

Spectrofluorometric, thermal, and molecular mechanics studies of the inclusion complexation of selected imidazoline-derived drugs with β -cyclodextrin in aqueous media

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Abstract The inclusion complexes of selected imidazoline-derived drugs, namely Antazoline (AN), Naphazoline (NP) and Xylometazoline (XM) with β -cyclodextrin (β -CD) were investigated using steady-state fluorescence spectroscopy, differential scanning calorimetry (DSC), and molecular mechanics (MM) calculations and modeling. The modified form of the Benesi-Hildebrand relation was employed for estimating the formation constant (K_f) of the 1:1 inclusion complexes, which was applied based on measuring the variation in the fluorescence intensity of the guest molecule as a function of growing β -CD concentration. On the other hand, the formation of the inclusion complexes was verified by analyzing solid samples of the complexes using DSC. The thermodynamics of the inclusion complexation, standard enthalpy (ΔH°) and entropy changes $-(\Delta S^\circ)$ were obtained from the temperature-dependence of K_f . Obtained values of ΔH° and ΔS° indicated that the inclusion process favorably proceeds through enthalpy changes that was sufficiently predominant to compensate for the unfavorable entropy changes. MM calculations revealed that the proposed drugs molecules can form 1:1 inclusion complexes with β -CD that are stabilized predominantly through van der Waals forces. In addition, MM calculation provided the energetically

favored configuration of the inclusion complexes, where NP and XM can be included inside the β -CD cavity through its wide rim, whereas AN can penetrate through the narrow rim of the β -CD cavity.

Keywords Fluorescence · Inclusion complex · β -cyclodextrin · Imidazoline-derived drugs · Differential scanning calorimetry · Molecular mechanics

Introduction

Cyclodextrins (CDs) are cyclic oligosaccharides that have the capability to form inclusion complexes with wide spectrum of molecules. There is three typical and native CDs that are well-known and widely used; namely, α -, β -, and γ -CD, which consist of 6, 7, and 8 glucopyranose units, respectively [1, 2]. Also, synthesizing modified CDs are of particular importance in order to enhance the original properties of the native CDs [3–5]. The unique capability of CDs to form inclusion complexes with various molecules is due to its characteristic features of possessing apolar cavity and hydrophilic outer surfaces; and thus, the inclusion process progresses via the insertion of the hydrophobic portion of a molecule, partially or entirely, inside the apolar cavity.

CDs have been employed in a variety of fields such as catalysis, pharmaceutical and food industries [6–8], separation sciences [9–14], and biotechnology [15, 16]. While CDs have a wide range of applications, using CDs as additives in various separation and pharmaceutical sciences is still the foremost application of them. Hence, adding the CDs to the separation media can significantly enhance the separation process, whereas employing CDs as additives to drugs formulation can promote the bioavailability through

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enhancing the stability and solubility of selected drugs [15–19].

It is noteworthy mentioning that significant alterations in the physicochemical properties of the included molecule are observed upon forming the inclusion complex with CDs. Accordingly, various techniques and methodologies have been employed in studying the CDs inclusion complexes; this includes absorption spectrophotometry [20, 21], fluorescence [22–25], mass spectrometry [26], electrochemistry [27, 28], nuclear magnetic resonance (NMR) [29–31], thermal [32–34], and infrared spectrophotometry (IR) [35, 36]. However, fluorescence measurements are considered as the primarily employed technique for studying CDs' inclusion complexes with molecules that possess chromophore groups, which is due to the significant variations in the fluorescence intensity of the guest molecules upon being included inside the CD cavity. On the other hand, thermal analysis, differential scanning calorimetry (DSC) in particular, is frequently used in parallel with other techniques to provide qualitative analysis for evidencing the formation of the inclusion complexes. Recently, there has been growing interests in utilizing molecular mechanics (MM) and modeling to study the inclusion complexes of CDs [37–39]. The MM calculation offers better understanding for the inclusion process by providing informative insights onto the energetically favored structure of the inclusion complexes, which is of particular importance in interpreting experimental results.

