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Binding Energy and Photostability of the β-cyclodextrin Encapsulates of Lornoxicam and Tenoxicam drugs:A combined Experimental and Theoretical Study

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Abstract

The lornoxicam (LRX) and tenoxicam (TNX) drugs form a stable 1:1 inclusion complex with b-cyclodextrin (β-CD) in aqueous solution. The experimentally determined association constants (K) of LRX- β-CD and TNX- β-CD are 13.4 and 10.3 M^{-1}, respectively. Quantum chemical computations simulated the preferred orientation of guest molecules in the host. Geometry optimized results using the ONIOM technique provided more in-depth insights and identified the structure and showed that both drugs were partially encapsulated within the β-CD cavity. The calculated inclusion binding energy (BE, kcal mol-1) reveals the noticeable thermal stability of LRX-β-CD (-24.19 kcal/mol) over the TNX-β-CD (-13.45 kcal/mol) capsulate. Furthermore, the photostabilities of the encapsulated drugs were tested. Drug encapsulation did not result in any additional photostability. Moreover, encapsulation of the drugs in the β-CD resulted in noticeable changes in the electronic characteristics of the drugs, as reflected in their reactivity indices. The fact that the water-soluble β-CD formed inclusion complexes with water-insoluble LRX and TNX enables the drug delivery vehicle for oral administration.

Keywords: Lornoxicam, Tenoxicam, Photostability, ONIOM, Binding Energy, Reactivity indices

1. Introduction

Photobreakdown of a drug is known to result in a loss of potency of the product and the development of antagonistic effects due to the formation of photodegradants during the storage or administration of the drug product. An increasing number of drugs are being found to undergo photodecomposition, which requires protection starting from handling until the final product. Photoprotection can be acquired through external or internal protection. External protection avoids radiation from reaching the formulation, by suitable packaging or by the use of a coating material, as in the case of capsules and tablets. Internal protection can be obtained through the addition of a stabilizer of the ability of absorb light photons more rapidly than the drug, or it can operate by suppressing the photoreaction [1]. Lornoxicam (LRX) [6-chloro-4-hydroxy-2-methyl-N-2-pyridyl-2H-thieno [2, 3-e] [1, 2]-thiazine-3 carboxamide-1, 1-dioxide] and tenoxicam (TNX) [(4-hydroxy-2-methyl-N-(pyridyl)-2H-thieno [2, 3-e] 1, 2-thiazine- 3- carboxamide 1, 1-dioxide)] are two analgesics belonging to the oxicam derivatives non-steroidal anti-inflammatory drugs (NSAIDs) (see Fig. 1). LRX is used in the management of musculoskeletal and joint disorders such as osteoarthritis and rheumatoid arthritis; it is also used in the treatment of other painful conditions, including postoperative pain [2]. TNX inhibits cyclooxygenase and subsequent prostaglandin formation. It is used in the treatment of rheumatological disorders [3].

It was reported that when the methanolic solutions of TNX and LRX were irradiated at 254 nm

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for 7 h, five main photodegradation products were identified [4, 5]. To the best of our knowledge, TNX was reported to present fast photochemical decomposition at pH 7.5. In the case of free molecules, increasing the concentration the photostability is enhanced. The effect of the encapsulation of TNX in CD on the photodegradation rate was studied [5]. TNX was also reported to undergo photodegradation when photoirradiation with 254 nm radiation in methanol for 7 hours [6].

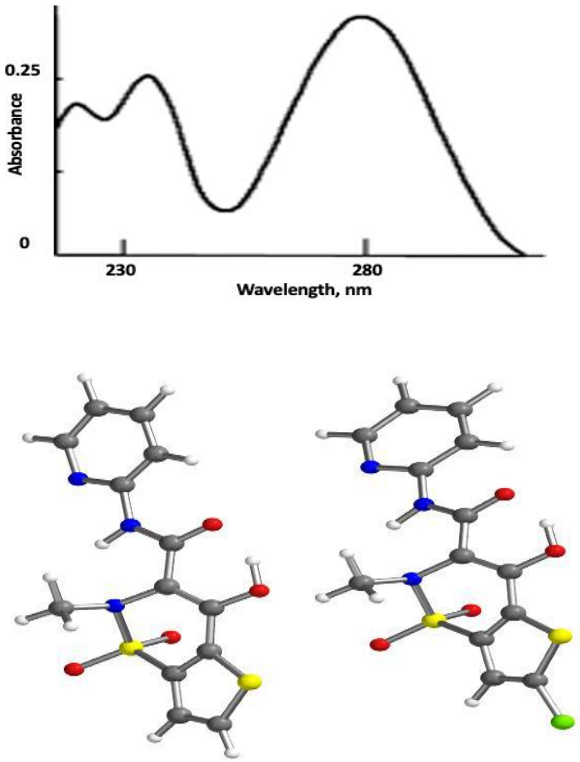

Figure 1. Absorption spectrum of LRX in ethanol and chemical structures of LRX (at the left side) and TNX (at the right side). Similar spectrum was obtained for TNX but blue shifted by 14 nm relative to that of LRX. (Color of the atoms = C: gray, H: white, N: blue, O: red, S: Yellow, Cl: green)

