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RESEARCH PAPER

Ofloxacin/β-Cyclodextrin Complexation

L. S. Koester,[1] S. S. Guterres,[1] M. Le Roch,[2] V. L. Eifler-Lima,[1] J. A. Zuanazzi,[1] and V. L. Bassani[1,*]

[1]*Curso de Pós-graduação em Ciências Farmacêuticas, Faculdade de Farmácia, Universidade Federal do Rio Grande do Sul, Av. Ipiranga, 2752, 90610-000, Porto Alegre, RS, Brasil*
[2]*UPRES Pharmacochimie de Molécules de Synthèse et de Substances Naturelles, UFR de Pharmacie 2, Av. du Pr. Léon Bernard, 35043 Rennes Cedex, France*

ABSTRACT

Ofloxacin (OFX) is a fluorquinolone characterized by photochemical instability. With the goal to improve its photostability in aqueous solutions, the complexation of ofloxacin with β-cyclodextrin was investigated. The complexes showed a water solubility enhancement of approximately 2.6 times; nevertheless, the photodegradation of ofloxacin was not reduced. The complexes obtained were characterized by thermal and ^1H nuclear magnetic resonance (NMR) analysis, which revealed an interaction between ofloxacin and β-cyclodextrin. The last analysis indicated that only partial inclusion of the N*-methylpiperazinyl moiety occurred, which can explain the fact that photostabilization was not improved. This partial inclusion phenomenon could be explained also by computer-aided molecular modeling.*

Key Words: *β-Cyclodextrin; Inclusion complex; Ofloxacin; Ofloxacin photostability.*

INTRODUCTION

Ofloxacin (OFX) belongs to the fluorquinolone antimicrobial class, which is widely used due to its excellent antimicrobial activity, wide spectrum of action, and good pharmacokinetics properties (1,2). In tuberculosis treatment, ofloxacin has been a first-line agent for the multidrug-resistant cases and has presented fewer adverse incidences and less severe adverse effects in long-term treatment than the standard agents (3–5). However, ofloxacin is characterized by photochemical instability (6,7). The β-cyclodextrin (β-CD) is a compound used to

* Corresponding author.

complex drugs to improve their solubility and stability profiles. In the present work, we investigated the effect of OFX:β-CD complexation on the photostability and water solubility of the drug. Considering that the piperazinyl ring at C-7 in the aromatic nucleus is probably one of the groups responsible for ofloxacin photodegradation (6), the protection of this part of the molecule by the hydrophobic cavity of β-CD was the hypothesis of our work.

EXPERIMENTAL

Materials

Ofloxacin was supplied by Sigma (St. Louis, MO); β-cyclodextrin was purchased from Merck (Darmstadt, Germany). Acetonitrile and methanol were high-performance liquid chromatography (HPLC) grade, and all other reagents used were of analytical quality.

Methods

Solubility Studies and Complex Preparation

In the solubility studies (8) and in the preparation of the complex, a neutralization method (9,10) was employed. Solubilities of OFX were determined by adding an excess amount of the drug (206.3 mg) to 25 ml of sodium hydroxide aqueous solutions (pH 8.3) containing increasing concentrations of β-CD. Five samples of the following molar ratios OFX β-CD were prepared: 1:1, 1:2, 1:3, 1:4, 1:5. The suspensions, contained in lightproof flasks, were deaerated by bubbling N_2 and were stirred for 5 days at 37°C, after which equilibrium was reached. After cooling to 25°C, the suspensions were neutralized with HCl 0.8 mol/L and filtered through a 0.45-μm membrane filter (Millipore HAWP, Bedford, MA). The drug present in the liquid phase was assayed by ultraviolet (UV) spectrophotometry at 293 nm (11). Preparation of the complex followed the same procedure as for the solubility study, using molar ratios of 1:1 and 1:3. The supernatants were dried in an Edwards freeze-dryer (West Sussex, UK) for further analysis by differential scanning calorimetry (DSC), solubility, photostability, and ^{1}H nuclear magnetic resonance (NMR).

