Characterization and Molecular Modelling of Cyclodextrin/Fluoroquinolone Inclusion Complexes

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Background: Cyclodextrins are widely used as complexing agents to increase the solubility of poorly water-soluble drugs, to improve their bioavailability and stability, to reduce or prevent gastrointestinal or ocular irritation, to reduce or eliminate unpleasant smells or tastes and to prevent drug-drug or drug-additive interactions. In recent years, cyclodextrins have been proven to be effective as host compounds in molecular recognition and chiral separation.
Aim: To evaluate the complexation role of cyclodextrins toward fluoroquinolones (FQ) in an attempt to assess their potential as new formulation additives for more efficient fluoroquinolone delivery and as chiral selectors in case of racemic mixture compounds.
Material and methods: Guest-host interactions of three second generation quinolones, ciprofloxacin, ofloxacin and norfloxacin with two parent cyclodextrins, beta-cyclodextrin (β-CD), gamma-cyclodextrin (γ-CD) and a beta-cyclodextrin derivative, 2-hydroxypropyl beta-cyclodextrin (HP-β -CD), were tested. Computer aided molecular modelling (ChemBio3D Ultra 12.0) was utilized to predict the preferred orientation of fluoroquinolones in the cyclodextrin cavity and the main structural features responsible for the enhancement of their solubility and photostability. Ciprofloxacin/β-cyclodextrin complex was prepared and the formation of inclusion complex was demostrated by IR spectroscopy.
Results: Our studies show that the orientation with the piperazinyl group included in the CD cavity is energetically more favorable.
Conclusions: The CDs act as complexing agents with the three FQ derivatives, which enter inside the CD torus, and interact with the hydroxyl groups of CD by Van der Waals, electrostatic forces ang hydrogen bonding. Our results suggest the 1:1 stoichiometry in the complex formation.

Keywords: cyclodextrin, fluoroquinolone, inclusion complex, molecular modelling

Introduction

In the present study we evaluated the complexation role of cyclodextrins toward fluoroquinolones in an attempt to assess their potential as new formulation additives for more efficient fluoroquinolone delivery and as chiral selectors in case of racemic mixture compounds. Cyclodextrins (CDs) are cyclic oligosaccharides composed of six, seven or eight α-1,4-linked glucose units and are characterized by a truncated cone shape. In their cavity, the CD can accommodate a wide class of organic molecules leading to inclusion complexes [1–3]. Fluoroquinolones are synthetic antibacterial agents with a broad spectrum of activity.

The second generation fluoroquinolones, which are the focus of this review have a 6-fluoro substituent on the quinolone ring structure, responsible for their broad gram negative activity and a 7-piperazinyl substituent, the last providing them a longer half-life [4–6]. Guest-host interactions of three second generation quinolones, ciprofloxacin, norfloxacin, ofloxacin and it's S (-) form levofloxacin with two parent cyclodextrins, beta-cyclodextrin (β-CD), gamma-cyclodextrin (γ-CD) and a beta-cyclodextrin derivative, 2-hydroxypropyl beta-cyclodextrin (HP-β-CD), were investigated by molecular modelling and physicochemical methods.

The values of the binding energy for the complexes for all models and complexes are presented and based on these values, the most probable inclusion geometries are indicated for each complex.

Material and methods

Computational methodology, construction of models: The molecular modelling of the host-guest interaction was performed as follows: the equilibrium structures of CDs and the four fluoroquinolones (FQ) were set up. The structures of CDs were taken from the ChemACX database. The initial structure of the fluoroquinolones was built up using the ChemBioDraw Ultra12.0 program. The S-enantiomer of ofloxacin (levofloxacin) was obtained from the reflection of the first one. The 1:1 inclusion complexes were constructed among CDs and each one of the FQs. The corresponding geometries were achieved with the aid of the docking module of ChemBioOffice Ultra 12.0 program package. In order to optimize the geometry in vacuum, molecular mechanics computations have been done with the ChemBio3D Ultra 12.0 software; MM2 method was used to energy-minimize the structures until the RMS gradient was lower than 0.010.

For all below presented calculations two initial relative orientations of the fluoroquinolone/CD complex were taken into account: (1) the carboxyl group of FQ was oriented to the centre of the CD and (2) the piperazinyl group of FQ was oriented to the centre of the CD [7].

