ORIGINAL ARTICLE

STUDY OF OFLOXACIN - RANDOM BY METHYLATED - β - CYCLODEXTRIN INCLUSION COMPLEX

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Abstract

Guest-host interactions of ofloxacin with randomly methylated beta-cyclodextrin (RAMEB) were assessed by complementary analytical techniques. Computer assisted molecular modelling (ChemBio3DUltra 12.0) was utilized for enantiomer-specific characterization of the complex. Ofloxacin - RAMEB complex was prepared by the kneading method and the formation of inclusion complex was confirmed by IR spectroscopy. Differences in their affinity to host molecules resulted in separation of the two enantiomers, thus capillary electrophoresis (CE) proved to be an eligible method for the chiral separation of ofloxacin. The best separation was achieved using a 50 mM phosphate buffer, at pH 3.1, applying a voltage of 20 kV at a temperature of 20°C and 40 mM RAMEB as chiral selector added to the background electrolyte. Under these experimental conditions the chiral separation occurred in 6 minutes.

Rezumat

Au fost investigate interacțiunile ofloxacinei cu beta-ciclodextrină metilată aleator (RAMEB) prin tehnici analitice complementare. S-a recurs la modelarea moleculară asistată de calculator (ChemBio3D Ultra 12.0) pentru caracterizarea enantiomer-specifică a complecșilor. S-a preparat complexul ofloxacină-RAMEB prin metoda malaxării și formarea complexului de incluziune a fost demonstrată prin spectroscopie în IR. Diferența în afinitatea lor față de molecula gazdă a rezultat în separarea celor doi enantiomeri, astfel electroforeza capilară (EC) s-a dovedit a fi o metodă eligibilă pentru separarea chirală a ofloxacinei. Separarea cea mai eficientă a fost realizată prin utilizarea soluției tampon fosfat pH 3,1 50 mM, în care s-a dizolvat RAMEB 40 mM ca și selector chiral, aplicând un voltaj de 20 kV la temperatura de 20°C. În aceste condiții experimentale separarea a avut loc în 6 minute.

Keywords: ofloxacin, ramdoly methylated beta-cyclodextrin (RAMEB), chiral selector

Introduction

Ofloxacin, a member of fluoroquinolone group is a synthetic antibacterial agent with a broad spectrum of activity. It is a chiral compound and the anti-bacterial activity of *S*(-) ofloxacin (levofloxacin) is 8 to 128 times higher than that of *R*(+) enantiomer and twice as higher than that of the racemate [8]. Cyclodextrins (CDs) are cyclic oligosaccharides composed of 6, 7 or 8 α-1,4-linked glucopyranose units, characterized by a truncated cone shape. They are widely used as complexing agents to improve properties of the guest compounds: to increase their solubility, bioavailability and stability, to reduce gastrointestinal or ocular irritation, to eliminate unpleasant smells or tastes and to prevent interactions. In recent years, cyclodextrins have been proved to be effective as host compounds in molecular recognition and chiral separation, as well [6]. The aim of this study was the assessment of ofloxacin complexation with a beta-cyclodextrin derivative, randomly methylated beta-cyclodextrin (RAMEB) and to develop an enantioselective separation method by capillary electrophoresis (CE).

Materials and Methods

Guest-host interactions of ofloxacin (Richter Gedeon, Hungary) with randomly methylated beta-cyclodextrin (RAMEB – Cyclolab, Hungary) were tested by molecular modelling, IR spectrophotometric and capillary electrophoresis (CE) studies.

The molecular modelling of the complex was performed as follows: the structure of RAMEB was taken from ChemACX database, the initial structure of *R*(+) ofloxacin was built by ChemBioDrawUltra 12.0 program, the *S*(-) enantiomer - levofloxacin was obtained from reflection of the *R*(+) one and 1:1 inclusion complexes were constructed between the cyclodextrin (CD) and both configurations. The models have been built by docking technique with the aid of ChemBioOfficeUltra 12.0 program package. Geometry optimization in vacuum through MM2 molecular mechanics was performed until a RMS

(root mean square) gradient lower than 0.010 was obtained and the potential energies for the host and guest molecules and for each inclusion complex were recorded [3].