Characterization of the Complexes

Thermal Analysis

Thermal analysis was performed using a Polymer Laboratories DSC system (Surrey, UK). So all samples would contain the same amount of OFX, different samples were weighed as follows: OFX (2.0 mg), β-CD (7.0 mg), OFX:β-CD 1:1 physical mixture (9.5 mg) and OFX:β-CD 1:1 complex (8.3 mg). The samples were heated at a scanning rate of 5°C/min from 30°C to 300°C. Temperature calibrations were performed using indium as a standard.

Proton Nuclear Magnetic Resonance Analysis

The samples of OFX:β-CD 1:1 and 1:3 complexes and the 1:1 physical mixture were analyzed by proton NMR at 500 MHz using a Bruker DMX 500 (500.1299) spectrometer (Lisle, IL). The probe temperature was regulated to 298 K. All the chemical shifts are given relative to D_2O (Aldrich, Milwaukee, WI, 99.9%) at 4.799 ppm. The conditions were as follows: 104 scans for the OFX sample, 128 scans for the β-cyclodextrin, 500 scans for 1:1 and 1:3 complexes, and 16 scans for the physical mixture; acquisition time 3.1457779 s. The precision in the chemical shift measurements was estimated to be $\pm 1 \times 10^{-3}$ ppm. ^{1}H-NMR AB systems were presented in the following order: H-α the more deshielded and H-β the more shielded.

Computer Graphics

Geometric optimization of OFX was performed at the self-consistent field (SCF) level using the Austin model 1 (AM1) Hamiltonian (12). Within the MOPAC version 6.00 package (13), on a 500-Hz personal computer with a Windows 98 operational system. The potential energy surface slices (PES) were pointwise calculated for the torsional angles, which were independently varied between 0° and 360°. We found that increments of 30° were sufficient for good coverage. Minimum energy structures were then reoptimized by adopting a norm of gradient of less than 0.1 kcal/(Å or rad). The visualization of the structure was done with HyperChem software (from Hypercube®, Inc., Scientific Software, Gainesville, FL).

Photostability Evaluation

The OFX and OFX:β-CD 1:1 and 1:3 complexes were accurately weighed and dissolved in water at a concentration around 0.5 mg/ml. The solutions were poured into quartz flasks and were exposed to UV light (HQV, 125 W, OSRAM, 320–400 nm).

Determination of OFX content in the samples exposed to photodegradation was carried out on a 2690 Waters Analytical Chromatography Alliance (Milford, MA) with a Nova-Pak C18 column, 5 μm (3.9 × 150 mm, Waters), using 5% acetic acid:acetonitrile:methanol (75:15:10) solution as the mobile phase at a flow rate of 1.0 ml/min. Eluting compounds were monitored with a Waters Millenium (version 2.15.01), which measured absorbance (200–800 nm) every 1 s with 4.8 nm resolution. OFX

obtained from Sigma was used as the external standard. The linearity of the assay was determined using the following OFX solutions in methanol: 1.6, 3.2, 4.8, 6.4, and 8.0 μg/ml. Evaluation of each point was repeated three times. The calibration curves were fitted by linear regression: $C = 4.2 \times 10^{-6}X + 0.23$, where C represents the ofloxacin concentration in micrograms per milliliter, and X is the peak area (mV/s). The coefficient of correlation was 0.999.

After 21 days of exposure, 0.2 ml of each sample was diluted to 5 ml with mobile phase, and OFX was assayed by HPLC. The absorption spectra of the same samples were performed in the same chromatographic system, with diode array scanning from 200 to 800 nm.

