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HOST–GUEST INCLUSION COMPLEX OF β-CYCLODEXTRIN AND CEPHALEXIN AND ITS ANALYTICAL APPLICATION

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Abstract
The host–guest inclusion complex between β-cyclodextrin (β-CD) and cephalexin was investigated spectrofluorometrically. The results showed that β-CD reacted with cephalexin to form 1:1 stoichiometry host-guest inclusion complex. Inclusion constant \(K = 5.33 \times 10^2 \text{ L mol}^{-1}\) was ascertained by the typical double reciprocal plots in pH 5.0 buffer solution. Furthermore, the thermodynamic parameters \(\Delta H^\circ, \Delta S^\circ\) and \(\Delta G^\circ\) associated with the inclusion process were also determined. The factors affecting the inclusion complex formation were carefully studied, incorporated in the procedure and optimized. A spectrofluorimetric method for the determination of cephalexin in solution in the presence of β-CD was developed based on the remarkable enhancement of the fluorescence intensity of cephalexin. Under the optimum reaction conditions, linear relationships with good correlation coefficients (0.9992) were in the concentration range of 1–10 µg/mL was obtained. The limit of detection (LOD) and limit of quantification (LOQ) were found to be 0.001 µg/mL and 0.036 µg/mL, respectively. The recovery was in the range of (104–124.5) %. The method has been successfully applied to the determination of cephalexin in pharmaceutical formulation with good accuracy.

Key word: cephalexin, inclusion complex; β-cyclodextrin; spectrofluorimetric

INTRODUCTION

Cyclodextrins (CDs) are water-soluble cyclic oligosaccharides composed of six (α–), seven (β–) and eight (γ–) units of D-(+)-glucopyranose arranged in a truncated cone shape structure. The hydrophobic cavity of CDs can host a large variety of organic and inorganic compounds of suitable size. The presence of CD and correctly-sized organic molecule in aqueous solutions results generally in the formation of inclusion complexes [1]. It is well documented that even a partial binding to the CD cavity is accompanied by noticeable changes in the photophysical properties of the guest compound. Among other interesting features, fluorescence intensity enhancement [2] and chiral activity induction [3] are obtained upon addition of CD. Accordingly, better enantiometric separations by High Performance Liquid Chromatography (HPLC), [4, 5] Thin-Layer Chromatography (TLC) [6] or capillary zone electrophoresis (CZE), [7-10] have been reported for various compounds. Analysts have used this property of CDs, and a lot of methods based on the fluorescence of inclusion complexes with CDs have been proposed for the determination of several pharmaceutical drugs, pesticides, and metal ions [11, 12].

Cephalexin, 7-(D-a-amino-phenylacetamido)-3-methyl-3-cephem-4-carboxylic acid (figure 1), is a second-generation cephalosporin and one of the most commonly used cephalosporin antibiotics. Cephalosporin antibiotic is an effective broad spectrum antibiotic that targets both Gram positive and Gram negative bacteria [13]. The widespread use...
of this compound requires fast and sensitive analytical methods.

![Chemical structure of cephalexin](image)

Figure 1. Chemical structure of cephalexin

A great variety of methods for determination cephalexin in pharmaceutical preparations have been reported, including spectrophotometry \[14-22\], spectrofluorometric \[23-29\], High Performance chromatography (HPLC) \[30-33\]. Most of the spectrophotometric methods are time-consuming and sometime required high temperature. US pharmacopeia recommended HPLC method for analysis of cephalexin \[34\], the method is accurate and effective means of determination of cephalexin, but they are time and solvent consuming and, therefore, disadvantageous for serial estimation for a large number of samples. Consequently, there is a demand for a rapid, efficient and inexpensive analytical method for cephalexin assay formulations during industrial process development and scale-up production.

In this paper, the host-guest inclusion interaction between \(\beta\)-CD and cephalexin was investigated by spectrofluorimetry. A series of conditions during the formation of the inclusion complex was studied. Based on the great enhancement of the fluorescence intensity of cephalexin, a novel method was developed to determine cephalexin in pharmaceutical formulation.

