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Study of the Inclusion Complexation of Sodium Diclofenac-3-amino-β-cyclodextrin and Determination of the Stability Constant by UV-Vis Spectroscopy

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Because of its therapeutical importance, sodium diclofenac has a widespread use. In this work, the complexation between sodium diclofenac and 3-amino-β-cyclodextrin was studied. The stability constant (K) of the complex was determined.

Key Words: 3-Amino-β-cyclodextrin, Sodium diclofenac, Inclusion complex, Stability constant.

INTRODUCTION

Cyclodextrins are cyclic oligosaccharides of α-(1→4) linked D-glucose residues. Three naturally occurring α-, β- and γ-cyclodextrins having six, seven and eight glucose residues, respectively, have been characterized (Fig. 1). They are also called cyclohexaamylose, cycloheptaamylose and cyclooctaamylose. Cyclodextrins are shaped like a hollow truncated cone due to a minor tilt in the glucose residues. The non-polar hydrogen atoms H-3 and H-5 point towards the inner cavity making it hydrophobic and, thus, capable of "hosting" non-polar "guest" molecules. The hydroxyl groups lying on the outside confer the molecule with a hydrophilic exterior. The O-2 and O-3 hydroxyl groups are on the wider side of the truncated cone whereas the hydroxymethyl groups are on the narrower side. The natural cyclodextrins (α-, β- and γ-cyclodextrins) have been extensively used in pharmaceutical formulation, but they have some undesirable properties as drug carriers[1, 2]. The limited application of natural cyclodextrins in the pharmaceutical formulation seems to be related to their relatively low aqueous solubility. Their ability to form inclusion complexes has led to a wide range of applications such as drug delivery systems and artificial enzymes. These molecules are capable of chiral recognition as well. Cyclodextrins, however, are often derivatized to alter their physico-chemical properties. Some modifications to the cyclodextrin structure have also been found to improve their complexing ability. The natural cyclodextrins can be chemically modified for many different purposes[3, 4]. The hydroxyl groups of cyclodextrins are available as starting points of structural modification and various functional groups have been incorporated into the cyclodextrin molecules. Numerous examples of modifications to the fundamental cyclodextrin structure have appeared in literature[7–10]. The aim of much of

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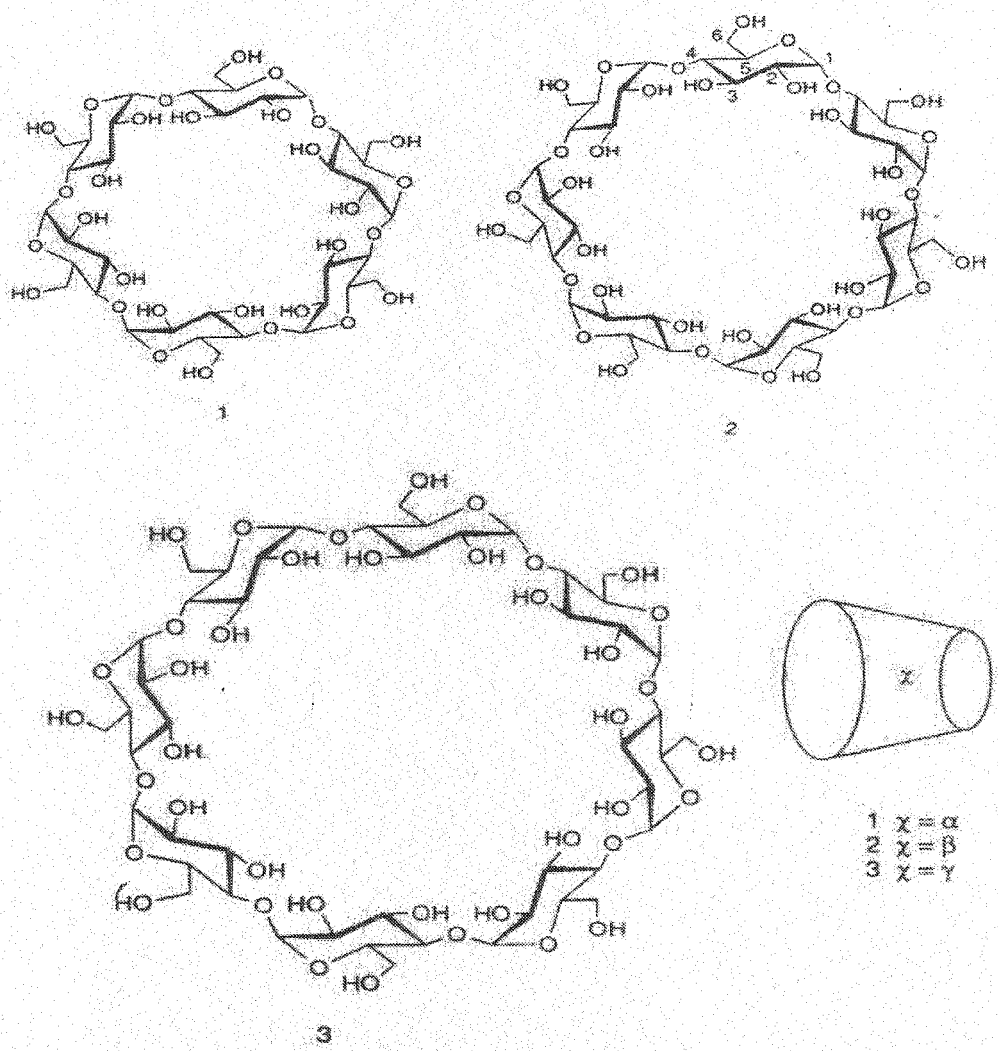


