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# Study of the Inclusion Complexation of β-Cyclodextrin-Chloramphenicol and Determination of its Stability Constant

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Because of the therapeutical significance of chloramphenicol, in the present work, its complexation with β-cyclodextrin is reported. The stability constant (K) of the complexes is determined by UV-Visible spectroscopy.

**Key Words: β-Cyclodextrin, Chloramphenicol, Inclusion complex, Stability constant**

## INTRODUCTION

Enzymes are very efficient biochemical catalysts, safe and easy to use. They operate under mild conditions, generally at room temperature and around neutral pH[1, 2]. While there are many different enzymes able to catalyze a wide range of reactions, individual enzymes are generally quite specific in terms of the reaction catalyzed, with respect to the structure of both the substrate and product. Given the particular properties of enzymes, they have considerable potential for use in organic chemistry[3], as tools for synthesis and resolution of mixtures but their widespread application is limited by a number of factors such as product inhibition and poor ability to discriminate between mixtures of non-natural substrates[3]. On the other hand, some guest molecules demonstrate enhanced reactivity when encapsulated in the hydrophobic cyclodextrin cavity. This behaviour implies an enzymatic role for cyclodextrins[4, 5]. Cyclodextrins are cyclic (1→4) linked oligomers of α-D-glucopyranose, each D-glucopyranosyl residue being in the $^4C_1$ conformation. The three most important cyclodextrins are the alpha, beta and gamma cyclodextrins (Schrödinger α-, β-, γ-dextrins), which respectively consist of six, seven and eight α-D-glucopyranosyl residues and are systematically named cyclomaltohexaose, cyclomaltoheptaose and cyclomaltooctaose. Because of its relative brevity, the term "cyclodextrin" will be used throughout this article. Higher homologues do exist; however, they are difficult

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to purify and their complexing ability appears to be poor[4]. Cyclodextrins having fewer than six α-D-glucopyranosyl residues are unknown, probably because of steric reasons. As a consequence of the $^4C_1$ conformation of the α-D-glucopyranosyl residues and the lack of free rotation about the glycosidic bonds, the compounds are not perfectly cylindrical molecules, but are somewhat cone-shaped , with all of the secondary hydroxyl groups situated at one end of the annulus and all of the primary hydroxyl groups at the other. The cavity is lined by a ring of hydrogen atoms (bonded to C-5), a ring of D-glucosidic oxygen atoms and another ring of hydrogen atoms (bonded to C-3), thus making the cavity relatively apolar. The shape of the molecule is stabilized by H-bonds between the secondary hydroxyl groups of adjacent D-glucopyranosyl residues. The numbering system and structure of β-cyclodextrin is shown in Fig. 1. Probably the most important property of the cyclodextrins is their ability to form complexes with a variety of organic and inorganic compounds. Inclusion complexes are formed when a molecule with a cavity or capable of forming such a cavity (the *host*), encapsulates a smaller molecule (the *guest*) by purely secondary forces. These complexes are quite stable. Formation of cyclodextrin inclusion complexes is characterized by the equilibrium between the cyclodextrin host, the guest and the complex. The stability or association or thermodynamic constant (K) of the