Inclusion complex of ofloxacin with RAMEB was prepared by the kneading method in 1:1 molar ratio. In comparison to the host, the obtained complex and guest components weres analysed using an IR spectrophotometer (Jasco FTIR 470 PLUS) using KBr pellet technique [4].

Capillary electrophoresis experiments were performed on an Agilent CE System apparatus with UV spectrophotometric detection at a 276 nm wavelength. Uncoated fused-silica capillary with a length of 40-50 cm was used. Before use, it was washed with 0.1 M sodium hydroxide solution for 5 minutes, followed by distilled water and running buffer for 5 minutes. As migration environment 0.025 M sodium tetraborate buffer solution and 0.05 M phosphate running buffer were chosen, prepared with phosphoric acid and both adjusted to the appropriate pH with NaOH 0.1 N. Ofloxacin samples were prepared in concentration of 100 ppm. The sample solutions were injected hydrodynamically in the capillary at 50 mbar for 5 seconds. The measurements were carried out at a potential difference of 20 - 30 kV, at temperatures of 15 to 20°C. In order to improve separation and detection, the pH and the running buffer concentrations (25 - 100 mM) and RAMEB concentrations (20 - 40 mM) were optimized [5, 11].

Results and Discussion

Molecular modelling study

The total molecularly potential energy, E, of a molecule can be described by the following summation of interactions (force-field):

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

The first three terms are the so-called bonded interactions. The last term includes van der Waals and electrostatic interactions (interactions from charges, dipoles, and quadrupoles) [3]. The molecular model of the two configurations (R and S) of ofloxacin molecule is presented below in Figure 1. The potential energies of the complex formed are listed in Table I.

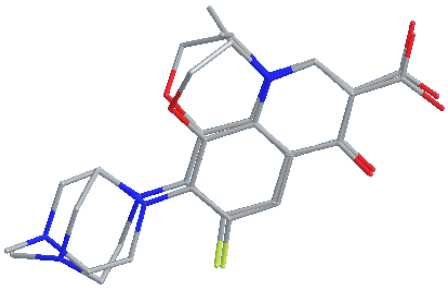

Figure 1.
The molecular model of ofloxacin *R*(+) and *S*(-)

Table I
Potential energies of RAMEB, ofloxacin *R*(+) and *S*(-) and their binding energies (E_{dif})

Interactions	Potential energies (kcal/mol)						
	RAMEB	OFL		OFL/ RAMEB		E_{dif}	
		R	S	R	S	R	S
Stretch:	257.82	3.42	3.35	260.40	260.72	-0.85	-0.46
Bend:	198.48	21.19	19.54	220.14	221.11	0.46	3.14
Stretch-Bend:	41.41	0.79	0.72	41.99	42.08	-0.21	-0.04
Torsion:	75.83	4.68	3.92	67.81	67.74	-12.71	-12.01
Non-1.4 VDW:	309.20	5.02	4.19	293.09	293.96	-21.14	-19.43
1.4 VDW:	126.86	27.19	27.54	155.73	155.37	1.67	0.96
Dipole/Dipole:	0.00	5.33	6.82	0.00	0.00	-5.33	-6.82
Total Energy:	1009.63	67.66	66.11	1039.18	1041.01	**-38.11**	**-34.74**

The investigated CD derivative interacted with the two configurations of ofloxacin mainly by Van der Waals forces. There was found a small difference in the binding energies of the two enantiomers.

IR spectrophotometric study

Having photo-protective properties, cyclodextrins are masking the characteristic peaks of functional groups included in their cavity. Figures 2 and 3 represent the IR spectra of ofloxacin and RAMEB, while Figure 4 shows the IR spectrum of their complex.