Imidazoline-derived drugs are a family of drugs that is structurally distinguished by the existence of the heterocyclic ring of imidazoline that enables these drugs to interact with α -adrenergic receptors via stimulating presynaptic and postsynaptic α -adrenoceptors [40–42]. The majority of the imidazoline-derived drugs are frequently used for their agonist activity, whereas others are used for either antihypertensive, antihistaminic, or antagonist activities [41]. However, the principle pharmaceutical applicability of these drugs is due to their vasoconstrictive effects. Three typical imidazoline-derived drugs were selected for this study; Naphazoline (NP) (4,5-Dihydro-2-(1-naphthalenylmethyl)-1H-imidazole), Antazoline (AN) (4,5-Dihydro-*N*-phenyl-*N*-(phenylmethyl)-1H-imidazole-2-methanamine, 2-[(*N*-benzyl anilino) methyl]-2-imidazole, and Xylometazoline (XM) (2-(4-*tert*-Butyl-2,6-dimethylbenzyl)-2-

imidazoline). The chemical structures of AN, NP, and XM are shown in Fig. 1. On the other hand, several studies have been conducted to detect and analyze various imidazoline-derived drugs using a variety of methods and techniques; this includes derivative spectrophotometry [43], potentiometric membrane sensors [44], fluorescence [45], high performance liquid chromatography (HPLC) [46], and capillary electrophoresis (CE) [47].

In this study, the inclusion complexation of selected imidazoline-derived drugs, NP, AN, and XM with β -CD is investigated. Fluorescence measurements were collected in order to calculate the binding constant of the inclusion complexation for each drug molecule with β -CD; in turn, inclusion complexations were performed at various temperatures to in order to determine the standard entropy changes (ΔS°) and enthalpy changes (ΔH°) of the inclusion process. Complementary results that confirm the formation of the inclusion complexes were obtained using DSC analysis. MM calculation were conducted based on vacuum analysis and used to predict the energetically favored structure of the inclusion complexes. Explanations that correlate the results obtained using various techniques for studying the inclusion complexes of imidazoline-derived drugs with β -CD are presented and discussed.

Experimental

Chemicals

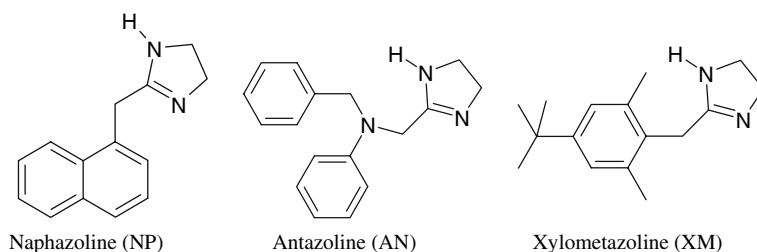
β -cyclodextrin (β -CD), sodium phosphate, and sodium 1-heptanesulfonate, were supplied by Sigma-Aldrich Co. The Antazoline sulfate and Naphazoline nitrate were provided as gift by Al-Hekma Pharmaceuticals (Amman, Jordan), and the Xylometazoline hydrochloride by Amman Pharmaceutical Industries (Amman, Jordan). Acetonitrile (ACN) and acetic acid (AA) were purchased from Fisher Scientific. All chemicals were used without further purification.

Measurements and methods

Preparation of the inclusion complexes

Buffer-free aqueous solutions of the inclusion complexes were prepared using in-house prepared deionized water

Fig. 1 Chemical structures of Antazoline (AN), Naphazoline (NP) and Xylometazoline (XM)



(18 M Ω) and were left for 2 h for equilibration before performing any further experimentation. As the drugs samples were received in the form of salted with acids (AN-H₂SO₄, NP-HNO₃, XM-HCl), high solubility in water was observed for each drug molecule, and hence no attempt was made to test enhancing the solubility of these drugs in the presence of CDs. On the other hand, a pH values in the range of 6.0–6.5 were observed for the drugs' solutions. Inclusion complexes in the solid state were prepared by freeze-drying a solution that contains approximately 1 mg of drug and 10 mg of β -CD in 10 mL of water. Reference samples of each drug were prepared similarly. Also, powder mixture that consisted of the same amount of the drugs and β -CD were prepared for referencing.

Spectrofluorometry measurements

The steady state emission measurements were obtained using Tegmenta SFM 25 spectrofluorimeter (Kontron Instruments) equipped with double monochromator and photomultiplier; slit width of 5 nm was used for excitation and emission. Fluorescence measurements were performed for 1×10^{-5} M of each drug molecule under complexation-free conditions and in the presence of the β -CD with concentration in the range of 0.4–2 mM. Emission spectra were collected at excitation wavelength (λ_{ex}) of 297, 288, and 285 for AN, NP and XM, respectively. All emission and absorption spectra were collected using quartz cells.