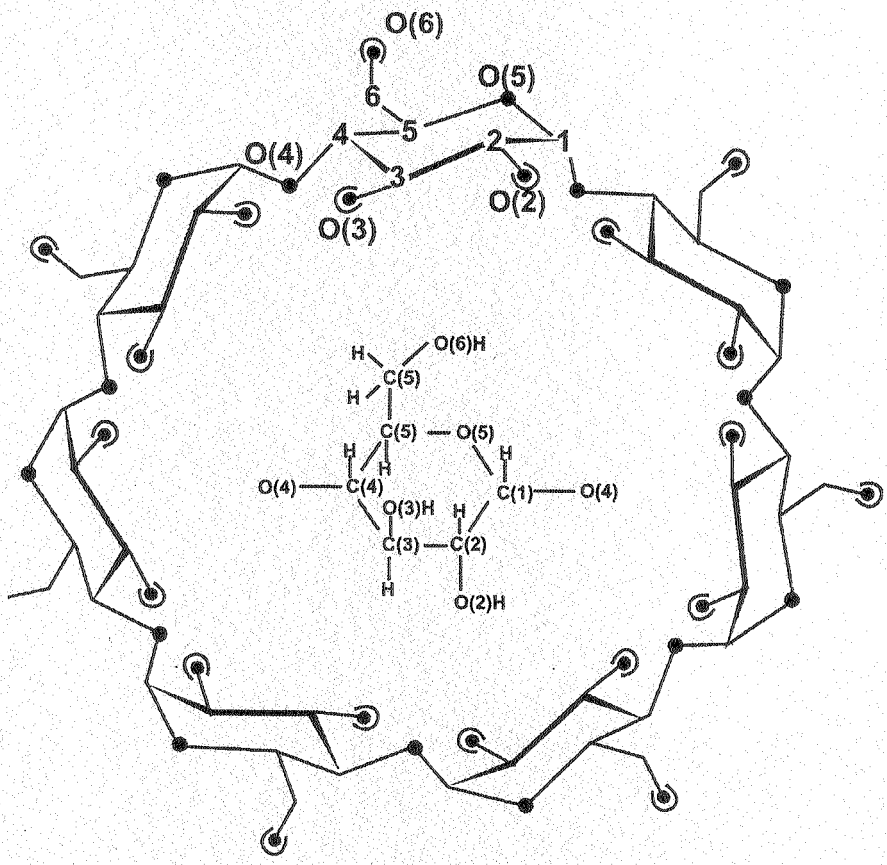
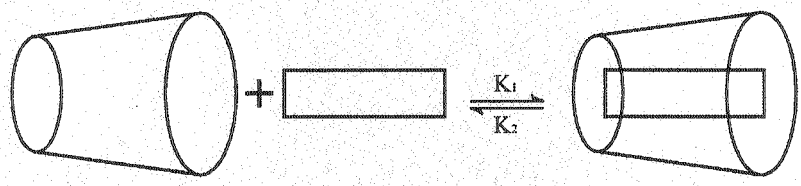



Fig. 1. Chemical structure and numbering of the atoms of β-cyclodextrin

complex defines the position of the equilibrium (Fig. 2)[7]. The stability of an inclusion complex depends to a large degree on the relative sizes of the cyclodextrin cavity and the portion of the guest molecule to be included. For example, a molecule may be too large to fit within the cavity of alpha cyclodextrin, but might form a stable complex with (the larger) β-cyclodextrin. In solution, the most common cyclodextrin-guest stoichiometric ratio is 1 : 1.




$$\text{Inclusion complex association constant} = K = \frac{\text{Inclusion complex}}{[\text{Cyclodextrin host}]\,[\text{Guest}]}$$

Fig. 2. Schematic representation of inclusion complex formation

## EXPERIMENTAL

**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:** 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 A).** 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 **(Solution B).** 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) was dissolved in deionized water in a 100 mL volumetric flask. Citric acid (29 mL of 0.1 M) was added to the flask and the volume was made up to 100 mL by adding more deionized water. The pH was measured and confirmed to be 6.5.

**Preparation of chloramphenicol solution:** Chloramphenicol (1. 7 mg, 5.32 $\times 10^{-3}$ mmol) was dissolved in the desired buffer solution in a 5 mL volumetric flask and the volume was made up to 5 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 β-cyclodextrin stock solution:** β-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:** This is the pH of the biological fluids. β-cyclodextrin (0.4494 g, 0.396 mmol) was dissolved in phosphate buffer solution with pH 7.4 in a 25 mL volumetric flask and the volume was made up to 25 mL by adding more of the buffer solution. Then, solutions with different molar concentrations of β-cyclodextrin were made (Table-1) in 5 mL volumetric flasks. Chloramphenicol (1.7 mg , $5.32 \times 10^{-3}$ mmol) was dissolved in phosphate buffer solution with pH 7. 4 in 5 mL volumetric flask as well and the volume was made up to 5 mL by adding more of the buffer solution. To each of the β-cyclodextrin concentration (Table-1) in a 5 mL volumetric flask, 166 μL of the chloramphenicol solution was added and the final volume was made up to 5 mL by adding more of the buffer solution (pH 7.4). The UV-Vis spectrum of each of the solutions was taken at wavelength 200–400 nm.