RESULTS AND DISCUSSION

The conditions for the complexation were chosen to include the *N*-methylpiperazinyl moiety in the cyclodextrin cavity and to protect it against photodegradation. Considering that ofloxacin is a zwitterionic molecule with an isoelectric point of 7.14, theoretically, in alkaline pH the ionic piperazinyl group should be present in lower concentration than the corresponding nonionic form. The higher concentration of piperazinyl group in the nonionic form would be a favorable condition to include this group in the hydrophobic cyclodextrin cavity. So, we chose pH 8.3 to carry out the inclusion procedure, which is close to the 8.22 pK_a (14) of the *N*-piperazinyl group, meaning that at least 50% of the piperazinyl moiety is in the nonionic form. To avoid the influence of the improvement of the solubility due to the carboxylic group ionization in pH 8.3, after complexation, the solutions were neutralized with HCl 0.8 mol/L.

Solubility Study

The OFX:β-CD solubility curve turned out to be a B_s-type phase diagram (Fig. 1), in which a water solubility increase of 2.6 times can be observed. Further addition of cyclodextrin did not result in an important solubility increase, meaning that the formation of more complex led to precipitation. The apparent 1:1 stability constant *Kc* of the complex was 152 M^{-1}, which characterizes a labile association.

Differential Scanning Calorimetry

Thermograms of OFX, β-CD, their physical mixture (1:1), and their complex (1:1) are shown in Fig. 2. The OFX thermogram shows an endothermic peak at 276°C,

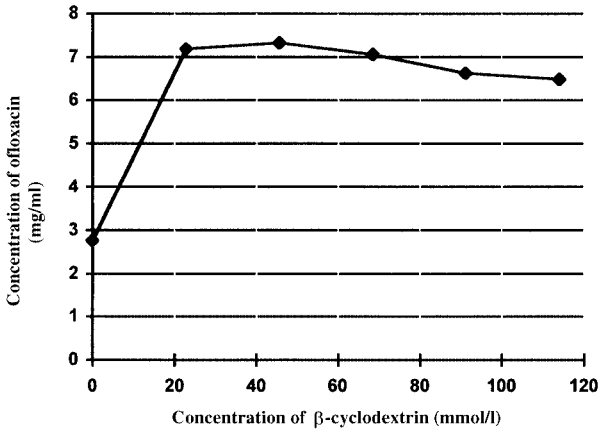


Figure 1. Phase solubility diagram of ofloxacin with β-cyclodextrin in aqueous media at 25°C.

which represents the melting point of the drug. The broad band from 50°C to 110°C observed in the β-CD thermogram corresponds to the loss of water. The DSC curve of the OFX:β-CD physical mixture (1:1) shows two peaks that correspond to the two separate components. The thermogram of the OFX:β-CD (1:1) complex shows that the peak corresponding to the melting point of ofloxacin is strongly reduced. This observation indicates an interaction of OFX and β-CD molecules without ruling out the fact that the freeze-dried product can be more or less amorphized. The broad band corresponding to β-CD loss of water is split, probably due to different degrees of hydration.

Photostability Evaluation

To investigate the photodegradation products, the evaluation in aqueous solutions was carried out by HPLC with diode array detection at wavelengths from 200 to 800 nm. Figure 3A shows the HPLC profile and the corresponding UV spectra of the OFX peak (retention time [RT] 5.34 min, UV_{max} 295 nm). Figure 3B shows that, after exposure of OFX to UV light, besides the OFX peak, there are two additional main peaks (RT 1.18 min., UV_{max} 237 nm, and RT 4.10 min, UV_{max} 290 nm), plus one less important peak, at 3.30 min and three shoulders (RT 1.5, 4.5, and 6.2 min.), all corresponding to the OFX photodegradation products. Similar profiles were obtained for the complexes of OFX:β-CD 1:1 and 1:3 (Figs. 3C and 3D). The complexation of OFX with β-CD did not change the scanner UV profile of the peaks corresponding to the OFX and to the main photodegradation products after 21 days of exposure to UV light. The