EXPERIMENTAL

Chemical and reagent

Pure cephalexin reference standard and Amilexin capsules (500 mg of cephalexin) were supplied from Amipharma pharmaceuticals, Khartoum, Sudan. \(\beta\)-cyclodextrin hydrate was supplied by Janssen Chimica, Beerse, Belgium. Doubly distilled water was used to prepare all solutions.

Instrumentation and apparatus

Fluorescence spectra and intensity measurements were made on a Shimadzu RF-1501 spectrofluorimeter equipped with a 150W xenon lamp. Slit widths for both monochromators were set at 10 nm. All of the spectrophotometric measurements were made with a double beam uv1800 ultraviolet-visible spectrophotometer provided with matched 1-cm quartz cell (Shimadzu, Japan). pH meter model pH 211 (Via dell’Artigianato 47 - 36043 Camisano Vicentino (VI), HANNA Italy) was used for adjusting the pH.

Stock and standard solutions

A 100 µg mL\(^{-1}\) stock standard solution of cephalexin was prepared by dissolving 0.0025 g of drug on distilled water and diluted to the mark in a 25 mL volumetric flask with water. Dilute the standard solutions were prepared daily in water just before use.

Procedure

Spectrophotometric method

Aliquots standard solution of cephalexin was transferred into a series of 10 mL volumetric flasks. A 2 mL of Britton Robinson (BR) buffer (pH 5.0), and appropriate amount of 10% of \(\beta\)-CD was added, and the solution was mixed well. The mixture was put at 30 °C for 10 min in thermostat, and then the solution was diluted to the mark with distilled water. Absorbance of solution was measured at 272 nm.

Spectrofluorimetric method

Into a 10 mL volumetric flask, solutions were added in the
following order: 1.0 mL (1.0 µg mL$^{-1}$) of cephalexin, 2 mL (pH 5.0) of BR buffer solution and appropriate amount of 10% β-CD. The mixture was put at 30 °C for 10 min in thermostat, and then the solution was diluted to the mark with distilled water. The fluorescence intensity of cephalexin-β-CD was measured at $\lambda_{ex}/\lambda_{em} = 272$nm/418 nm.

Buffer solutions
Britton- Robinson buffer solution.— Stock solution of (0.04 mol L$^{-1}$acetic acid, 0.04 mol L$^{-1}$ phosphoric acid and 0.04 mol L$^{-1}$ boric acid) was prepared and appropriate amount of 0.2 mol L$^{-1}$ of sodium hydroxide was added to prepare solution of pH range from 1.0 - 13.0.

Spectrofluorimetric determination of stoichiometry and inclusion constant
1.0 mL of 10 µg mL$^{-1}$ and 2.0 mL (pH 5.0) of BR buffer solution were added to a volumetric flask, then the varied amounts of β-CD (0.0, 1.0, 2.0, 3.0, 4.0, and 6.0 mL of 5.0×10$^{-3}$ mol L$^{-1}$) were added sequentially. The mixture was diluted to 10.0 mL with water and was put for 10 minutes in thermostat 30°C.

RESULT AND DISCUSSION
Absorption spectra
According to the procedure the absorption spectrum of products produced by the reaction of cephalexin with β-CD are recorded in Figure 2.

Figure 2. Absorption spectra of cephalexin-β-CD complex, pH = 5.0, T= 30 °C absorption spectra of [cephalexin (a) and; absorption spectra of cephalexin-β-CD (b). The concentration of cephalexin] = 1.0×10$^{-5}$ mol L$^{-1}$, [β-CD]=2.5×10$^{-3}$ mol L$^{-1}$, $\lambda_{max}$ is 272 nm.
Emission spectra
The spectral characteristics of cephalexin were studied and the result showed that the wavelengths of maximum emission of cephalexin at pH 5.0 were 418 nm Figure 3. When β-CD was added into the cephalexin solution, the wavelength of maximum of emission did not change but the fluorescence intensity dramatically increased.