TABLE-1
SOLUTIONS OF THE HOST (β-CYCLODEXTRIN)

| Volume of stock solution taken to make 5 mL final solution (mL) | Molar concentration of diluted β-cyclodextrin (mol $L^{-1}$) |
|---|---|
| 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 |

**(ii) pH 7 and (iii) pH 6.5:** Exactly the same amounts were used and the same procedure was carried out as in (i).

**Determination of $\lambda_{max}$:** The measurements were made with pH 7.4 buffer solution. Exactly experiment 6 (i) above was repeated and the UV-Vis spectra were taken at wavelength 200–400 nm at 25°C. From the recorded spectra, it was found that $\lambda_{max}$ is 278 nm.

**Determination of the stability constant (K):** To each of the diluted solutions of β-cyclodextrin given in Table-1 in 5 mL volumetric flasks, chloramphenicol (1.7 mg , $5.32 \times 10^{-3}$ mmol) was added 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 h, then filtered by filter paper. Each time 50 μL of the filtrate and 2.95 mL of the buffer solution with pH 7.4 were placed in a UV-Vis cell at 25°C and finally the UV-Vis spectrum was taken at wavelength 200–400 nm. The absorbance at $\lambda_{max} = 278$ nm of each of the various concentrations of β-cyclodextrin added to the chloramphenicol solution was recorded (Table-2) and then plotted *vs.* β-cyclodextrin (Fig. 3). For each entry of Table-1, the measurements were repeated 3 times. On the basis of $\lambda_{max} = 278$ nm of chloramphenicol and various concentrations of β-cyclodextrin, the stability constant was calculated as given in the results and discussion section of this article.

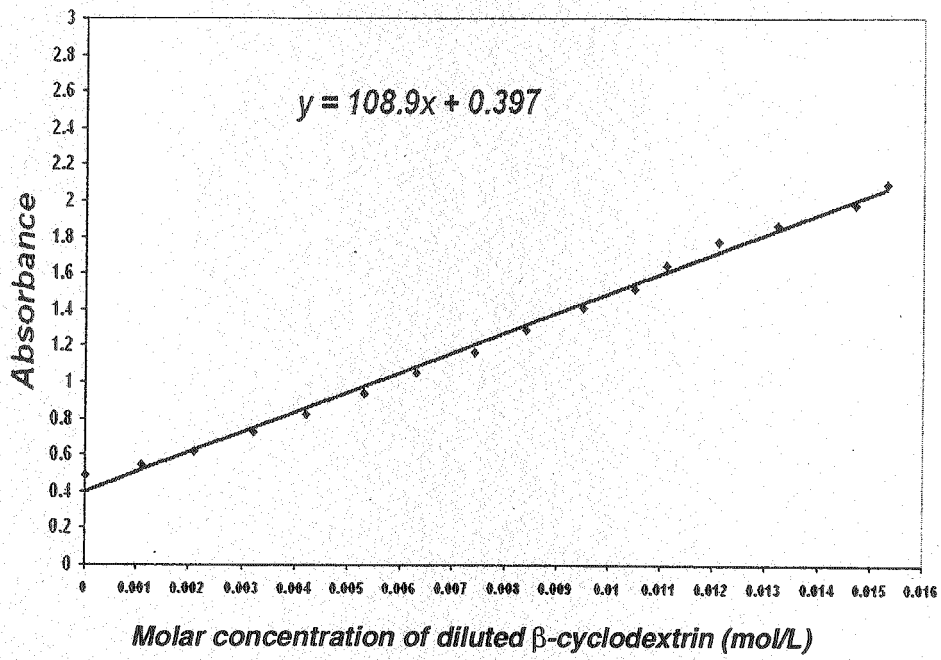


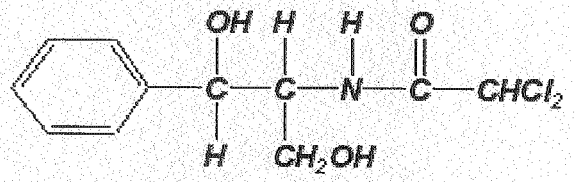

Fig. 3. Absorbance of the inclusion complex between β-cyclodextrin and chloramphenicol at various β-cyclodextrin concentrations at $\lambda_{max} = 278$ nm

