | 70.66                        | 67.0                         | 59.78                        |

The work was carried out at three different pHs of 6.5, 7 and 7.4. At each of the pHs, various concentrations of  $\beta$ -cyclodextrin (Table 2) were added to a constant amount of the piroxicam drug and then the UV-visible spectrum was recorded at 200-400 nm at 25°C. The results are given in Table 4. Based on these results, it was concluded that the absorbance for the complex formation was highest in the phosphate buffer solution with pH 7.4, which is the pH of biological fluids. Therefore, it can be concluded that the proper

**Scheme 1.** Piroxicam, [4-hydroxy-2-methyl-N-(2-pyridyl)-2H-1,2-benzothiazine-3-carboxamide,1,1-dioxide].

pH for the inclusion complex formation between  $\beta$ -cyclodextrin and piroxicam was pH 7.4.

The second task in this research was to find out the optimum  $\lambda_{\max}$ . Since the proper pH was found to be 7.4, all the experiments for the determination of  $\lambda_{\max}$  were carried out in buffer solution with pH 7.4. After adding various concentrations of  $\beta$ -cyclodextrin solutions (Table 2) to a constant amount of piroxicam, the UV visible spectra were recorded at 200-400 nm. On the basis of the results obtained, it is concluded that the  $\lambda_{\max}$  is 352, 285 and 251 nm.

The third and main objective of this research was to determine the stability constant or association constant, thermodynamic constant ( $K$ ) of the inclusion complex between  $\beta$ -cyclodextrin and piroxicam at  $\lambda_{\max} = 352$  nm in a phosphate buffer solution with pH 7.4. Since two different concentrations of piroxicam were used, different values of  $K$  were determined, which are explained below.

#### *i) Calculation of the Stability Constant ( $K$ ) by Using Diluted Solution of Piroxicam (I)*

As explained in the previous section (Determination of  $\lambda_{\max}$ ), the stability constant can be calculated based on the results given in Table 3 and also from the graph in Figure 3. The ( $K$ ) value can be calculated, as follows.

In accordance with the Beer-Lambert law:

$$A = \epsilon.l.c.$$

Then, the following steps were considered:

1. The initial concentration of piroxicam used was  $1.024 \times 10^{-3}$  mol.L<sup>-1</sup>, after dilution in the UV-visible cell:

$$[\text{Piroxicam}] = C = 3.39 \times 10^{-5} \text{ mol.L}^{-1}.$$

2. For calculating  $\epsilon$ , by using Figure 4, when no  $\beta$ -cyclodextrin was used, i.e.  $[\beta\text{-cyclodextrin}] =$

0.000, the absorbance could be read to be 0.595, therefore:

$$A = 0.595, \quad l = 1 \text{ cm},$$

$$[\text{Piroxicam}] = C = 3.39 \times 10^{-5} \text{ mol.L}^{-1},$$

$$\varepsilon = \frac{0.595}{(1 \text{ cm} \times 3.39 \times 10^{-5} \text{ mol.L}^{-1})},$$

$$\varepsilon = 17551.62 \text{ mol}^{-1} \cdot \text{cm}^{-1} \cdot \text{L}.$$

3. The stability constant can be determined from the following formula;

$$K = \frac{A_{\text{complex}}}{A_{\text{pure Guest}} \times [\beta \text{ cyclodextrin}]_{\text{Free}}}.$$

From Figure 4,  $A_{\text{complex}}$ , for various concentrations of  $\beta$ -cyclodextrin, can be calculated, e.g., for the second entry of Table 3 at  $\lambda_{\text{max}} = 352 \text{ nm}$ , one will have:

$$[\beta \text{ cyclodextrin}] = 0.0011 \text{ mol.L}^{-1},$$

$$A_{(\text{guest}+\beta \text{ cyclodextrin})} = 0.611.$$

Therefore:

$$\begin{aligned} A_{\text{complex}} &= A_{(\text{guest}+\beta \text{ cyclodextrin})} - A_{\text{pure Guest}} \\ &= 0.611 - 0.595 = 0.016, \end{aligned}$$

$$A_{\text{complex}} = 0.016.$$

4. Now, the concentration of the complex can be calculated:

$$C_{\text{complex}} = \frac{0.016}{17551.62 \text{ mol}^{-1} \cdot \text{cm}^{-1} \cdot \text{L} \times 1 \text{ cm}},$$

So:

$$C_{\text{complex}} = 9.11 \times 10^{-7} \text{ mol.L}^{-1}.$$

- 5.

$$[\beta \text{ cyclodextrin}]_{\text{Free}} = [\beta \text{ cyclodextrin}]_{\text{initial}} - [\beta \text{ cyclodextrin}]_{\text{used}}.$$