Experimental study, preparation of inclusion complex: Inclusion complex of Ciprofloxacin with βCD was prepared by a kneading method in 1:1 molar ratio. The ac-

Table I. Potential energies of cyclodextrins

Interactions	Energy (kcal/mol)		
	β-CD	γ-CD	HP-β-CD
Stretch	8.7959	14.7511	33.9581
Bend	54.7193	85.4575	157.0844
Stretch-Bend	5.0858	7.6115	14.9238
Torsion	28.8268	71.7611	106.0259
Non-1,4 VDW	-50.0546	-54.4156	-113.1712
1,4 VDW	89.3795	109.5288	200.6465
Dipole/Dipole	-23.5402	-4.9422	4.0155
Total Energy	113.2124	229.7521	403.4829

Table II. Potential energies of fluoroquinolones

Interactions	Energy (kcal/mol)			
	Ciprofloxacin	Ofloxacin		Norfloxacin
		R	S	
Stretch	2.2924	3.4287	3.3566	3.2763
Bend	11.6702	21.1976	19.5468	25.9809
Stretch-Bend	0.5704	0.797	0.7231	1.2376
Torsion	-0.5299	4.6884	3.9245	-6.4489
Non-1,4 VDW	5.4043	5.0253	4.1914	9.3026
1,4 VDW	22.3317	27.1922	27.548	21.7912
Dipole/Dipole	6.0064	5.3379	6.823	6.4397
Total Energy	47.7455	67.667	66.1133	61.5794

quired complex was analyzed in comparison to the host and guest components by IR spectrophotometer (Jasco FTIR 470 PLUS) using KBr pellet technique [8–11].

Results

The models were built by docking technique starting by locating each FQ molecule at the larger side of the CD cavity first with the carboxyl group of FQ oriented to the CD and than with the piperazinyl group of FQ oriented to the CD. The process was repeated by locating the FQ molecules at the narrow side of the CD cavity [12-14]. Geometry optimization in vacuum through MM2 molecular mechanics was performed and the potential energies for the host and guest molecules and for each inclusion complex were recorded. The total potential energy, E, of a molecule can be described by the following summation of interactions:

E = Stretching Energy + Bending Energy +Torsion Energy + Non-bonded Interaction Energy

The first three terms are the so-called bonded interactions. In general, these bonded interactions can be viewed as strain energy imposed by a model moving from some ideal zero strain conformation. The last term, which represents the non-bonded interactions, includes van der Waals interaction (repulsion for atoms that are too close and attraction at long range from dispersion forces) and electrostatic interactions (interactions from charges, dipoles, quadrupoles). The potential energy functions and the parameters used for their evaluation are known as a "force-field" [7]. The potential energies of each CD molecule and each FQ are listed in Tables I and II.

The molecular models of ciprofloxacin/CD inclusion complexes with the lowest energies are presented in Fig. 1–3.

The potential energies of the presented inclusion complexes are listed in Table III.