TABLE-2
ABSORBANCE OF THE INCLUSION COMPLEX FORMED BY ADDITION OF VARIOUS CONCENTRATIONS OF β-CYCLODEXTRIN TO THE CHLORAMPHENICOL SOLUTION WITH pH 7.4

| Molar concentration of diluted β-cyclodextrin (mol $L^{-1}$) | Observed average absorbance (A) |
|---|---|
| 0.0000 | 0.485 |
| 0.0011 | 0.545 |
| 0.0021 | 0.621 |
| 0.0032 | 0.725 |
| 0.0042 | 0.818 |
| 0.0053 | 0.937 |
| 0.0063 | 1.052 |
| 0.0074 | 1.165 |
| 0.0084 | 1.286 |
| 0.0095 | 1.403 |
| 0.0105 | 1.511 |
| 0.0111 | 1.646 |
| 0.0121 | 1.772 |
| 0.0132 | 1.863 |
| 0.0147 | 1.979 |
| 0.0153 | 2.091 |

## RESULTS AND DISCUSSION

Upon inclusion within the cyclodextrin cavity, a guest molecule experiences changes in its physico-chemical properties, as well as changes in its chemical reactivity, due to changes in its environment on its removal from the bulk solution. These changes in behaviour have great practical significance; for example, for the stabilization of reactive substances, reduction in volatility and increase in solubility. In research, however, the changes in physico-chemical properties of the guest provide an easy method of detecting inclusion-complex formation. A variety of techniques can be used for studying inclusion-complex formation. In this research work, UV-Vis spectroscopy technique is used due to its easy accessibility, low cost and simplicity. Chloramphenicol with synonyms as chloranfenicol or cloranfenicol, systematically named as 2,2-dichloro-N-[(αR, βR)-β-hydroxyl-α-hydroxymethyl-4-nitrophenethyl]acetamide (1) and has a molecular formula of $C_{11}H_{12}Cl_2N_2O_5$ (m.w. 323).




Chloramphenicol (1)

The work was carried out at three different pH, *i.e.*, 6.5, 7, 7.4. At each of these pH, various concentrations of β-cyclodextrin (Table-1) were added to a constant amount of chloramphenicol drug and then the UV-Vis spectrum was recorded at 200–400 nm at 25°C. Based on these results, it was concluded that the absorbance for the complex formation was highest in phosphate buffer solution with pH 7.4 . Therefore, it can be concluded that the proper pH for the inclusion complex formation between β-cyclodextrin and chloramphenicol was 7.4. Similarly, after addition of various concentrations of β-cyclodextrin solutions (Table-1) to a constant amount of chloramphenicol, the UV-Vis spectra were recorded at 200–400 nm. On the basis of the results obtained, it was concluded that the $\lambda_{max}$ is 278 nm (Table-3).

The stability constant can be calculated on the basis of the results given in Table-2 and also from the graph in Fig. 3. The K value can be calculated as follows:

In accordance with the Beer-Lambert law:

$$A = \varepsilon \cdot l \cdot c$$

where $A = 0.485$, $l = 1$ cm, $c = [\text{Chloramphenicol}] = 1.77 \times 10^{-5}$ mol $L^{-1}$, $\varepsilon = 0.485/(1\text{ cm} \times 1{:}77 \times 10^{-5}\text{ mol L}^{-1}) = 27401.1\text{ mol}^{-1}\text{ cm}^{-1}\text{ L}$.

The stability constant can be determined from the following formula:

$$K = \frac{A_{complex}}{A_{pure\ Guest} \times [\beta\text{-cyclodextrin}]_{Free}} \quad (1)$$

TABLE-3
COMPARISON OF ABSORBANCES OF THE INCLUSION COMPLEX FORMED BY ADDITION OF VARIOUS CONCENTRATIONS OF β-CYCLODEXTRIN TO THE CHLORAMPHENICOL SOLUTION AT THREE DIFFERENT pH (7.4, 7, 6.5)