On the other hand:

$$[\beta \text{ cyclodextrin}]_{\text{used}} = [C_{\text{complex}}].$$

Therefore:

$$[\beta \text{ cyclodextrin}]_{\text{Free}} = [\beta \text{ cyclodextrin}]_{\text{initial}} - [C_{\text{complex}}],$$

$$\begin{aligned} [\beta \text{ cyclodextrin}]_{\text{Free}} &= 0.0011 - 9.11 \times 10^{-7} \\ &= 1.09 \times 10^{-3} \text{ mol.L}^{-1}. \end{aligned}$$

6. Now,  $K$  can be calculated by substituting the values into the equation given in Step 3:

$$K = \frac{0.016}{0.595 \times 1.09 \times 10^{-3} \text{ mol.L}^{-1}}.$$

Therefore, one would have:  $K = 24.5 \text{ mol}^{-1} \cdot \text{L}$ .

Then, the  $K$  value for each entry of Table 2 and Figure 4, was calculated as above and the results summarized in Table 4.

According to the data given in Table 4, the range of the stability constant is:

$$30.64 - 18.87 = 11.77.$$

Therefore:

$$\bar{K} = 24.75 \pm 5.89 \text{ mol}^{-1} \cdot \text{L}.$$

#### ii) Calculation of the Stability Constant ( $K$ ) by Using Saturated Solution of Piroxicam (II)

It is well known that when the absorption spectrum of a guest molecule does not change significantly, due to inclusion complexation, then, stirring the host molecule into an excess amount of the guest molecule results in the enhancement of the absorption of the final solution relative to the saturated solution of the guest molecule. Therefore, it was decided to investigate this fact and calculate the stability constant of the inclusion complex at a higher concentration of piroxicam.

Exactly the same procedure as in the previous section was carried out for calculating the new stability constant:

$$\begin{aligned} [\text{piroxicam}]_{\text{initial}} \\ &= 3.62 \times 10^{-3} \text{ mol.L}^{-1} \text{ almost saturated solution.} \end{aligned}$$

After diluting twice:

$$[\text{piroxicam}]_{\text{diluted}} = 3.02 \times 10^{-5} \text{ mol.L}^{-1},$$

$$[\beta \text{ cyclodextrin}] = 0.000 \text{ mol.L}^{-1},$$

$$A = 0.525, \quad l = 1 \text{ cm},$$

$$\varepsilon = 17384.1 \text{ mol}^{-1} \cdot \text{cm}^{-1} \cdot \text{L}.$$

For example, for the second entry, when:

$$[\beta \text{ cyclodextrin}]_{\text{Free}} = 0.0011 \text{ at } \lambda_{\text{max}} = 352 \text{ nm},$$

$$A = 0.560, \quad K = 66.8 \text{ mol.L}^{-1}.$$

The  $K$  values are given in Table 5. The range was:

$$85.12 - 61.26 = 23.86 \text{ mol.L}^{-1},$$

$$\bar{K} = 73.19 \pm 11.93 \text{ mol}^{-1} \cdot \text{L},$$

$K$  values at other  $\lambda_{\max} = 285$  and  $251$  nm in pH 7.4 buffer solution were calculated as well and the results are given in Table 8.

$$K = 69.35 \pm 5.65 \text{ mol}^{-1} \cdot \text{L at } \lambda_{\max} = 285 \text{ nm,}$$

$$K = 56.34 \pm 8.34 \text{ mol}^{-1} \cdot \text{L at } \lambda_{\max} = 251 \text{ nm.}$$

On the basis of the small values of the stability constant of the inclusion complex, it can be suggested that the interaction between  $\beta$ -cyclodextrin and piroxicam molecule is weak. This may be due to either the cavity size of  $\beta$ -cyclodextrin annulus, which cannot admit the piroxicam molecule properly, or the UV-visible spectroscopy technique, which may not be an accurate technique for this purpose. Therefore, it is suggested that the inclusion complex between the piroxicam molecule and modified  $\beta$ -cyclodextrins, such as those having polar and ionic functional groups attached to the  $\beta$ -cyclodextrin molecule or with the linked  $\beta$ -cyclodextrin molecules, be investigated. Also, other more accurate techniques, such as High Performance Liquid Chromatography (HPLC), HNMR or CNMR should be used for the determination of the stability constant of the inclusion complexation. The results show that the stability constant ( $K$ ) at a saturated solution of Piroxicam is almost two to three times greater than at low concentrations of piroxicam and, also, the amount of  $K$  at  $\lambda_{\max} = 352$  nm is the highest.

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