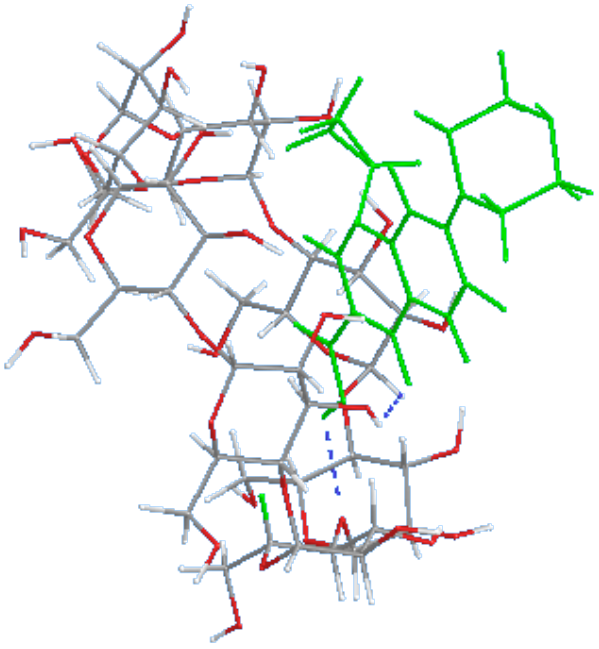

Fig. 1. Molecular model of ciprofloxacin/β-CD inclusion complex

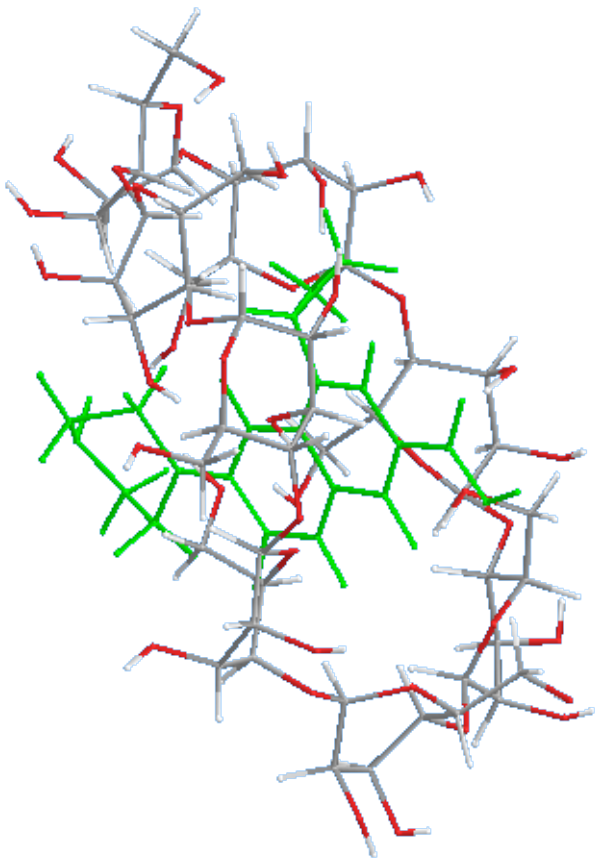

Fig. 2. Molecular model of ciprofloxacin/γ-CD inclusion complex

Table III. Potential energies of ciprofloxacin/CD inclusion complexes

Interactions	Energy (kcal/mol)		
	CIP/β-CD	CIP/γ-CD	CIP/HP-β-CD
Stretch	11.1529	14.7234	30.6638
Bend	72.4843	80.8928	152.3012
Stretch-Bend	5.3416	7.2038	13.3959
Torsion	27.4036	66.8572	105.1581
Non-1,4 VDW	-74.6069	-92.1448	-156.1508
1,4 VDW	112.1959	130.8572	225.576
Dipole/Dipole	-18.6529	-0.9619	5.4712
Total Energy	135.3185	207.4277	376.4154

Table IV. Potential energies of norfloxacin/CD inclusion complexes

Interactions	Energy (kcal/mol)		
	NOR/β-CD	NOR/γ-CD	NOR/HP-β-CD
Stretch	11.0832	15.7219	15.4234
Bend	68.9072	98.9869	84.5814
Stretch-Bend	5.9792	7.8079	7.6658
Torsion	27.7527	63.8623	64.0026
Non-1,4 VDW	-77.0854	-93.4668	-77.3756
1,4 VDW	113.2283	129.7773	132.1443
Dipole/Dipole	-20.657	-14.153	1.7426
Total Energy:	129.2082	208.5364	228.1845