Binding constant and thermodynamic parameters calculation

The variations in the fluorescence intensity of each drug molecule upon forming inclusion complex with β -CD were used to calculate the formation constant (K_f). An average of three measurements was used for each data point. A modified form of the Benesi-Hildebrand relation that implies 1:1 inclusion complex was applied to calculate the formation constant:

$$\frac{1}{(F - F_0)} = \frac{1}{(F_\infty - F_0)} + \frac{1}{(F_\infty - F_0) \cdot K_f \cdot [\text{CD}]_0} \quad (1)$$

where F_0 , F , and F_∞ correspond to the fluorescence intensities in the absence of β -CD, in the presence of β -CD, and when all the guest's molecules are included inside the β -CD cavity, respectively; K_f is the formation constant; and $[\text{CD}]_0$ refers to the initial concentration of β -CD. Emission wavelengths maxima ($\lambda_{\text{em}}\text{max}$) of 333, 330, and 325 nm were recorded for AN, NP and XM, respectively. Standard thermodynamics parameters of the inclusion

process, ΔS° and ΔH° , were calculated by applying the van't Hoff equation:

$$\ln(K) = \frac{-\Delta H^\circ}{RT} + \frac{\Delta S^\circ}{R} \quad (2)$$

where, T and R correspond to the temperature in Kelvin and gas constant, respectively. A linear correlation is expected upon plotting $\ln(K)$ against the reciprocal of temperature; hence, ΔS° and ΔH° are obtained from the slope and intercept of the linear plot.

Thermal analysis

The DSC measurements were carried out using DSC 910S Differential Scanning Calorimeter (TA instrument) equipped with vented aluminum pans. Thermograms of ~ 5 mg samples were obtained by scanning within a temperature range of 50–250 °C and scanning rate of 10 °C/min.

MM calculations

MM calculations were performed in vacuum using Hyperchem software (release 4, Hyperchem Inc., Waterloo, Canada) and MM⁺ force field. A relative permittivity of 1.5 and conjugate gradient algorithm of 0.1 Kcal/mol Å were applied for electrostatic interaction and minimization, respectively. The β -CD structure was built using previously published x-ray diffraction data [48]. Geometrical energies for β -CD and drugs' structures were minimized using the MM+ force field. The inclusion process was simulated by positioning the O₄ glucosides atoms at the Cartesian coordinates' origin; the β -CD was held fixed at the original while allowing the guest molecule to approach and penetrate inside the β -CD cavity. Simulations were performed while the guest molecule approaches toward the wide and narrow rim, alternatively. Since each molecule may approach the β -CD cavity through different parts of the molecule, MM calculations were performed based on the inclusion of different parts of each guest molecule.

Results and discussion

Spectrofluorometric investigations

There is a variety of techniques and methodologies that can be employed for analyzing inclusion complexes of CDs with wide range of guest molecules via recording variations in the physicochemical characteristics of the guest molecules during the complexation process. Accordingly, the inclusion process can be evident through justifying

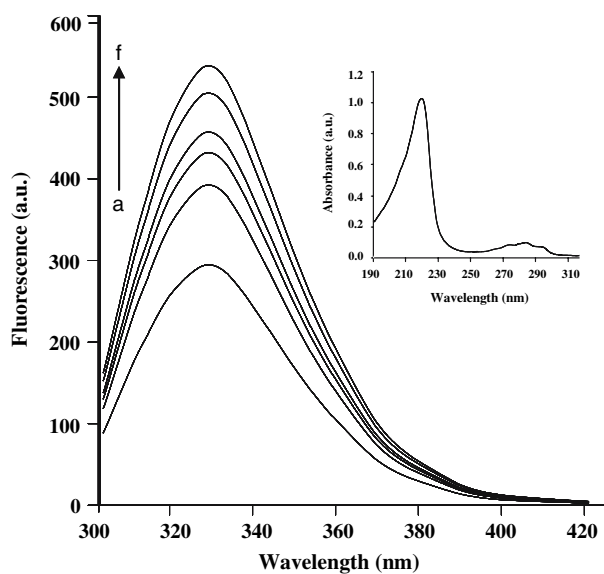


Fig. 2 Fluorescence spectra of 1×10^{-6} M of Naphazoline in the presence of growing concentration of β -CD: (a) 0; (b) 0.4; (c) 0.7; (d) 1.0; (e) 2; and (f) 4 mM. The inset shows the absorption spectrum of 1×10^{-5} M of NP