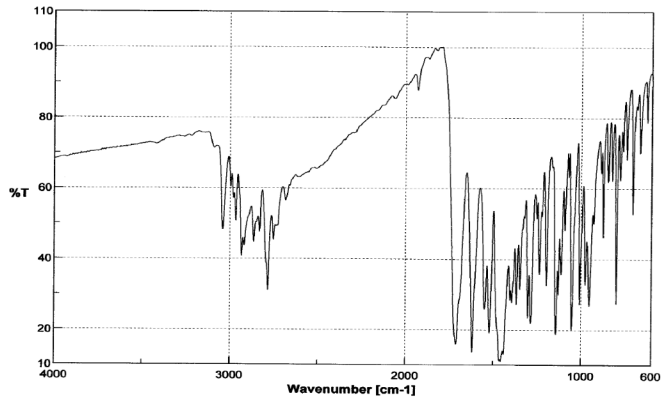

Figure 2.
IR spectra of ofloxacin

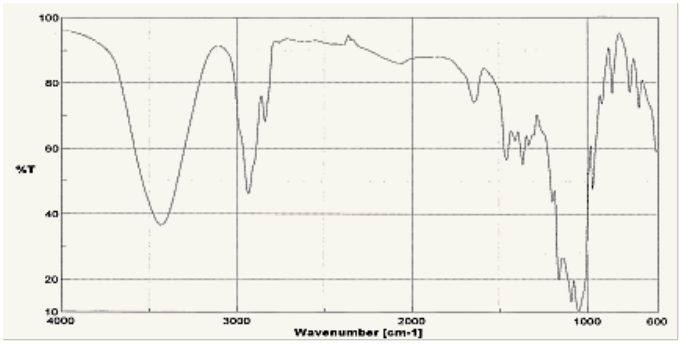

Figure 3.
IR spectra of RAMEB

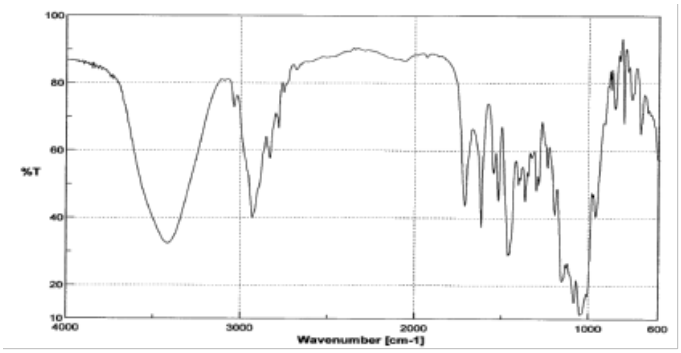

Figure 4.
IR spectra of ofloxacin-RAMEB inclusion complex

Comparing the main IR frequencies of complex with those of the guest, the following was found: the spectrum of ofloxacin shows a strong absorption peak for C = O at 1714 cm^{-1} as the fraction of the carboxylic group. The presence of the characteristic band in absorption spectrum of the complex indicates that this moiety has not been encapsulated. Some peaks assigned to C-O bond in the structure of ofloxacin disappear from the spectrum of the complex formed with RAMEB. Based on these results we assume that a part of the oxazine ring has been embedded in the cavity [4].

Capillary electrophoresis experiments

RAMEB was tested for the enantio-separation of ofloxacin and in order to demonstrate the inclusion complex formation, in different experimental conditions. Ofloxacin, like most of the fluoroquinolone derivatives has two relevant ionisable functional groups, the 3-carboxyl group and N-4 of the piperazine ring, which determine its acid-base properties.