Fig. 1. Natural (native) cyclodextrin

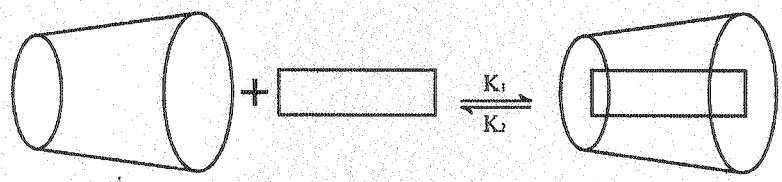
these works has been to improve the catalytic properties of the cyclodextrins and thus to develop so-called "artificial enzymes". Cyclodextrins themselves have long been known to be capable of catalyzing such reactions as ester hydrolysis[11, 12], by interaction of the guest with the secondary hydroxyl groups around the rim of the cyclodextrin cavity. The replacement, by synthetic methods, of the hydroxyl groups with other functional groups has been shown, however, to improve remarkably the number of reactions capable of catalysis by the cyclodextrins. Some modifications to the cyclodextrin structure have also been found to improve their complexing ability.

Modified cyclodextrins and their complexing characteristics usually involve substitution of one or more of the C-2, C-3 and C-6 hydroxyl groups. The modifications may be divided into two categories:

1. In one, the hydroxyl substituents are substituted in a symmetric fashion to give a single modified cyclodextrin (*i.e.*, all the hydroxyl groups may be substituted) or at random to give a complex mixture of cyclodextrins in which the average effect is that of a symmetric substitution. This tends not to alter the symmetry of the cyclodextrin or the enantioselectivity that it displays.

2. With the other type of modified cyclodextrin, either a single substituent or a specific combination of substituents is introduced. This may induce substantial changes in the asymmetry of the cyclodextrin and result in additional and more specific interactions between the chiral area of the guest and the asymmetry of the host, which restrict the geometry of binding, leading to greater enantioselectivity.

Chemically modified cyclodextrins have been shown to have increased aqueous solubility and also alter the thermodynamic stability of any complexes formed, relative to the case when the host is the parent cyclodextrin (Fig. 2). There is a potential to exploit the effects of these modifications to increase or decrease the degree to which a particular guest can be sequestered by a modified cyclodextrin.



Cyclodextrin host Guest Inclusion complex

$$\text{Inclusion complex association constant (K)} = \frac{\text{Inclusion complex}}{\text{(Cyclodextrin host)(Guest)}}$$

Fig. 2. Schematic of inclusion complex formation

In summary, the use of inclusion complexation of cyclodextrins or modified cyclodextrins with different drugs has the following considerable pharmaceutical potentials:

1. Increase of solubility and dissolution rate, 2. deceleration of chemical reaction (hydrolysis, oxidation, photo-decomposition, dehydration), 3. enhancement of drug absorption, 4. suppression of volatility, 5. powdering of liquid drugs, 6. suppression of unpleasant tastes, 7. reduction of local irritancy and hemolysis.

EXPERIMENTAL

UV-Visible spectra were recorded using a Jasco 810-UV spectrophotometer. All glasswares wre pre-dried in an oven. All the chemicals were obtained from Darou-Pakhsh Pharmaceutical Company, Iran.

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.