these variations. Amongst these techniques, spectrofluorometry is considered as powerful methodology because of its comprehensivity in providing qualitative and quantitative details regarding the inclusion process. Hence, adding CDs to the solutions of wide range of fluorophores results in dramatic alterations within the emission properties of these fluorophores (fluorescence intensity) because of including the guest molecule inside the apolar cavity of the CD molecule. On the basis of such observation, the inclusion complexation of imidazoline-based drugs with β -CD was investigated employing the routinely used technique of spectrofluorometry. Figure 2 illustrates the influence of increasing concentration of β -CD on the fluorescence spectrum of NP at room temperature (25 °C); the inset shows the absorption spectrum for the same solution of NP. Notably, adding β -CD to the solution of NP has significantly increased the fluorescence intensity of NP. Such observation could be attributed mainly to the role of the apolar cavity of β -CD in stabilizing the NP molecules during the excitation-relaxation phenomenon. In particular, the apolar cavity provides protection for the NP molecule at the excited state against various deactivation processes, such as collisions with water molecules in the bulk solutions and internal conversion that may reduce the fluorescence intensities of the NP molecules. Similarly, enhancement in the fluorescence intensities of the other two drugs, AN and XM, was observed (data not shown). These observations suggest that stable inclusion complexes between β -CD and these three imidazoline-derived drugs were formed.

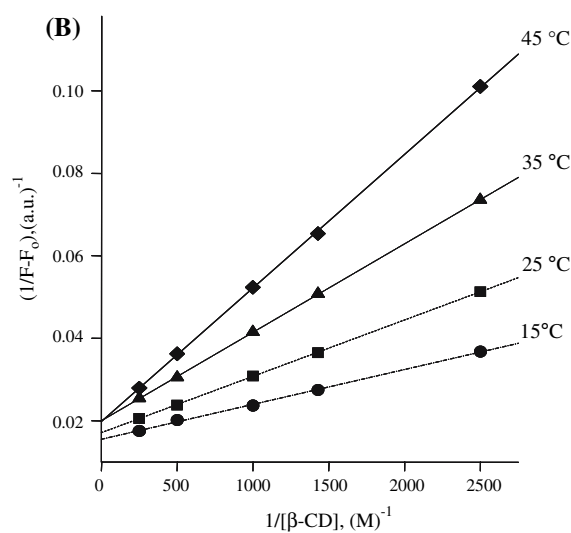
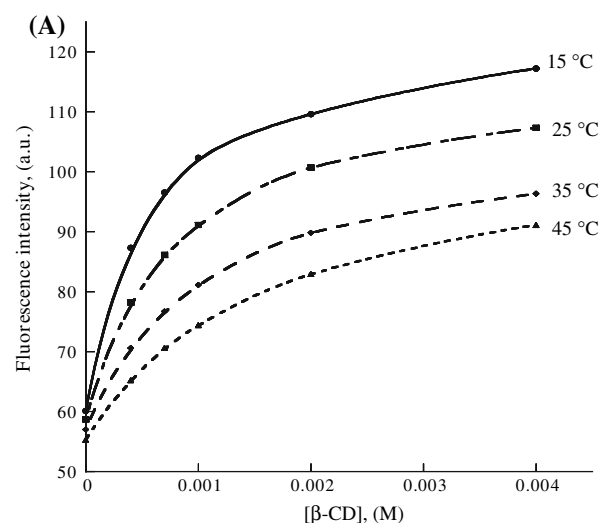


Fig. 3 Plot of fluorescence intensity of 1×10^{-5} M of Naphazoline as a function of β -CD concentration (a), and double-reciprocal plot of the fluorescence intensities and β -CD concentrations at 25 °C. Other conditions are the same as in Fig. 2

It is well known that the efficiency of the complexation process of β -CD with various guest molecules depends mainly on their molecular sizes and polarities, which in turn determine the deriving forces that lead to the formation of inclusion complexes of CDs with various molecules [49]. Therefore, it is essential to perform quantitative analysis that can provide some insights on the extent of the complexation process. Hence, the formation constant (K_f) is considered as the most important parameter in studying the inclusion complexation process of CDs, which in turn provides extensive details about the efficiency of the inclusion process. Thus, the enhancement in the fluorescence intensities of the guest molecules upon forming inclusion complexes with β -CD was used for determining K_f . It worths mentioning that using acid-salted form of the