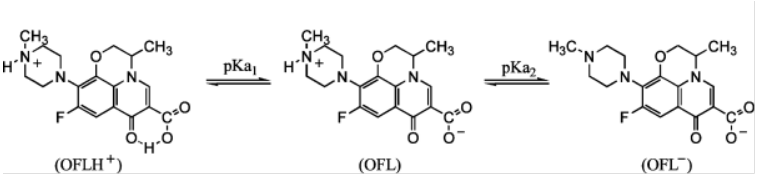

Figure 5.
The ionization equilibria of ofloxacin (pKa_1 = 6.10 and pKa_2 = 8.28) [13]

When pH values are set between pK_1 and pK_2, the major form of quinolones is the zwitterionic one, so they migrate with electroosmotic flow and their electrophoretic mobility values are nil. At pH values greater than the pK_2, quinolones have a negative net charge and are detected after the electroosmotic flow marker, their mobility values are negative. When pH values are below pK_1, quinolones have a positive net charge and migrate faster than the electroosmotic flow marker, which gives positive mobility values. Buffer pH may affect mobility and electroosmotic flow (EOF) not only by changing dissociation constant of analyte but even by affecting ionization of silanol groups on the capillary wall [1, 17].

Electrophoretic properties of the racemic mixture have been investigated at pH values greater than the pKa_2 of the analite. For this purpose, 0.025 M borate running buffer was chosen adjusted to the appropriate pH with 0.1 N NaOH. RAMEB was added to the background electrolyte in concentrations of 20 - 40 mM. Figure 6 shows the migration of racemic mixture at pH = 9.3 in the presence of 40 mM RAMEB.

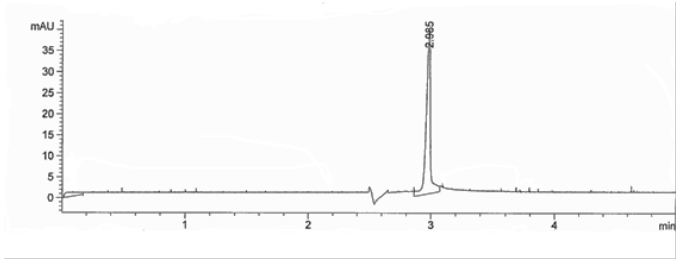

Figure 6.
Electropherogram of ofloxacin in 25 mM borate running buffer after addition of RAMEB 40 mM

It is to be noted, that in alkaline pH range, using borate buffer as supporting electrolyte, enantio-separation did not occur. In the next step electrophoretic behaviour of the racemic mixture has been studied at pH values lower than the first dissociation constant. Further experiments were performed using 0.05 M phosphate buffer at different pH values in the range of 2.2 - 5.8, which contained 20 - 40 mM RAMEB. Under these conditions two characteristic peaks of *S*(+) and *R*(-) enantiomers appeared.

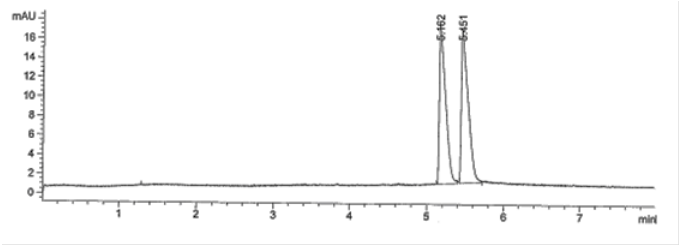

Figure 7.
Electropherogram of ofloxacin *S*(+) and *R*(-) in 50 mM phosphate running buffer after addition of RAMEB 40 mM

The best separation was achieved using 0.05 M phosphate buffer, at pH 3.1, applying a voltage of 20 kV at a temperature of 20°C and 40 mM RAMEB as chiral selector added to the background electrolyte, as shown in Figure 7. Under these experimental conditions, the chiral separation occurred in 6 minutes.

Conclusions

The interactions of ofloxacin with RAMEB were characterized by molecular modelling, IR spectro-

photometric and CE studies. Differences in their affinity to host molecules resulted in the separation of the two enantiomers, thus CE proved to be an eligible method for the chiral separation of ofloxacin.

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