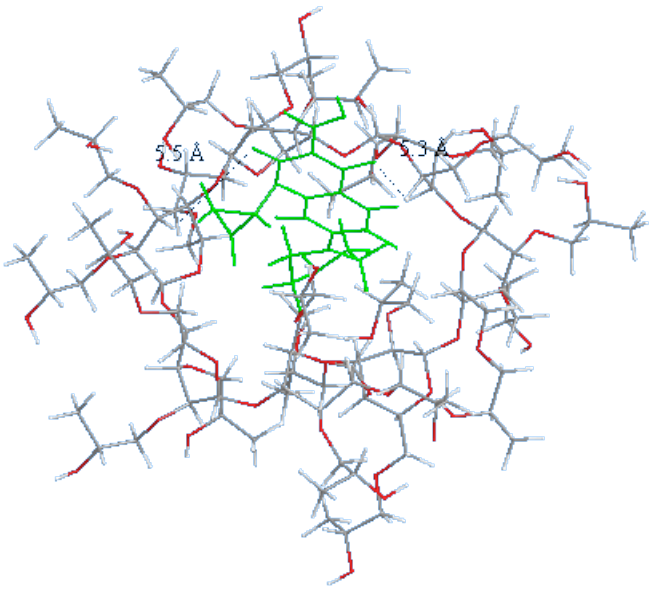

Fig. 3. Molecular model of ciprofloxacin/HP-β-CD inclusion complex

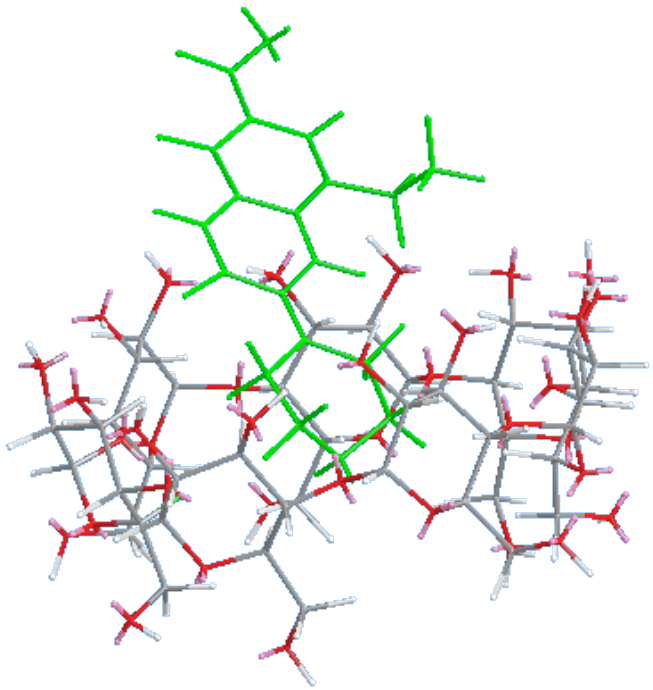

Fig. 4. Molecular model of norfloxacin/β-CD inclusion complex

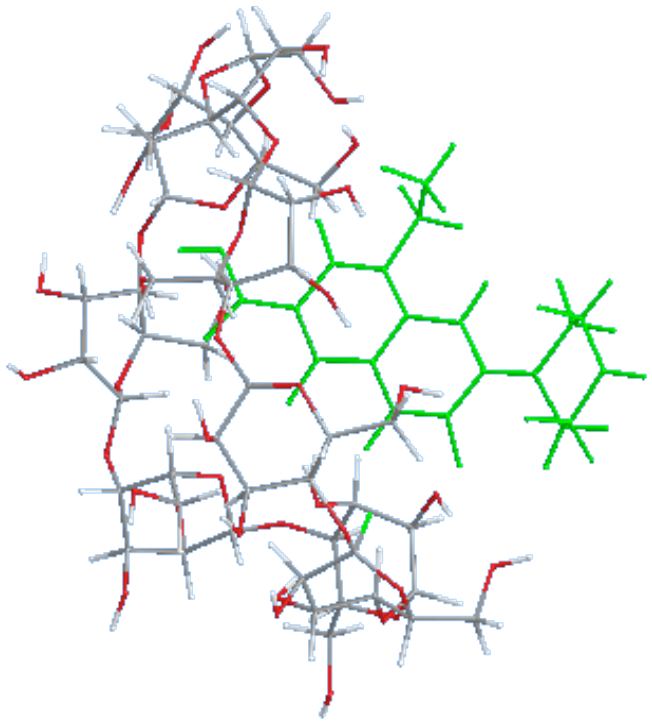

Fig. 5. Molecular model of norfloxacin/γ-CD inclusion complex

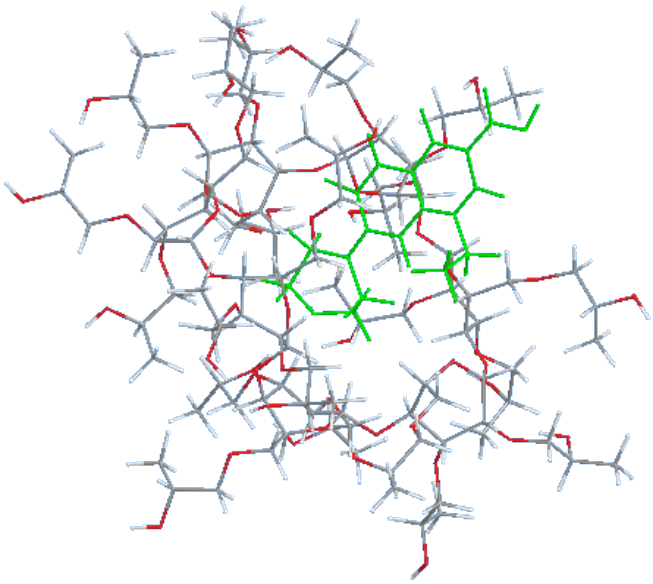

Fig. 6. Molecular model of norfloxacin /HP-β-CD inclusion complex

Table V. Potential energies of ofloxacin(R,S)/CD inclusion complexes

Interactions	Energy (kcal/mol)					
	Ofloxacin/β-CD		Ofloxacin/γ-CD		Ofloxacin/HP-β-CD	
	R	S	R	S	R	S
Stretch	12.1976	12.4494	14.9655	15.379	28.3302	28.1233
Bend	76.9176	79.0495	82.2611	83.511	143.2104	141.6003
Stretch-Bend	5.8605	5.9734	7.3026	7.4657	12.8087	12.6758
Torsion	35.8849	33.7181	71.6776	71.1702	95.291	95.4184
Non-1,4 VDW	-80.1898	-80.6001	-86.0637	-84.0826	-155.1353	-156.7871
1,4 VDW	118.558	118.518	140.0707	139.4585	231.0059	231.5657
Dipole/Dipole	-25.5433	-25.4931	3.6111	3.8632	6.0687	5.3222
Total Energy	143.6855	143.6151	233.8248	236.7651	361.5795	357.9185

The models of norfloxacin/CD inclusion complexes with the lowest energies are presented in Fig. 4–6.

The potential energies of the presented inclusion complexes are listed in Table IV.

The models of ofloxacin/CD inclusion complexes with the lowest energies are presented in Fig. 7–9.

The potential energies of the presented inclusion complexes are listed in Table V.