drugs proposed in this study disregarded the potential of using phase-solubility method for studying the inclusion complexes. Importantly, performing inclusion experimentation using molecules of the same family with different structures are of particular importance in providing better understanding of the inclusion complexation process. Figure 3a shows typical plot for the fluorescence intensity of NP as a function of β -CD concentration. According to the modified form the Benesi-Hildebrand relation (Eq. 1), the K_f was calculated based on double-reciprocal plotting of variation in fluorescence intensities of the guest molecule as a function of β -CD concentration. Figure 3b illustrates a typical plot for the variations in NP fluorescence intensities against β -CD concentration in the range 0.4–4.0 mM. The correlation coefficient (R^2) of the linear fits was >0.99 , which implies that a 1:1 inclusion complex was formed between NP and β -CD. K_f was calculated from the ratio of the intercept to the slope of the linear fit. Similarly, identical procedure was applied for the other two drugs (AZ and XY), where the formation of a 1:1 inclusion complexes with β -CD was confirmed. Furthermore, a plot for the reciprocal of the variations in the fluorescence intensities against the reciprocal of $[\beta\text{-CD}]^2$ was constructed, which in turn revealed poor R^2 ; and thus, the possibility of forming 2:1 inclusion complex with β -CD for all drugs molecules was eliminated. In view of that, it is eminent that the concept size/shape-fit plays critical role in the inclusion process, which in turn could be judged through comparing the values of K_f . Accordingly, a K_f values of 1031, 1250, and 691 M^{-1} were obtained at room temperature (25°C) for AN, NP, and XM, respectively; hence, these values of K_f reveal a size/shape-fit in the order $\text{NP} > \text{AN} > \text{XM}$. Interestingly, K_f values obtained in this study are relatively close to those obtained for the inclusion complexation of β -CD with drugs molecules that exhibit some structural similarities to NP, such as Nabumetone and Naproxen, using the spectrofluorometric technique [50].

Thermodynamics of inclusion process

The inclusion process between imidazoline-derived drugs and β -CD was investigated at different temperatures in order to determine the thermodynamics parameters accompanying the inclusion process. Figure 3b shows the plots of the double-reciprocals of the variations in fluorescence intensity of NP as a function of β -CD concentration within a temperature range of $15\text{--}45^\circ\text{C}$. Interestingly, significant increase in the slope of the linear fits can be observed as the temperature increases with minimal changes in the intercepts, which is an indication for the decrease in the value of the K_f as the temperature increases. This observation can be attributed to the

dissociation of the inclusion complex at higher temperatures, which in turn can be attributed to the excessive molecular motions at higher temperatures. Accordingly, plotting the natural logarithm of the formation constant ($\ln(K_f)$) against the reciprocal of temperature (in Kelvin) according to Vant Hoff relation (Eq. 2) provides direct method for determining the changes in ΔH° and ΔS° during the complexation process. These plots were constructed for all drugs (data not shown) and the thermodynamics parameters for the inclusion complexation with β -CD are summarized in Table 1. In addition, the changes in standard Gibb's free energy (ΔG°) at room temperature (25°C) were calculated based on the Gibbs equation: $\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ$, where T corresponds to the room temperature in Kelvin (298 K). As can be noticed from Table 1, the enthalpy and entropy changes have negative values, which suggests that the inclusion reaction is exothermic and more ordered-system is obtained after complexation. However, the high ΔH° value in comparison to ΔS° value indicates that the inclusion process is predominantly driven through enthalpy changes, which in turn can compensate for the unfavorable entropy changes. Furthermore, a negative ΔG° was obtained for all drugs molecules that were investigated (AZ, NP and XM), which implies that the inclusion complexation reaction between these molecules and β -CD proceed spontaneously. Additionally, it is worth pointing out that AN exhibited the lowest ΔH° and ΔS° upon forming inclusion complex with β -CD in comparison to NP and XM, which could be attributed to the large geometrical size of the molecule; and thus, the higher steric hindrance could have retarded the inclusion process.

Thermal characterization

Thermal analysis, DSC in particular, is one of the most frequently used techniques that can be utilized to evident the formation of inclusion complexes of CDs with various guest molecules. Typically, a shift or disappearance of the endothermic peak that corresponds to the melting or sublimation points of pure guest molecule is observed upon performing similar DSC analysis of the inclusion complex

Table 1 Thermodynamic parameters for the inclusion complexation of Naphazoline, Antazoline, and Xylometazoline with β -cyclodextrin at 25°C

Drug	ΔH° (kJ/mol)	ΔS° (J/Kmol)	ΔG 25°C (kJ/mol)
Naphazoline	-27.5	-33.0	-17.7
Antazoline	-21.6	-14.6	-17.2
Xylometazoline	-23.1	-23.4	-16.1