The effect of encapsulating materials in the β-CD cavity has been extensively investigated. Encapsulation remarkably alters the physicochemical properties of the guest materials, allows the encapsulated molecules to overcome biological barriers and protect the encapsulate [7]. Noteworthy mentioning that, (β-CD) (among other types of cyclodextrins (CDs)) is a well-known molecular host for forming stable host–guest inclusion complexes or supramolecular species because of their extraordinary hydrophobic internal cavity and hydrophilic external surface. The excellent ability of CDs or their derivatives to form inclusion complexes is widely used in numerous areas, e.g. analytical chemistry, pharmaceutical fields, food, agriculture, textiles, for removing environmental impurities, host guest systems, supramolecular chemistry, catalysis, drug carrier, and cosmetics industries [8-12]. This work aimed to study the effect of the encapsulation of LRX and TNX within the β-CD cavity and the impact on their photostabilities. It is also interesting to validate the experimental results by theoretical investigations for interpreting the results more deeply. The encapsulation – induced changes in the electronic characteristics of both drugs will also be investigated. The ONIOM multilayer modeling method will be applied for the first time with encapsulated LRX and TNX drugs in the β-CD cavity. Moreover, theoretically predicted drug- β-CD binding energies of LRX and TNX drugs will be computed and vis-à-vis the experimental values of the association constants. Although the literature shows the importance of inclusion complexes ability to help produce innovative biotechnological substances, we still need more studies to develop and expand their therapeutic properties. It is, therefore, very important to gather confirmation of the efficacy of inclusion complexes with cyclodextrins in order to assist a better understanding of research on this area and inspire further reports.

2. Material and Methods

2.1. Chemicals and reagents

Lornoxicam (LRX) (99.5%) and tenoxicam (TNX) (100.2%) were obtained from Glenmark Generics Limited and Marcyrl Pharmaceutical Industries Company, Egypt, respectively.

2.2. Instrumentation

A home-made photoreactor with 120 W UVA lamps was used for irradiation. A Shimadzu UV-Vis spectrophotometer Model UV-2450 (Helios Company, Japan) was used for the measurements.

2.3. Sample preparation and irradiation tests

The absorption spectra of LRX and TNX were measured in ethanolic solution (Fig. 1). Samples were irradiated within the air-cooled UVA home-made photoreactor (120 W) and the effect of irradiation was monitored spectrophotometrically. LRX and TNX inclusion complexes with β-cyclodextrin prior to irradiation were prepared as follows: Weigh approximately 0.3718 gm and 0.337 gm of LRX and TNX, respectively and 1.1350 gm of β-CD and grind them in mortar. Then incubate the mortar for 24 h at 60 ºC. Add a few drops of distilled water to form the paste. Incubate the mortar again for 24 h at 60 ºC. Transfer a suitable weight of (LRX- β-CD) and (TNX- β-CD) dried paste complexes to a 250 mL

volumetric flask and complete with distilled water and sonication for 24 h at 60 ºC. Transfer a constant volume of the complex's solutions and different volumes of (2×10-3 M) β-CD aqueous solution separately to volumetric flasks and complete with distilled water.

2.4. Computational Methods

Density functional theory (DFT) was applied using the APDF/6-31G(d,p) method to optimize the geometries of both LRX and TNX. Then, we utilized the ONIOM multilayer technique [13, 14] with (APFD/6-31G(d,p):PM6) methods included in Gaussian 16 version A.03 [15] for the inclusion complexes. We employed the Austin-Frisch-Petersson functional with dispersion APFD/6-31G(d,p) [16] method for the higher level layer in the ONIOM job. The APFD hybrid-DFT functional is selected because at present it represents the best balance between accuracy and computational cost for many molecular systems [17]. Semi-empirical PM6 method was used for the outer (low) layer (β-CD) and using the solute mechanical density (SMD: water) model [18]. Frequency calculations yield real frequency modes, which reveal that molecules are at a minimum global energy. A Broadberry Workstation (40 cores; 80 threads) (UK) was used.