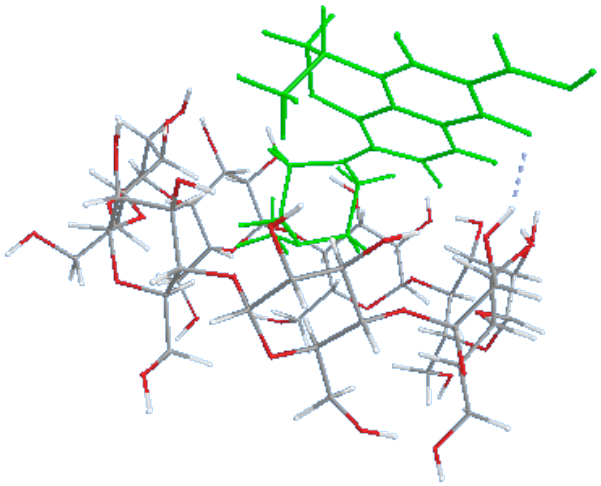

Fig. 7. Molecular model of ofloxacin/β-CD inclusion complex

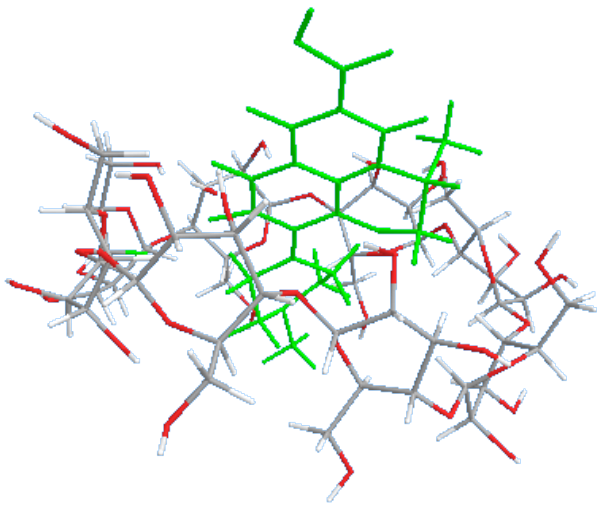

Fig. 8. Molecular model of ofloxacin/γ-CD inclusion complex

Discussions

The molecular modelling of the host-guest interaction was performed by semiempirical methods similar to the method used by Bogdan et al. [12], but without including any water molecules. Their results show that the number of water molecules are larger in size and the heat of formation is smaller in size. They explain this outcome by the polarization induced by the water molecules into the electronic density of the complexes. Based on the predicted models obtained with docking simulations in vacuum, the relative orientation of FQ molecule with respect to the CD cavity allows us to separate them into two groups. The first group constitutes of inclusion complexes with the FQ carboxyl group oriented towards the centre of the CD (1) and the second group (2) having opposite orientation. Pornthep et al [13] have also found that two arrangements of Lphe with almost equal statistic and energetic values may be possible, they presume the need for helper-water molecules to stabilize the complex by hydrogen bonding [12–14]. Our studies show that orientation (2) is energetically more fa-

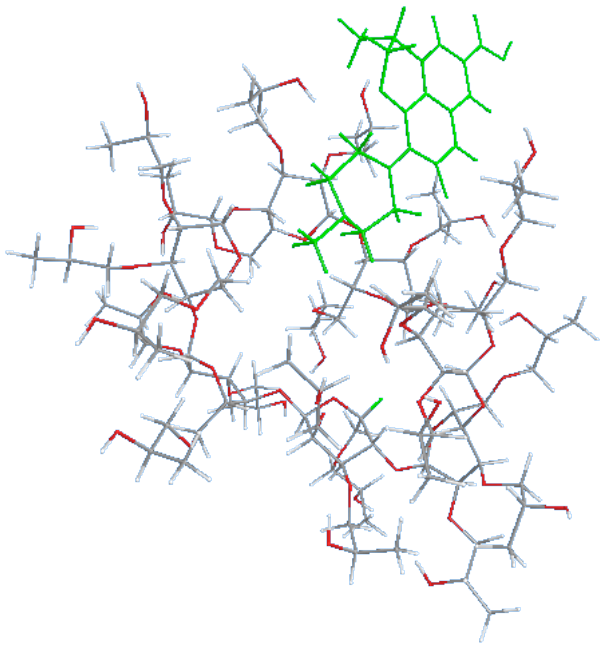

Fig. 9. Molecular model of levofloxacin/HP-β-CD inclusion complex

vourable. However, the predicted results for orientation (1) should not be neglected either because their energetic values were insignificantly different from that of orientation (2). Jianbin Chao et al. proposed similar spatial configuration of complexes based on the two dimensional NMR technique. They studied the interaction of ciprofloxacin with β-CD and HP-β-CD by several analytical techniques. Their results confirmed the existence of 1:1 inclusion complex. In their study regarding norfloxacin with 2-methyl-b-cyclodextrin complexes (Me-β-CD) results indicate that the piperazine ring is out of the Me-β-CD cavity [8–10]. Comparing the main IR complex frequencies to that of ciprofloxacin, we found the followings: there was a strong absorption peak in the spectrum of the guest ciprofloxacin, i.e. 1706.39 cm^{-1} due to the carboxylic group, this band was also detected in the spectrum of the inclusion complex, indicating that this moiety was not included in the CD cavity. Based on these results we presume, that the piperazinyl group was included in the CD cavity, however the IR spectra does not permit a definitive answer, since the characteristic bands for the piperazinyl group are in the same region as for the β-CD.

Conclusions

CDs act as complexing agents with the three FQ derivatives, which enter inside the CD torus, and interact with the hydroxyl groups of CD via the Van der Waals electrostatic forces and hydrogen bonding. The Van der Waals forces have a significant contribution to the total binding energy of the complex. For Ciprofloxacin and β-CD the formation of inclusion complex was also confirmed by IR spectroscopy. Our results suggest the 1:1 stoichiometry in the complex formation.

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