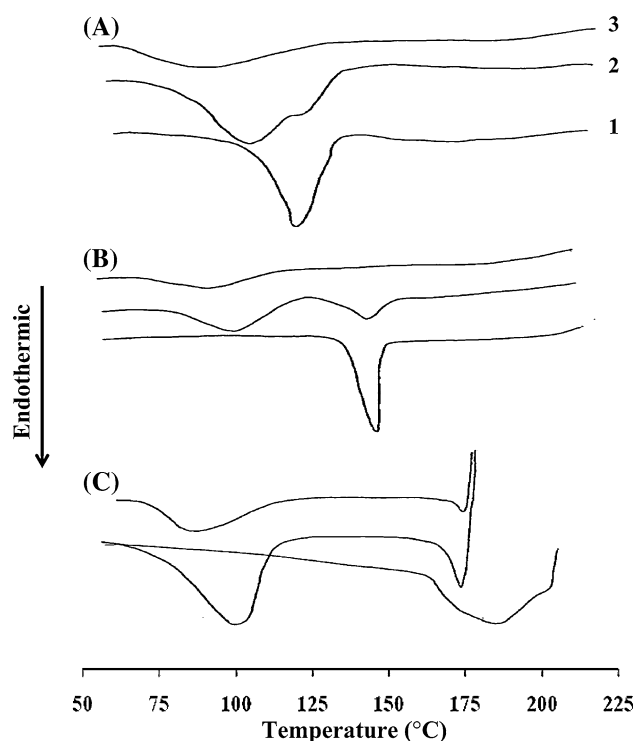


Fig. 4 DSC thermograms of Antazoline (a), Naphazoline (b) and Xylometazoline (c); curves 1, 2 and 3 correspond to drug sample, physical mixture of drug sample and β -CD, and drug: β -CD inclusion complex, respectively

[32–34]. Figure 4 shows three DSC thermograms for the imidazolines drugs before and after complexation with β -CD, where frames A, B, and C correspond to thermograms of AN, NP and XM, respectively. On the other hand, thermograms were obtained for pure drug (curve 1), physical mixture of pure drug and β -CD (curve 2), and inclusion complex of the drug molecule with β -CD (curve 3). As can be noticed from Fig. 3, the DSC thermograms of

AN (frame A) and NP (frame B) exhibit two sharp endothermic peaks at approximately 120 and 150 °C that disappeared for the inclusion complexes. This observation confirms the formation of the inclusion complexes of the imidazolines drug with β -CD. Furthermore, reduction in the ΔH values was observed for the melting peaks of AN and NP for the physical mixtures with β -CD in comparison with pure drugs. Unfortunately, it was challenging to obtain further informative thermograms for XM, which can be attributed to the high melting point of XM (~ 278 °C) that falls within the temperature range where decomposition of β -CD is observed. Additionally, it is worth mentioning that a broad endothermic peak was obtained for the β -CD within the range 65–110 °C, which corresponds to the desolvation of water molecules from the β -CD cavity. Such observation is highly consistent with results reported by other research groups [32–34].

Molecular modeling

All calculations were performed using the Hyperchem software package. Importantly, the focal benefit of performing MM calculations is to provide some insights on the inclusion process that can support the experimental findings. In order to provide comprehensive computational analysis, two principal modes were considered: inclusion through the wide narrow rims of β -CD cavity. In addition, various parts of the proposed drugs molecules were allowed to penetrate inside the β -CD cavity. Thus, each drug molecule would form inclusion complex with β -CD via a variety of configurations; e.g. NP has two parts via which it can form the inclusion complex, the naphthyl and imidazoline groups. Therefore, a total of four possible configurations are expected for NP- β -CD complex.

Fig. 5 Schematic representations for the coordinate system used for the inclusion complexation of different parts of NP molecule inside the β -CD cavity. (a) and (c) correspond to inclusion of the naphthyl and imidazoline groups through the wide rim of β -CD, respectively; and (b) and (d) correspond to inclusion through the narrow rim of β -CD, respectively