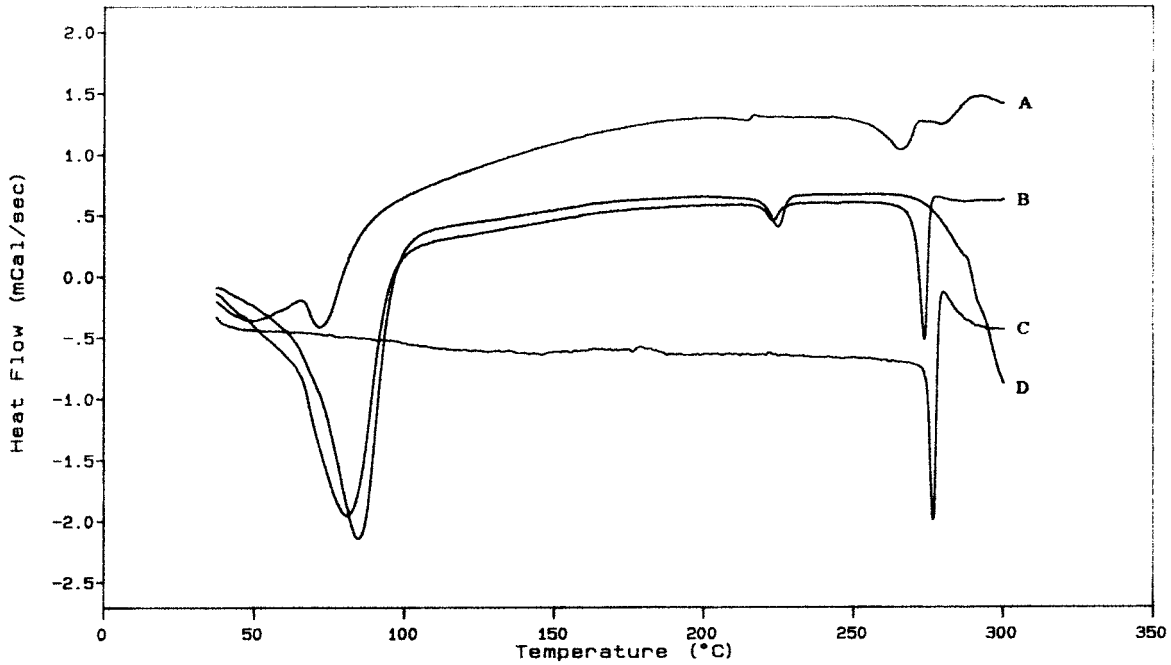


Figure 2. DSC analysis: (A) ofloxacin:β-cyclodextrin 1:1 complex; (B) ofloxacin:β-cyclodextrin 1:1 physical mixture; (C) ofloxacin; and (D) β-cyclodextrin.

quantitative analysis of OFX by HPLC demonstrated that, after 21 days, the remaining amounts of OFX in the OFX:β-CD 1:1 and 1:3 mixtures were, respectively, 3% and 6%. This more accurate photodegradation analysis did not show important differences between the profiles of OFX:β-CD 1:1 and 1:3 ratio complexes, as indicated in our preliminary studies (15).

RMN and Computer Graphic Analysis

The complexation of *N*-methylpiperazinyl moiety with β-CD would be theoretically observed using ^{1}H-NMR, by which it changes would be expected in the H-3 and H-5 chemical shifts of β-CD, while the other protons of this molecule (H-1, H-2, H-4, and H-6) should not be affected. Concerning the *N*-methylpiperazinyl moiety, changes would be expected in the chemical shifts of the protons of N-CH_3, H-3′, H-5′, H-2′, and H-6′.