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) 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 sodium diclofenac solution: Sodium diclofenac (0.1 g, 0.314 mmol) was dissolved in the desired buffer solution in a 250 mL volumetric flask and the volume was made up to 250 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 3-amino-*p*-cyclodextrin stock solution: 3-Amino-β-cyclodextrin (0.8988 g, 0.7815 mmol) 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).

Determination of the proper pH:

pH 7.4: This is the pH of the biological fluids. To various concentrations of 3-amino-β-cyclodextrin (0.8988 g, 0.7815 mmol) (Table-1) in 5 mL volumetric flasks, 166 μL of sodium diclofenac 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 (3-AMINO-β-CYCLODEXTRIN)

Volume of stock solution taken to make 5 mL final solution (mL)	Molar concentration of diluted 3-amino-β-cyclodextrin (mol L^{-1})	Volume of stock solution taken to make 5 mL final solution (mL)	Molar concentration of diluted 3-amino-β-cyclodextrin (mol L^{-1})
0.662	0.0021	2.990	0.0095
1.010	0.0032	3.314	0.0105
1.325	0.0042	3.500	0.0111
1.672	0.0053	3.810	0.0121
1.980	0.0063	4.160	0.0132
2.330	0.0074	4.640	0.0147
2.650	0.0084	4.820	0.0153

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 λ_{max}: The measurements were made with pH 7.4 buffer solution. Exactly experiment 6 (i) was repeated and the UV-Vis spectra were taken at wavelengths 200–400 nm at 25°C. From the recorded spectra, it was found that λ_{max} is 275 nm.

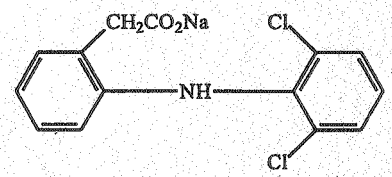
Determination of the stability constant (K): To each of the diluted solutions of 3-amino-β-cyclodextrin given in Table-1 in 5 mL volumetric flasks, sodium diclofenac solution (166 μL, 1.26×10^{-3} mol L^{-1}) was added and the volume was made up to 5 mL by adding more of the buffer solution with pH 7.4. The flasks were kept at room temperature for 2 h, then filtered on filter paper. Each time the filtrate was placed in a UV-visible cell at 25°C and finally the UV-Vis spectrum was taken at wavelength 200–400 nm. The absorbance at $\lambda_{max} = 275$ nm of each of the various concentrations of 3-amino- β-cyclodextrin added to the sodium diclofenac solution was recorded (Table-2) and then plotted *vs.* 3-amino-β-cyclodextrin (Fig. 3). For each entry of Table-1, the measurements were repeated 3 times at 15 min intervals. On the basis of $\lambda_{max} = 275$ nm of sodium diclofenac solution and various concentrations of 3-amino-β-cyclodextrin, the stability constant was calculated as given in the results and discussion section of this article.

TABLE-2
ABSORBANCE OF THE INCLUSION COMPLEX FORMED BY ADDITION OF VARIOUS CONCENTRATIONS OF 3-AMINO-β-CYCLODEXTRIN TO THE SODIUM DICLOFENAC SOLUTION WITH pH 7.4

Molar concentration of diluted 3-amino-β-cyclodextrin (mol L^{-1})	Observed average absorbance (A)	Molar concentration of diluted 3-amino-β-cyclodextrin (mol L^{-1})	Observed average absorbance (A)
0.0000	661	0.0084	776
0.0011	676	0.0095	799
0.0021	697	0.0105	812
0.0032	707	0.0111	824
0.0042	718	0.0121	836
0.0053	729	0.0132	849
0.0063	742	0.0147	859
0.0074	768	0.0153	870

RESULTS AND DISCUSSION

In this research work, UV-Vis spectroscopy technique was used due to its ease of accessibility, low cost and simplicity. Sodium diclofenac with synonym as voltaren which is systematically named as sodium [2-(2,6-dichloro-aniline)-phenyl] acetate (**I**), is a derivative of phenyl acetic acid and has the molecular formula of $C_{14}H_{10}Cl_2NNaO_2$ (m.w. 318.1 g/mol).