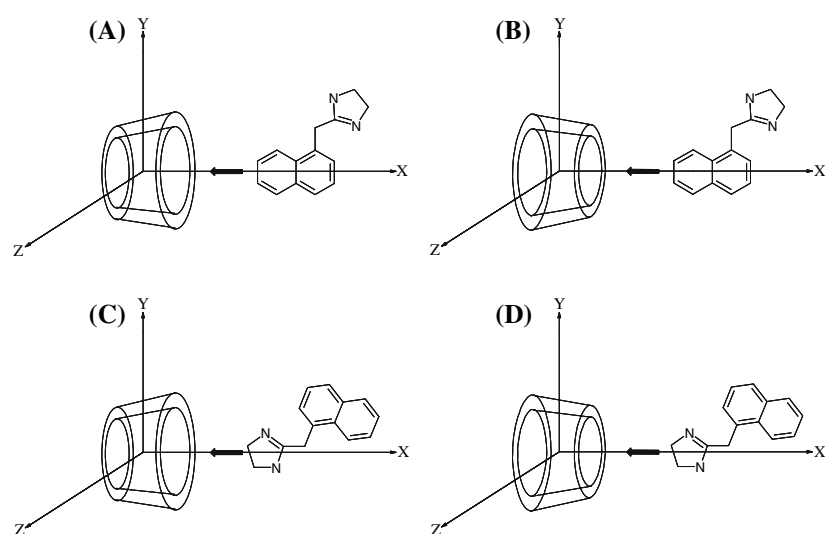


Table 2 Energy changes and energy of binding accompanying the formation of inclusion complexes between β -CD and imidazoline derived drugs

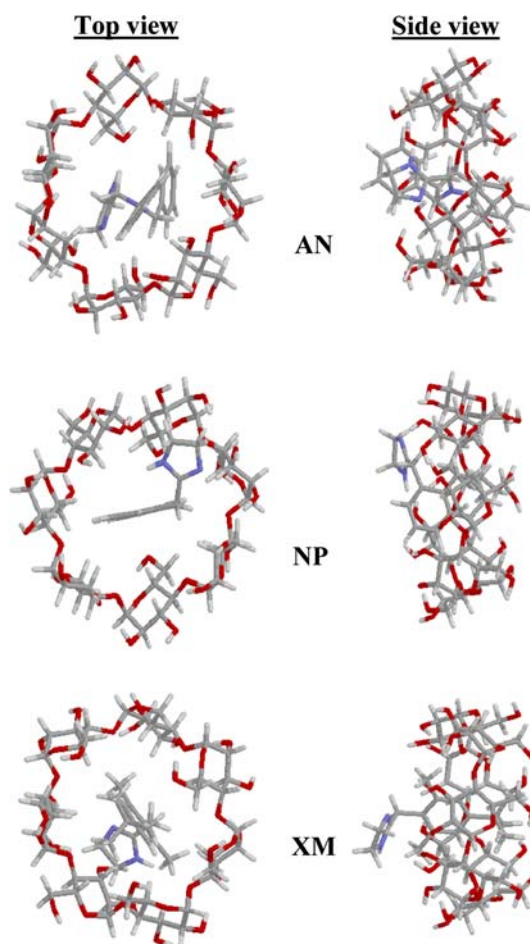
Drug	Approach	E-binding (kcal/mol)	E-vdw (kcal/mol)	E-static (kcal/mol)
<i>Naphazoline</i>				
Naph. wide		-23.6	-23.0	-0.61
Naph. narrow		-22.2	-20.8	-1.40
Imide. wide		-23.6	-23.3	-0.27
Imide. narrow		-18.4	-18.8	0.41
<i>Antazoline</i>				
Phenyl. wide		-26.7	-27.1	0.37
Phenyl.narrow		-34.0	-30.0	-3.98
Benzyl. wide		-25.1	-23.5	-1.61
Benzyl. narrow		-24.1	-23.0	-1.17
Imide. wide		-29.0	-25.7	-3.24
Imide. narrow		-24.6	-25.9	1.33
<i>Xylometazoline</i>				
Phenyl. wide		-28.1	-26.8	-1.30
Phenyl. narrow		-25.4	-25.7	0.34
Imide. wide		-27.7	-27.0	-0.71
Imide. narrow		-27.7	-28.2	0.51

Likewise, AZ- β -CD and XM- β -CD could have six and four configurations, respectively; where AZ could penetrate the β -CD cavity through the phenyl, benzyl and imidazoline groups, whereas XM could penetrate through the t-butyl-phenyl and imidazoline groups. The energy changes (ΔE) or Energy of binding (E_{binding}) accompanying the formation of 1:1 inclusion complex between the proposed drugs molecule and β -CD was considered for judging the most stable configurations of the complex. The ΔE was estimated based on the following equation:

$$\Delta E = E_{\text{drug}:\beta\text{-CD}} - (E_{\text{isolated drug}} + E_{\text{isolated } \beta\text{-CD}})$$