The possible reason is as follows: cephalexin can enter the hydrophobic cavity of β-CD under the affection of non-covalent bond including Van der Waals bond and hydrogen bond. In the cavity, the degree of motion freedom of cephalexin molecule reduced so that the probability of cavity can shield the excited signal of cephalexin from quenching by quencher in the aqueous solution. So the fluorescence intensity increased when the cephalexin-β-CD inclusion complex was produced.

Optimization of experimental variables
In order to optimize the reaction conditions between the cephalexin and β-CD the following parameters were investigated: pH of the buffer, the volume of the buffer, reaction time, temperature and β-CD concentration.

Effect of pH on the fluorescence intensity
The effect of pH on the fluorescence intensity of the cephalexin-β-CD complex was studied in the pH range 1.0-8.0, the solutions were prepared as described in the general procedure and the results obtained are presented in Figure 4. It was found that for cephalexin-β-CD complex the fluorescence intensity is increased as pH increase up to pH 5.0 and then decrease, so this pH was selected as an optimum pH.

Figure 3. Emission spectra of cephalexin (a) of cephalexin in the absence of β-CD. (b) Emission spectrum of cephalexin in the presence of β-CD. Concentration of cephalexin: 1.0 µg mL⁻¹. \( \lambda_{em} = 418 \) nm.
The influence of β-CD concentration on the fluorescence intensity of cephalexin was also investigated by keeping its concentration constant at 1.0 µg mL⁻¹ and varying the β-CD concentration from 2.0 to 14.0 % (w/v). The fluorescence intensity reached its maximum when the concentration of β-CD is 10 % and then decrease as shown in Figure 5. So 10% concentration of β-CD was quite appropriate for further studies.
**Effect of Reaction Temperature and Time**

The effect of temperature on the reaction was also studied by varying the temperature from 20 °C to 80 °C for cephalaxin Figure 6. The inclusion complex does not proceed at high temperature and the highest fluorescence intensity is obtained at 30°C for 10 min. This can be ascribed to the drug degradation in high temperature and increase in the kinetic energy of the molecules and hence the probability of their colliding; as a result, radiationless deactivation through the internal conversion prevailed and the fluorescence quantum efficiency decreased, furthermore, it is also observed that the absorbance remain constant for 20 min. Figure 7.

![Graph](image1.png)

Figure 6. Influence of the temperature on the fluorescence intensity of cephalaxin-β-CD complex; [cephalexin] =1.12×10^{-6} mol L^{-1}, pH 6.0, [β-CD] = 7.0× 10^{-3} mol L^{-1}, \( \lambda_{ex} = 272 \text{ nm} \), \( \lambda_{em} = 418 \text{ nm} \). Both excitation and emission slits widths were set at 10 nm, reaction time: 10 minutes.

![Graph](image2.png)

Figure 7. Effect of reaction time on fluorescence intensity of the inclusion complex of cephalaxin with β-CD, [cephalexin] =1.12×10^{-6} mol L^{-1}, pH 6.0, [β-CD] = 7.0× 10^{-3} mol L^{-1}, \( \lambda_{ex} = 272 \text{ nm} \), \( \lambda_{em} = 418 \text{ nm} \). Both excitation and emission slits widths were set at 10 nm.
The stoichiometry and apparent association constant of the inclusion complex were studied under the established experimental condition: assuming that the composition of the complex was 1:1, the following expression can be written:

\[
\text{Cephalexin} + \beta-\text{CD} \rightleftharpoons \beta-\text{CD}-\text{Cephalexin}
\]  

(1)

Where \( \beta-\text{CD}, \) [cephalexin] and \( \beta-\text{CD}-\text{cephalexin} \) are equilibrium concentrations. The apparent association constant value for the inclusion complex can be determined by the typical double reciprocal (or Benesi–Hildebrand) plots:

\[
\frac{1}{(F - F_0)} = \frac{1}{(F_\infty - F_0) K [\beta-\text{CD}]_0} + \frac{1}{F_\infty - F_0}
\]  

(3)