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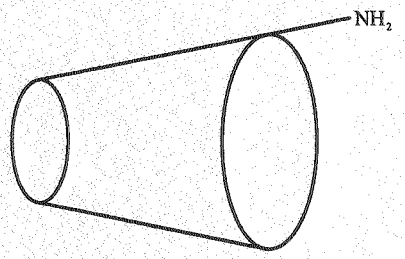

II

Sodium diclofenac

3-amino-β-cyclodextrin

The work was carried out at three different pHs, *i.e.*, 6.5, 7, 7.4. At each of the pHs used, various concentrations of 3-amino-β-cyclodextrin (Table-1) was added to a constant amount of sodium diclofenac solution and immediately, the UV-Vis spectrum was recorded at 200–400 nm at 25°C. The results are given in Table-3. Based on these results, it was concluded that the absorbance for the complex formation was highest in phosphate buffer solution with pH 7.4. Since the proper pH was found to be 7.4, therefore all the experiments for the determination of λ_{max} were carried out in the buffer solution with pH 7.4. After addition of various concentrations of 3-amino-β-cyclodextrin solutions (Table-1) to a constant amount of sodium diclofenac solution, the UV-Vis spectra were recorded at 200–400 nm. On the basis of the results obtained, it was concluded that the maximum absorbance wavelength (λ_{max}) was 275 nm.

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

[3-amino-β-cyclodextrin] (mol L^{-1})	Average absorbance at pH 7.4	Average absorbance at pH 7	Average absorbance at pH 6.5
0.0000	0.661	0.596	0.569
0.0011	0.676	0.610	0.592
0.0021	0.697	0.635	0.617
0.0032	0.707	0.649	0.630
0.0042	0.718	0.662	0.641
0.0053	0.729	0.674	0.650

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 = 0.661, l = 1 cm, c = [sodium diclofenac] = 4.1832×10^{-5} mol L^{-1}.

$$\varepsilon = \frac{0.061}{1\ \text{cm} \times 4.1832 \times 10^{-5}\ \text{mol L}^{-1}} = 15801.3\ \text{mol}^{-1}\ \text{cm}^{-1}\ \text{L}$$

The stability constant can be determined from the following formula:

$$K = \frac{A_{complex}}{A_{pure\ guest} \times [3\text{-amino-}\beta\text{-cyclodextrin}]_{free}}$$

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

$$[3\text{-amino-}\beta\text{-cyclodextrin}] = 0.0011\ mol\ L^{-1};\quad A_{(guest + \beta\text{-cyclodextrin})} = 0.676$$

Therefore:

$$A_{complex} = A_{(guest + 3\text{-amino-}\beta\text{-cyclodextrin})} - A_{pure\ guest} = 0.676 - 0.66 = 10.015$$

Now, the concentration of the complex can be calculated quite easily:

$$C_{complex} = \frac{0.015}{15801.3\ mol^{-1}\ cm^{-1}\ L \times 1\ cm} = 9.5 \times 10^{-7}\ mol\ L^{-1}$$

$$[3\text{-amino-}\beta\text{-cyclodextrin}]_{Free} = [3\text{-amino-}\beta\text{-cyclodextrin}]_{initial} - [3\text{-amino-}\beta\text{-cyclodextrin}]_{used}$$

On the other hand

$$[3\text{-amino-}\beta\text{-cyclodextrin}]_{used} = [C_{complex}]$$

Therefore

$$[3\text{-amino-}\beta\text{-cyclodextrin}]_{Free} = [3\text{-amino-}\beta\text{-cyclodextrin}]_{initial} - [C_{complex}]$$
$$= 0.0011 - 9.5 \times 10^{-7} = 1.1 \times 10^{-3}\ mol\ L^{-1}$$

Now, K can be calculated by substituting the values

$$K = \frac{0.015}{0.061 \times 1.1 \times 10^{-3}\ mol\ L^{-1}}$$

Therefore, we would have:

$$K = 20.65\ mol^{-1}\ L$$

Then, the K value for each entry of Table-2 and Fig. 3 was calculated as above; the results are summarized in Table-4.

TABLE-4
CALCULATED STABILITY CONSTANT (K) OF THE INCLUSION COMPLEX BETWEEN 3-AMINO-β-CYCLODEXTRIN AND SODIUM DICLOFENAC AT VARIOUS 3-AMINO-β-CYCLODEXTRIN CONCENTRATIONS AT $\lambda_{max} = 275$ nm

Molar concentration of diluted 3-amino-β-cyclodextrin (mol L^{-1})	Calculated stability constant (K) (mol^{-1} L)	Molar concentration of diluted 3-amino-β-cyclodextrin (mol L^{-1})	Calculated stability constant (K) (mol^{-1} L)
0.0011	20.65	0.0095	22.00
0.0021	25.96	0.0105	21.80
0.0032	21.80	0.0111	22.23
0.0042	20.50	0.0121	21.90
0.0053	19.43	0.0132	21.56
0.0063	19.50	0.0147	20.30
0.0074	21.90	0.0153	20.70
0.0084	20.72		

According to the data given in Table-4, the range of the stability constant is 25.96 – 19.43 = 6.53. Therefore, K = 22.695 ± 3.265 mol^{-1} L.