3. Results and discussion

3.1. Determination of the association constant (K)

First, we examined the possibility of the formation of inclusion complexes between LRX, TNX and β- CD. The UV-Vis absorption spectra of aqueous solutions of LRX and TNX in the presence of β-CD were measured. Similar to the general observation reported in support of the formation of inclusion complexes [8-12], the addition of different concentrations of β-CD to a constant concentration of the aqueous solution of LRX or TNX (2×10^{-5} M) induced a small decrease in the absorbance value of the LRX or TNX with increasing the concentrations of β-CD. The association constants for the inclusion complexes of LRX, TNX and β-cyclodextrin were calculated by applying the Benesi–Hildebrand treatment to the UV absorption [19]. The association of the drug with β-CD depends on the formation of energetically favorable weak bonding interactions between the two partners. Keq indicates how much inclusion complex is formed:

[Drug] + [β-CD] ⇋ [Drug.β-CD]

$K\ (M^{-1})$ = [Drug.β-CD]/[Drug]x[β-CD]

The well-known Benesi– Hildebrand [19] relation is used for the evaluation of the association constant K at equilibrium by assuming the formation of a 1:1 host–guest complex:

where [Drug] is the initial concentration of LRX and TNX, [β-CD] is the concentration of β-CD added, Δε is the difference between the molar absorptivity of solutions of associated and non-associated drugs, ΔA is the difference between the absorbance of solutions of associated and non-associated LRX and TNX and K is the association constant of the complex between the drugs and β-CD. Plotting of 1/ΔA vs 1/ [(β-CD] results in straight lines (with correlation coefficient r = 0.99 or better), which confirms the 1:1 complex formation. Dividing the slope by the intercept yields the K value (13.4 M^{-1} for LRX and 10.3 M^{-1} for TNX). The experimentally obtained K values refer to a weak inclusion complex formation in solution, which provides an explanation of the observed negligible change in the photodegradation rates of both drugs (about 0.12 hr^{-1}) compared to the presence of β-CD (0.11 hr-1). Figure 2 shows the fading of the absorption spectrum of LRX associated with the β-CD aqueous solution over time of irradiation. Similar results were obtained in the case of TNX.

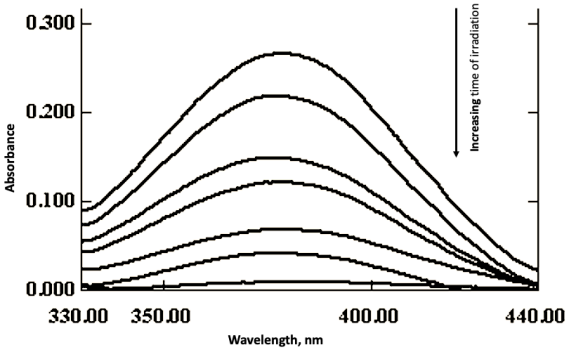

Figure 2. Effect of increasing light exposure time up to 21 hours on the absorption spectra of LRX-β-CD inclusion complexes. Similar behavior was noticed for the TNX- β-CD complex.

3.2. Theoretical determination of binding energy (ΔE_B)

To validate the experimental results obtained, we carried out theoretical computations using two different strategies described below.

3.2.1. DFT computations

From a theoretical point of view, the well-known stabilization energy ΔEB (or the binding energy) is defined:

$\Delta E_B = E_{Drug\text{-}(\beta\text{-}CD)} - (E_{(\beta\text{-}CD)} + E_{Drug})$

where $E_{Drug\text{-}(\beta\text{-}CD)}$, $E_{(\beta\text{-}CD)}$ and E_{Drug} are the (formation energy) or total energy of the geometry-optimized complex, free (β-CD) and the guest drug molecules, respectively. Table 1 summarizes the collected data. Inspection of Table 1 indicates that the LRX-(β-CD) encapsulate has $\Delta E_{B(LRX\text{-}(\beta\text{-}CD))}$ = -24.19 kcal/mol, which is pointing to its high thermal stabilization relative to the TNX-(β-CD) encapsulate of $\Delta E_{B(TNX\text{-}(\beta\text{-}CD))}$ = -13.45 kcal/mol. The calculated reactivity indices from the frontier orbitals [20-23] show the effect of encapsulation on the chemical potential, hardness, electrophilicity and nucleophilicity. The

encapsulation results in increasing the chemical potential and electrophilicity of the drug, while the hardness and nucleophilicity values decrease. Figure 3 depicts the structure of the complexes.

Figure 3. Inclusion complexes of LRX (left side) and TNX (right side) (space filling model) with β-CD (tube model). Black dotted lines represent the hydrogen bonds between the drug molecule and the β-CD.