where $E_{\text{drug}:\beta\text{-CD}}$ corresponds to the potential energy of the inclusion complex, and $E_{\text{isolated drug}}$ and $E_{\text{isolated } \beta\text{-CD}}$ correspond to the potential energy of the free drug molecule and β -CD molecules, respectively. Also, the calculations were performed in vacuum phase without considering the solvation effect. Typical example for the inclusion process of NP with illustrated coordinate system used in the complexation process is shown in Fig. 5. The setting for the coordinate system was as the following: the center of the glycosidic oxygen atoms of β -CD located at the origin of the Cartesian coordinates, whereas the tertiary inertial axis and secondary inertial axis were set at the x-axis and y-axis, respectively; the guest molecule was coincident with the x-axis.

It is worth mentioning that the inclusion complexation process is driven via a combination of forces, this includes electrostatic, van der Waals, bond angle bending and

**Fig. 6** Side and top views for the proposed models of the energetically favored configurations of drug: β -CD inclusion complexes

dihedral angle bending forces. The predominant contribution to the E_{binding} comes from the van der Waals forces, whereas the electrostatic contribution is very small. Contribution from other were neglected.

Table 2 summarizes the values for the E_{binding} , $E_{\text{van der Waals}}$, and E_{static} for all possible configurations of the inclusion complexes of the proposed drugs with β -CD. The E_{binding} for the approach of each part in the drug molecule with β -CD were computed and summarized. The optimal E_{binding} was determined as the minima of the plot of E_{binding} vs. the x-coordinate. Accordingly, the favorable configuration for the inclusion complex of each drug molecule with β -CD is shown in Fig. 6 with side and top views. The calculations were carried out for the three drugs based on their tendency to form 1:1 inclusion complexes. However, calculations were also performed for the possibility of forming 1:2 complexes, where results revealed very diminutive E_{binding} , which suggests ruling out the possibility of forming 1:2 inclusion complexes. This finding is consistent with the results obtained using the spectrofluorometric investigations. For NP, the results in Table 2 show

that the inclusion of naphthalene moiety is slightly favored over the inclusion of the imidazoline ring, due to the difference in the polarity of each group. When the naphthalene moiety is included, the non-substituted ring is included entirely, whereas imidazoline is totally included inside the cavity. For AN, the difference in energy between the inclusion of the phenyl group from the narrow rim is significant due to steric hindrance. The inclusion of the phenyl group is entirely and this is due to the directed position of phenyl group inside the cavity. In contrast, the inclusion of benzyl group and the imidazoline group is partially and has equal probability to occur. For XM, as it appears from Table 2, the inclusion of the t-butyl-phenyl group from the wide rim is the favorable one. The small difference between this inclusion and the other three approaches gives them the possibility for inclusion, which in turn is similar to NP where the inclusion of imidazoline ring occurs entirely. In summary, the results obtained from the MM calculations suggest that the inclusion of NP and XM proceeds favorably from the side of β -CD's wide rim through the naphthyl and t-butyl-phenyl groups, respectively; whereas inclusion of AZ proceeds from the narrow rim of β -CD through the phenyl group. It is noteworthy that the method applied here for calculating the E_{binding} was conducted using commercially available software (Hyperchem[®] molecular modeling software), which offers direct methodology for optimizing the E_{binding} of the inclusion complexation process. Interestingly, results obtained herein are consistent with those reported by Mura et al. [51] and Mattice et al. [52], who utilized INSIGHT II 95.0 program and Sybyl 6.3 software, respectively, for calculating the E_{binding} of naphthalene-based-molecules: β -CD inclusion complexes in vacuum.

Concluding remarks

In the present study, we have demonstrated the ability of β -CD to form 1:1 inclusion complexes with selected imidazoline-derived drugs, namely AZ, NP and XM. The formation of the inclusion complexes was confirmed experimentally using steady-state fluorescence and DSC, and theoretically using MM calculations and modeling. The thermodynamics parameters accompanying the formation of the inclusion complexes have affirmed that the inclusion process is driven predominantly by enthalpy changes with minimal entropy retardation. Importantly, the geometrical size and polarity of various substituents, such as phenyl and naphthyl groups, have dramatically altered the stability and geometrical configuration of the inclusion complexes. Hence, MM calculations revealed that NP and XM exhibited slight preference for inclusion through the wide rim over the narrow rim of the β -CD, whereas AZ exhibited significant preference to penetrate through the

narrow rim, which could be mainly attributed to the large geometrical size of the AN molecule.

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