Where \( [\beta-\text{CD}]_0 \) denotes the initial \( \beta-\text{CD} \) concentration; \( F_0 \) the fluorescence intensity of cephalexin in the absence of \( \beta-\text{CD} \); \( F_\infty \) the fluorescence intensity when all of the cephalexin molecules are essentially complexed with \( \beta-\text{CD} \); and \( F \) the observed fluorescence intensity at each \( \beta-\text{CD} \) concentration tested. When a plot of \( 1/F - F_0 \) vs. \( 1/[\beta-\text{CD}] \) is constructed, a straight line is obtained which is indicative of a 1:1 stoichiometry for \( \beta-\text{CD}-\text{cephalexin} \) complex. Good linear relationship \( (r = 0.9992) \) is observed in Figure 8. On the other hand, assuming the stoichiometry of the inclusion complex was 1:2, the following expression is obtained:

\[
\frac{1}{(F - F_0)} = \frac{1}{(F_\infty - F_0) K' ( [\beta-\text{CD}]_0 )^2} + \frac{1}{F_\infty - F_0}
\]  

(4)

When making a plot of \( 1/F - F_0 \) against \( 1/([\beta-\text{CD}]_0)^2 \), nonlinear relationship is obtained Figure 9, which indicated that the stoichiometry of the inclusion complex is not 1:2. These confirmed that \( \beta-\text{CD} \) and cephalexin formed host–guest complex in 1:1 stoichiometry. The inclusion constant \( (K) \) was calculated to be \( 5.33 \times 10^2 \) L mol\(^{-1}\) and the relative standard deviation (RSD) was 3.45% (\( n = 5 \)).
Figure 8. Plot of $1/(F - F_0)$ vs. $1/[\beta-CD]$ of cephalxin-β-CD complex; [cephalexin] = 2 μg mL$^{-1}$.

Figure 9. Plot of $1/(F - F_0)$ vs. $1/[\beta-CD]^2$ of cephalxin-β-CD complex; [cephalexin] = 2 μg mL$^{-1}$.

**Inclusion complex thermodynamics**

The thermodynamic parameters ($\Delta H^\circ$, $\Delta S^\circ$ and $\Delta G^\circ$) for the formation of inclusion complex were determined from temperature dependence of apparent association constants, by using classical van’t Hoff equation ($\ln K = -\Delta H^\circ/RT + \Delta S^\circ/R$), and plotting $\ln K$ versus $1/T$ [35, 36]. The corresponding enthalpy and entropy can be obtained from the slope and intercept.
respectively, which indicate the marked tendency of cephalexin to complex with β-CD. \( \Delta G^\circ \) was obtained according to the equation: \( \Delta G^\circ = \Delta H^\circ - T \Delta S^\circ \). The results were shown in Table 1. The stability constant of the complexes of cephalexin with the β-CD at different temperature (303, 313, 323 and 333 K) are shown in Table 1. We note, that the association constant for cephalexin -β-CD decreases as the temperature rises.

Thermodynamic parameters were calculated based on the temperature dependence of the association constant for cephalexin-β-CD binding. The thermodynamic parameters such as the enthalpy changes (\( \Delta H \)) and entropy changes (\( \Delta S \)) of the binding reaction are important to confirm the force of interactions of cephalexin with β-CD. Four driving forces for the inclusion of CDs with substrates were proposed, including: hydrogen bonding between the hydroxyl groups of CDs and the guest, van der Waals interactions between host and guest molecules, hydrophobic interaction, and the release of ‘high-energy water’ molecules from the cavities of β-CDs to the bulk water. Hydrophobic interaction essentially involves favourable positive entropy together with a slightly positive enthalpy change, whereas the other forces involve negative \( \Delta H \) and \( \Delta S \) [37].

Upon complexation both positive enthalpic changes and positive entropic values are obtained, indicating that this inclusion is mainly entropically driven. Apparently, when cephalexin is free in solution, it seems to have a strong interaction with its solvent shell. Upon binding, this solvent shell is broken up, leading to the partly unfavorable enthalpic change.