The ^{1}H-NMR spectra of OFX dissolved in D_2O in the absence and the presence of β-CD show a singlet at δ 2.94 corresponding to N-Me protons and a broad peak resonance centered at δ 3.38, attributed to CH_2-3′ and CH_2-5′ of the piperazinyl ring. When complexed with β-CD, the signals of N-CH_3 and CH_2-3′ and CH_3-5′ protons were shifted upfield, indicating the shielding of these protons when this part of the drug was inside the cavity, for both 1:1 and 1:3 OFX:β-CD ratios. Table 1 shows the differences between proton shifts of the OFX and OFX:β-CD complexes in 1:1 and 1:3 ratios ($\Delta\delta = \delta_{complex} - \delta_{OFX\text{-}free}$). In ^{1}H-NMR analysis (Table 2), the shifts experienced by the protons of the β-CD and OFX:β-CD 1:1 and 1:3 complex formation showed slight differences, mainly upfield for both cyclodextrin and OFX. For the 1:3 complexes, an upfield shift was observed for the N-CH_3, 3′-H, and 5′-H proton signals of OFX, as well as a modification in the signal observed of the H-3 of β-CD. These effects were more pronounced for the 1:3 complex, indicating a complexation of the drug into the β-CD cavity. These differences for the extent of this phenomenon have been recently reported for ibuprofen/2-hydroxypropyl-β-cyclodextrin complex (IBU/HP-β-CD) by Oh and colleagues (16). They also observed that the chemical shifts differences of IBU/HPβ-CD complex depended on the concentration of HP-β-CD.

These results suggest a partial inclusion of the piperazinyl moiety in the β-CD cavity. Two hypothetical reasons can explain this limited inclusion, one related to the steric hindrance and repulsive effect due to the fluorine presence and the other related to the conformational effect caused by the hindrance effect of the piperazinyl ring, which seems to be placed in the same plane of the benzoxacine ring (17). According to Mucci and colleagues (17), both

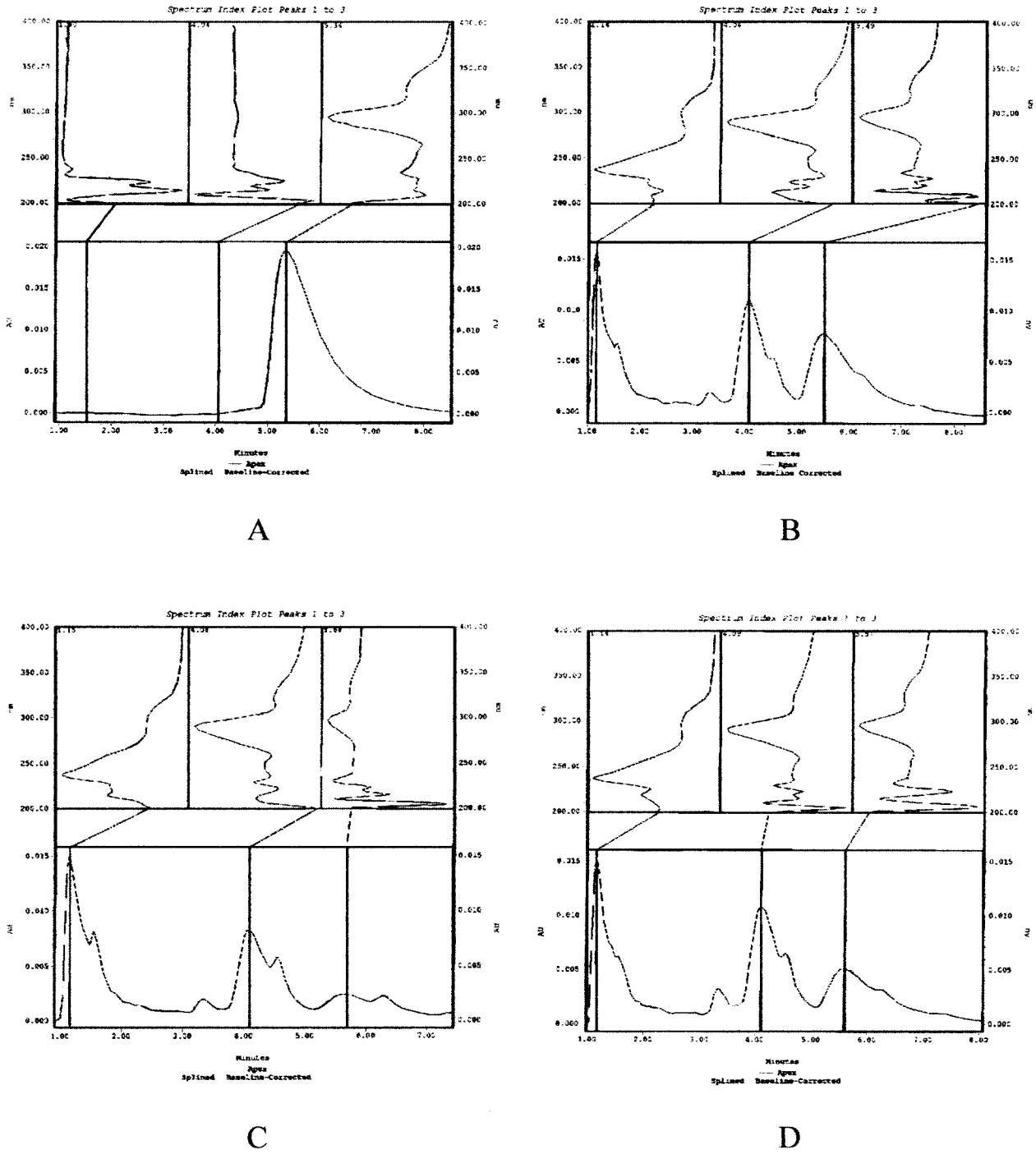
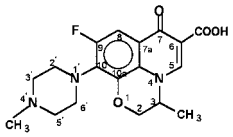