Table 1. Calculated total molecular energy, HOMO, LUMO, binding energy and reactivity indices of the molecules studied (DFT using APDF/6-31(d,p) method)

Molecule	Energy (au)	E HOMO (eV)	E LUMO (eV)	BE kcal/mol	μ	η	ω	N
LRX	-2222	-8.2	-0.8	---	-4.5	3.7	2.77	2.97
TNX	-1763	-8.2	-0.6	---	-4.4	3.77	2.61	2.99
CD	-4274	8.8	3.4	---	-2.6	6.15	0.59	2.36
LRX-CD	-6496	-8.2	-0.9	-24.1	-4.5	3.68	2.85	2.93
TNX-CD	-6037	-8.2	-0.7	-13.4	-4.4	3.74	2.68	2.98

3.2.2. The ONIOM computations

The supportive result of the formation of inclusion complexes between the drugs and β-CD was obtained from computations using the multilayer technique included in the Gaussian package [15] by using the two-layered ONIOM method [13, 14]. In this method, the encapsulated drug is divided into inner and outer layers (see Figures 2 and 3). The inner layer consists of the **LRX** or **TNX** complexes calculated at a higher level of theory (APFD/6-31G(d,p)), and (β-CD) comprises the outer layer calculated at a lower level of theory (semi-empirical PM6), which yields a consistent energy expression with similar accuracy to a higher-level calculation in the full system. The full system is called ''real'' treated with a lower level of theory; the inner layer is termed ''model'' treated with both the lower and the higher levels of theory. The total stabilization energy (EONIOM) is expressed:

$$E^{ONIOM} = E_{(high,\ model)} + E_{(low,\ real)} - E_{(low,\ model)}$$

where $E_{(high,\ model)}$ is the energy of the inner layer guest molecule at a higher level (here DFT model, $E_{(low,\ real)}$ is the energy of the full system (inclusion complex) at the lower level (here at PM6), and $E_{(low,\ model)}$ is the energy of the outer layer (β-CD) at the lower level. The binding energy and the total stabilization energy (EONIOM) were used to confirm the most favorable inclusion complex structure starting from optimized equivalent geometries of LRX and TNX drugs to treat the two drugs equivalently and to check the trend obtained by APFD/6-31G(d,p) computations. As seen from the data in Table 1, the inclusion reaction is exothermic for both inclusion complexes. LRX-(β-CD) is much more thermodynamically stable than TNX-(β-CD). To clarify the calculations, we follow the same method of data presentation [24, 25] and as summarized in Table 2.

Table 2: Thermodynamic data for the inclusion complexes of the molecules studied with (β-CD) using ONIOM (APFD/6-31G(d,p):PM6) in water*

complex	(high, model)/au	(low, real)/au	(low, model)/au	E^{ONIOM}/ kcal/mol
LRX-(β-CD)	-2222.02	-2.697	-0.125	-1.397
TXN-(β-CD)	-1762.54	-2.694	-0.124	-1.108

* E^{ONIOM} is given for the sake of clarity and not for comparison.

Thermodynamic results obtained from the oniom computations favor the trend LRX-(β-CD) > TNX-(β-CD). The formation of the inclusion complex of LRX-(β-CD) was stabilized relative to TNX-(β-CD) by -2.02 kcal/mol (using higher level ONIOM (APFD/6-31G(d,p):PM6)), which is calculated from the difference between E(low, real) of LRX-(β-CD) and E(low, real) of TNX-(β-CD) given in Table 2.

Finally, the source of stability of inclusion complexes stems from the interplay between H-bonding forces between the complexes and the (β-CD) molecule and other weak forces (such as van der Waals and dispersion forces), which are mainly the electronic factors dependent.

4. Conclusion

The two drugs investigated, namely **LRX** and **TNX**, form a stable 1:1 inclusion complex with betacyclodextrin (β-CD) in aqueous solution. The experimentally determined association constants (K) of LRX- β-CD and TNX- β-CD refer to stable complexes. The preferred orientation of guest molecules into the host was simulated by ONIOM quantum chemical computations, which identified the structure and showed that both drugs were partially encapsulated within the β-CD cavity. The calculated inclusion binding energy (BE, kcal mol-1) reveals the noticeable thermal stability of LRX-β-CD (-24.19 kcal/mol) over the TNX-β-CD (-13.45 kcal/mol) capsulate. Furthermore, the photostabilities of the encapsulated drugs were tested. Drug encapsulation did not result in any additional photostability. Furthermore, encapsulation of the drugs in the β-CD resulted in noticeable changes in the electronic characteristics of the drugs, as reflected in their reactivity indices. The encapsulation results in a slight enhancement of the chemical potential and the electrophilicity of the drug, while the hardness and nucleophilicity values slightly fall. The fact that β-CD formed inclusion complexes with water-insoluble LRX and TNX indicates its utility to enable the drug delivery vehicle for oral administration.

Conflict of interest

There are no conflicts of interest to declare. Data availability The corresponding author is ready to provide any detailed data upon request.

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