With the debates above, \( \Delta G^\circ \) obtained in Table 1 are negative, which display the inclusion process proceeded spontaneously at experimental temperature. The positive \( \Delta H^\circ \) together with positive \( \Delta S^\circ \) suggested that the inclusion process is an enthalpy controlled process in the case of the cephalexin and cephalexin-β-CD complex.

### Table (1) Thermodynamic parameter

<table>
<thead>
<tr>
<th>Temperature (K)</th>
<th>303</th>
<th>313</th>
<th>323</th>
<th>333</th>
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</thead>
<tbody>
<tr>
<td>Ln K_(\alpha)</td>
<td>6.279</td>
<td>5.999</td>
<td>5.347</td>
<td>4.078</td>
</tr>
<tr>
<td>(\Delta G^\circ)  (kJ mol(^{-1}))</td>
<td>-44.59</td>
<td>-29.12</td>
<td>-20</td>
<td>-19.05</td>
</tr>
<tr>
<td>(\Delta H^\circ)  (kJmol(^{-1}))</td>
<td>0.00928</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\Delta S^\circ)  (Jmol(^{-1}) K(^{-1}))</td>
<td>99.37</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a Apparent formation constant(K), b Standard free energy(\(\Delta G^\circ\)), c Enthalpy(\(\Delta H^\circ\)), d Entropy(\(\Delta S^\circ\)).

### 3.7. Validation of the Methods

#### 3.7.1 Linearity and Limit of Detection.

In the proposed methods, linear plot (n =5) the regression for the results was A=0.1684+0.01624C (r=0.9992), where A is the absorbance at 272 nm, C is the concentration of cephalexin in µg/mL in the range of 1-10 µg/mL, and r is correlation coefficient. The limit of detection (LOD) and limit of quantification (LOQ) were determined according to the following formula LOD=3.3×SDa/b, and LOQ =10 × SDa/b, SDa is the standard deviation of intercept; b is the slope [38]. The LOD and LOQ were 0.001 and 0.036 µg/mL.
Selectivity
The effect of the presence of common excipients such as; starch, magnesium stearate, iron oxide yellow, titanium dioxide and gelatin was studied. It was found that no interference was introduced by any of them.

Recovery of cephalixin
To a fixed amount of the drug in the dosage form, pure drug was added at three different levels and the total was found by the proposed methods. Each test was performed in triplicate. The recovery of each was calculated by comparing the concentration obtained from the spiked mixtures with those of the pure drug. The percentage recoveries are revealing good accuracies and non-interference from any excipients, which are present in capsules, Table 2.

**Applications of the methods**
In order to study the validity of the proposed method, the pharmaceutical dosage forms (Amilexin capsule) was subjected to the analysis of their cephalixin content by the proposed method. The percentages were found to be 104.6 ± 0.81, Table 3. Indicate the high accuracy of the proposed method for the determination of the studied drug. The proposed method has the advantage of being virtually free from interferences by excipients.

<table>
<thead>
<tr>
<th>Table 2. Recovery of the spectofluorometric methods</th>
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<tbody>
<tr>
<td>Sample content (µg mL⁻¹)</td>
</tr>
<tr>
<td>--------------------------</td>
</tr>
<tr>
<td>0.01</td>
</tr>
<tr>
<td>0.01</td>
</tr>
<tr>
<td>0.01</td>
</tr>
</tbody>
</table>

* Recovery was calculated as the amount found/amount taken × 100. Values are mean for three determinations

<table>
<thead>
<tr>
<th>Table 3. Applications of the methods</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug</td>
</tr>
<tr>
<td>-------</td>
</tr>
<tr>
<td>Amilexin</td>
</tr>
</tbody>
</table>

*Values are mean of five determinations

**CONCLUSIONS**
The supramolecular interaction of cephalixin with β-CD has been investigated by spectrofluorimetry. The results showed that β-CD reacted with cephalixin to form a 1:1 (host:guest) complex with inclusion constant \( K = 5.33 \times 10^2 \) L mol⁻¹. Based upon the enhancement effect observed, a sensitive spectrofluorimetric method for the determination of cephalixin was proposed. Under optimized experimental conditions, the method was applied to the detection of cephalixin in pharmaceutical formulations.
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