Figure 3. HPLC profile at 295 nm and diode array spectra 200–400 nm: (A) ofloxacin before UV light exposure; and (B), (C) and (D) after 21 days of UV light exposure for (B) ofloxacin, (C) ofloxacin:β-cyclodextrin 1:1 complex, and (D) ofloxacin:β-cyclodextrin 1:3 complex.

Table 1

^{1}H-NMR Chemical Shifts (D_2O) of the Ofloxacin (OFLX) Protons Before and After Complexation with β-Cyclodextrin in 1:1 and 1:3 Ratios and Structure and Numbering of Ofloxacin



Proton	OFLX (ppm)	1:1 (ppm)	Δδ (ppm)	1:3 (ppm)	Δδ (ppm)
3-CH_3	1.5199 (d/3H/J = 6.67)	1.525 (d/3H/J = 6.68)	−0.0051	1.5282 (d/3H/J = 6.67)	−0.0083
N-CH_3	2.9414 (s/3H)	2.8957 (s/3H)	0.0457	2.8620 (s/3H)	0.0794
3′-H and 5′-H	3.3797 (m/4H)	3.3368 (m/4H)	0.0429	3.2955 (m/4H)	0.0842
2′-H and 6′-H	3.5758 (m/4H)	3.572 (m/4H)	0.0038	3.5656 (m/4H)	0.0102
2-Hα	4.4126 (dd/2H/^{1}J = 11.44/^{2}J = 2.22)	4.4259 (d/J = 11.13)	−0.0133	4.4285 (d/2H/J = 11.13)	−0.0159
2-Hβ	4.5527 (dd/2H/^{1}J = 11.45/^{2}J = 1.91)	4.5502 (d/J = 11.44)	0.0025	4.5499 (d/2H/J = 11.12)	0.0028
3-H	4.6608 (m/1H)	4.6583 (m/1H)	0.0025	4.6589 (m/1H)	0.0019
8-H	7.5395 (d/1H/J = 12.71)	7.5281 (d/J = 12.71)	0.0114	7.5455 (d/J = 12.4)	−0.006
5-H	8.4071 (s/1H)	8.4129 (s/1H)	−0.0058	8.4116 (s/1H)	−0.0045

Table 2

^{1}H-NMR Chemical Shifts (D_2O) of the β-Cyclodextrin (β-CD) Protons Before and After Complexation with Ofloxacin at 1:1 and 1:3 Ratios