| [β-cyclodextrin] (mol $L^{-1}$) | Average absorbance at pH 7.4 | Average absorbance at pH 7 | Average absorbance at pH 6.5 |
|---|---|---|---|
| 0.0000 | 0.485 | 0.420 | 0.381 |
| 0.0011 | 0.545 | 0.474 | 0.415 |
| 0.0021 | 0.621 | 0.532 | 0.462 |
| 0.0032 | 0.725 | 0.618 | 0.501 |
| 0.0042 | 0.818 | 0.672 | 0.545 |
| 0.0053 | 0.937 | 0.724 | 0.598 |
| 0.0063 | 1.052 | 0.786 | 0.649 |
| 0.0074 | 1.165 | 0.839 | 0.683 |
| 0.0084 | 1.286 | 0.902 | 0.721 |
| 0.0095 | 1.403 | 0.964 | 0.758 |
| 0.0105 | 1.511 | 1.031 | 0.794 |
| 0.0111 | 1.646 | 1.069 | 0.838 |
| 0.0121 | 1.772 | 1.125 | 0.881 |
| 0.0132 | 1.863 | 1.198 | 0.915 |
| 0.0147 | 1.979 | 1.253 | 0.948 |
| 0.0153 | 2.091 | 1.304 | 0.995 |

From Fig. 3, $A_{complex}$ for various concentrations of β-cyclodextrin can be calculated, *e.g.*, for the second entry of Table-1 we will have:

$$[\beta\text{-Cyclodextrin}] = 0.0011 \text{ mol } L^{-1}; \quad A_{(guest + \beta\text{-cyclodextrin})} = 0.545$$

Therefore,

$$A_{complex} = A_{(guest + \beta\text{-cyclodextrin})} - A_{pure\ guest} = 0.545 - 0.485 = 0.06 \qquad (2)$$

Now, the concentration of the complex can be calculated:

$$C_{complex} = \frac{0.06}{27401.1 \text{ mol}^{-1} \text{ cm}^{-1} \text{ L} \times 1 \text{ cm}} = 2.19 \times 10^{-6} \text{ mol L}^{-1}$$

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

On the other hand:

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

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

$$[\beta\text{-cyclodextrin}]_{free} = 0.0011 - 2.19 \times 10^{-6} = 1.1 \times 10^{-3} \text{ mol L}^{-1}$$

Now, K can be calculated by substituting the values into the equation given in step 1:

$$K = \frac{0.06}{0.485 \times 1.1 \times 10^{-3} \text{ mol L}^{-1}} = 112.46 \text{ mol}^{-1} \text{ L} \qquad (3)$$

Then, K value for each entry of Table-2 and Fig. 3 was calculated the same as above; the results are summarized in Table-4. According to the data given in Table-4 , the range of the stability constant is:

$$221.11 - 112.46 = 108.67$$

Therefore:

$$K = 166.79 \pm 54.34 \text{ mol}^{-1} \text{ L}$$

On the basis of the relatively small values of the stability constant of the inclusion complex, it can be suggested apparently that the interaction between β-cyclodextrin and chloramphenicol molecule is not strong. This may be due to either the cavity size of β-cyclodextrin annulus which cannot admit chloramphenicol molecule properly or the UV-Vis spectroscopy technique which may not be an accurate technique for this purpose. Therefore, it is suggested that the inclusion complex between chloramphenicol molecule and modified β-cyclodextrins like those having polar and ionic functional groups attached to the β-cyclodextrin molecule be investigated and also other more accurate techniques such as high performance liquid chromatography (HPLC), $^{1}H$ NMR or $^{13}C$ NMR be used for the determination of the stability constant of the inclusion complexation.

TABLE-4
CALCULATED STABILITY CONSTANT (K) OF THE INCLUSION COMPLEX BETWEEN β-CYCLODEXTRIN AND CHLORAMPHENICOL AT VARIOUS β-CYCLODEXTRIN CONCENTRATIONS AT $\lambda_{max}$ = 278 nm

| Molar concentration of diluted β-cyclodextrin (mol $L^{-1}$) | Calculated stability constant (K) ($mol^{-1}$ L) |
|---|---|
| 0.0011 | 112.46 |
| 0.0021 | 133.53 |
| 0.0032 | 159.62 |
| 0.0042 | 163.47 |
| 0.0053 | 175.84 |
| 0.0063 | 186.15 |
| 0.0074 | 191.07 |
| 0.0084 | 197.31 |
| 0.0095 | 200.08 |
| 0.0105 | 202.24 |
| 0.0111 | 217.92 |
| 0.0121 | 221.13 |
| 0.0132 | 215.24 |
| 0.0147 | 211.00 |
| 0.0153 | 217.85 |

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