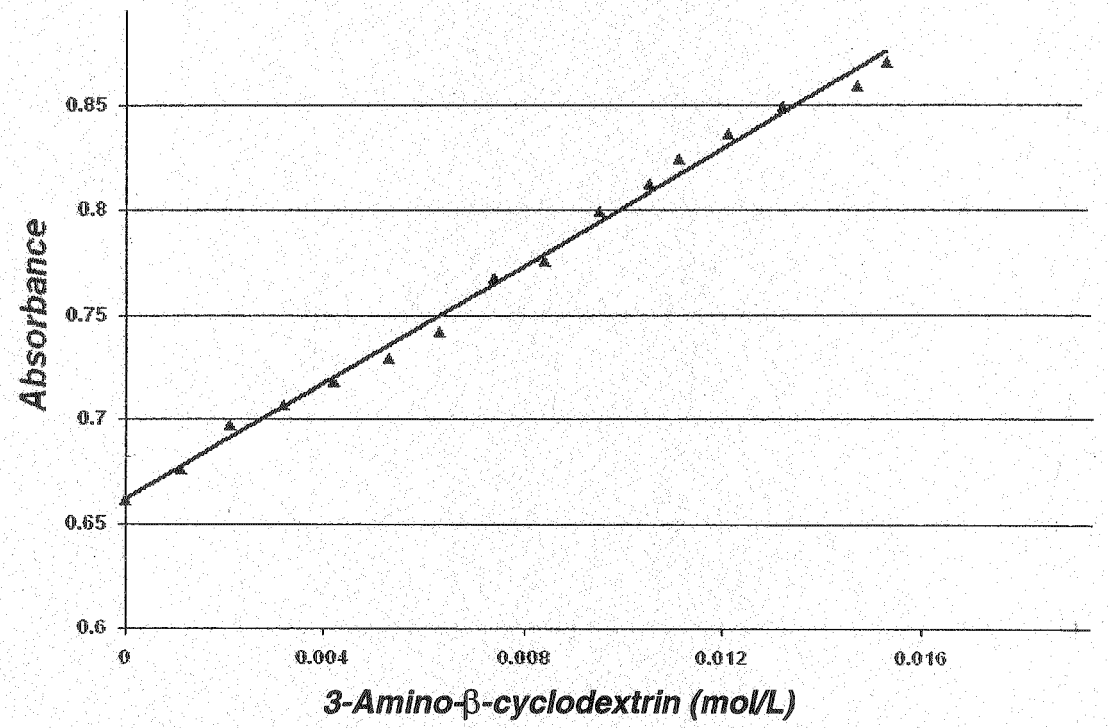

Fig. 3. Absorbance of the inclusion complex between 3-amino-β-cyclodextrin and sodium diclofenac molecule at various 3-amino-β-cyclodextrin concentrations at λ_{max} = 275 nm

On the basis of the relatively small values of the stability constant of the inclusion complex, it can be suggested that the interaction between 3-amino-β-cyclodextrin and sodium diclofenac molecule is quite weak. This may be due to either the cavity size of β-cyclodextrin annulus which cannot admit sodium diclofenac molecule properly and also no interaction between the amino substituent group on the β-cyclodextrin rim with the guest molecule or the UV-Vis spectroscopy technique, which may not be an accurate technique for this purpose. On the basis of relatively small values of the stability constant of the inclusion complex, it can be suggested that either the cavity size of β-cyclodextrin annulus cannot admit sodium diclofenac molecule properly and also no interaction between the amino substituent groups on the β-cyclodextrin rim eith the guest molecule is existing, or the UV-Visible spectroscopy technique may not be an accurate technique for this purpose. Therefore, it is suggested that the inclusion complex between sodium diclofenac molecule and other modified β-cyclodextrins like those having polar and ionic functional groups attached to the β-cyclodextrin molecule and also with α-cyclodextrin and modified α-cyclodextrin be investigated and finally other more accurate techniques such as high performance liquid chromatography, ^{1}H NMR or ^{13}C NMR be used for the determination of the stability constant of the inclusion complexation.

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