Proton	β-CD (ppm)	Complex 1:1 (ppm)	Δδ (ppm)	Complex 1:3 (ppm)	Δδ (ppm)
H-1	5.0902 (d/J = 3.5)	5.0876 (d/J = 3.5)	0.0026	5.0879 (d/J = 3.81)	0.0023
H-2	3.6699 (dd/^{1}J = 9.86/^{2}J = 3.5)	3.6673 (dd/^{1}J = 9.86/^{2}J = 3.5)	0.0026	3.6673 (dd/^{1}J = 9.85/^{2}J = 3.49)	0.0026
H-4	3.6044 (t/J = 8.9)	3.6038 (t/J = 9.22)	0.0006	3.6041 (t/J = 9.22)	0.0003
H-3	3.9855 (t/J = 9.53)	3.9731 (t/J = 9.53)	0.0124	3.9737 (t/J = 9.54)	0.0118
H-5	3.8765 (m)	3.8695 (m)	0.0070	3.8688 (m)	0.0077
H-6	3.8981 (2H/m)	3.8949 (m/2H)	0.0032	3.8936 (2H/m)	0.0045

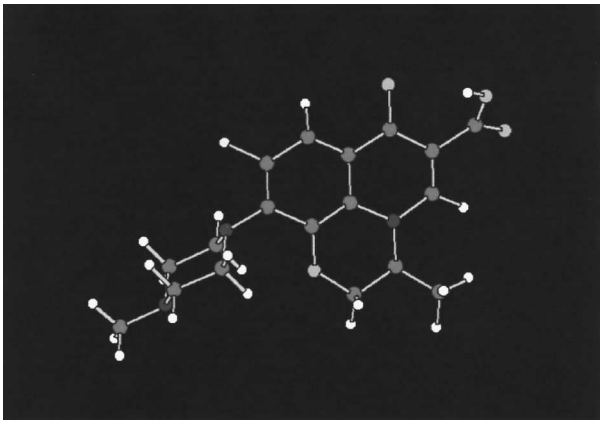


Figure 4. Computer-generated minimal energy structure of ofloxacin (see Experimental section).

the CH_2-2′ and CH_2-6′ protons are close to the fluorine atom. By computer-aided molecular modeling (Fig. 4), we found that the interatomic distance between H-2′ and F-6 is 3.66 Å and between H-6′ and O-oxacine ring is 3.65 Å; for a torsion angle of C(2′)-N-C(7)-C(8) of 65.9°, this conformer presents a $\Delta Hf = 8.84$ kcal/mol (the conformer most stable), confirming the coplanarity between piperazinyl and aromatic rings and the consequent limitation of OFX inclusion into the β-CD cavity.

Taken together, the goal of the first part of the work was to investigate the potential of the ofloxacin/β-cyclodextrin complexation for improvement of the photostability of the drug in aqueous solution to prepare oral dosage forms for pediatric or elderly patients. However, the low extent of the photostabilization obtained by OFX:β-CD complexation led to investigations of the physicochemical phenomena involved in the complexation of this zwitterionic molecule. The RMN and computer graphic analyses demonstrated that the *N*-methylpiperazinyl moiety was not completely included in the β-cyclodextrin cavity, and that this effect was not due only to the presence of a fraction of the moiety in ionic form (50% in pH 8.3), as we thought at the beginning. The conformational effect and the steric hindrance of the piperazinyl ring and the repulsive effect of the H-2′ and the fluorine atom are also responsible for the limited ofloxacin/β-cyclodextrin inclusion. This new information can contribute significantly to the understanding of the complexation of this type of molecule with cyclodextrins.

CONCLUSIONS

In conclusion, the complexation method using controlled pH allowed us to obtain complexes of ofloxacin: β-cyclodextrin with a water solubility enhancement for ofloxacin of 2.67 times. However, the complexation did not extend enough to protect the ofloxacin against photodegradation. The inclusion of only one part of the *N*-methylpiperazinyl moiety was demonstrated by ^{1}H-NMR analysis and by computer-aided molecular modeling to be related to the steric hindrance effect and to the nonfavorable energy for total *N*-methylpiperazinyl inclusion.

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