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RESEARCH ARTICLE

Study of Cyclodextrin/Fluoroquinolone Inclusion Complexes by Capillary Electrophoresis

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Introduction: In the present work 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 selectors in capillary electrophoresis.

Material and method: Guest-host interactions of two second generation quinolones, ciprofloxacin and norfloxacin with four cyclodextrins, beta-cyclodextrin (β-CD), gamma-cyclodextrin (γ-CD) and two beta-cyclodextrin derivatives, 2-hydroxypropyl beta-cyclodextrin (HP-β-CD) and randomly methylated beta-cyclodextrin (RAMEB), were tested by capillary electrophoresis in borate running buffer. Experimental parameters like buffer concentration, pH, organic modifier, voltage and cyclodextrin concentration have been varied for a better resolution.

Results: In capillary zone electrophoresis ciprofloxacin and norfloxacin are migrating together, a difference in their migration times and thus separation occured by the addition of cyclodextrins.

Conclusion: Our results suggest formation of inclusion complexes between fluoroquinolones and cyclodextrins. Differences in their affinity to host molecules resulted in separation of the two fluoroquinolones.

Keywords: cyclodextrin, fluoroquinolone, selector, capillary electrophoresis

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Introduction

In the present work 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 selectors in capillary electrophoresis. 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. They 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 proved to be effective as host compounds in molecular recognition, chiral and achiral separation [1–3]. Fluoroquinolones (FQs) 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 two quinolones, ciprofloxacin and norfloxacin with four cyclodextrins, beta-cyclodextrin (β-CD), gamma-cyclodextrin (γ-CD) and two beta-cyclodextrin derivatives, 2-hydroxypropyl beta-cyclodextrin (HP-β-CD) and randomly methylated beta-cyclodextrin (RAMEB), were tested and their separation was achieved by capillary electrophoresis. Experimental parameters like buffer concentration, pH, organic modifier, voltage and cyclodextrin concentration are discussed.

Material and method

Capillary electrophoresis (CE) experiments were performed on Agilent CE System apparatus with UV spectrophotometric detection at a wavelength of 276 nm. Uncoated fused-silica capillary was used with a length of 40–50 cm. 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 sodium tetraborate buffer solution was chosen. Ciprofloxacin and norfloxacin samples were prepared in concentrations of 100 ppm. The sample solutions were injected in the capillary hydrodynamically at 50 mbar for 5 seconds. The measurements were carried out at a voltage of 20–30 kV, at temperatures of 15 to 20 °C. In order to improve separation and detection the following experimental parameters were optimized: the concentration of running buffer (25–100 mM) and its composition by adding organic solvents (methanol 5–15%) and cyclodextrins in concentrations of 20–40 mM, at different pH values in the range of 7.2–12. Cyclodextrin derivatives used in this study were beta-cyclodextrin (β-CD), gamma-cyclodextrin (γ-CD), 2-hydroxypropyl beta-cyclodextrin (HP-β-CD) and randomly methylated beta-cyclodextrin (RAMEB).

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Table I. Influence of pH on migration time of the two analytes

pH		8.8	9.3	10.5	11	12
Migration time (min)	Norfloxacin	2.110	2.286	2.677	2.724	3.079
	Ciprofloxacin	2.207	2.382	2.770	2.841	3.394

Table II. Influence of methanol concentration on migration time of the mixture

Methanol conc. (%)	0	5	10	15
Migration time (min)	2.612	3.512	3.641	4.384

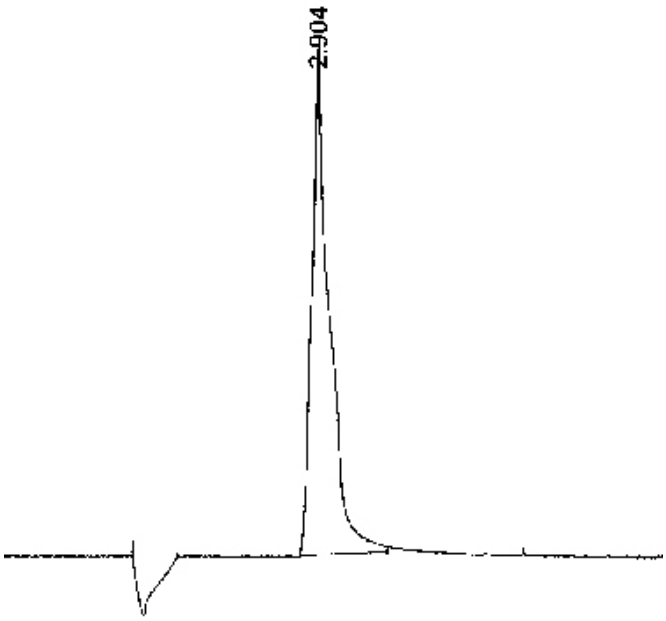

Fig. 1. Electropherogram of the mixture of norfloxacin and ciprofloxacin, 25 kV

Results

To test the behaviour of individual quinolones in CZE, in the first part of the investigation no CD derivatives were added. The effect of buffer concentration on migration of the two analytes, ciprofloxacin and norfloxacin, was studied. There was a slight increase in analyte migration times due to a decrease in EOF (Electroosmotic flow) with increasing buffer concentration. At all buffer concentrations norfloxacin migrated first followed by ciprofloxacin. Applying the investigated fluoroquinolones in mixture the two analytes migrated together. Further experiments were performed using 25 mM borate buffer at different pH values in the range of 7.2–12. Results are shown in Table I.

The effect of organic modifier was analized by adding methanol to the 25 mM running buffer in a 5–15% range, at pH 9.3. Migration times of the mixture of the two analytes are listed in Table II.

Subsequent experiments took place under similar conditions with addition of different cyclodextrin derivatives to background electrolyte (BGE). Figure 1 presents migration of the mixture at pH 9.3, without addition of cyclodextrins.

Figures 2 and 3 show migration and separation of the two FQs in the presence of RAMEB in the running buffer in different concentrations and at two different pH values.

Discussions

Cyclodextrins are known as chiral selectors in enantiomeric separation of chiral drugs, but these are eligible for separation of other drugs in CE, as well. The application of CDs as chiral and achiral selectors in CE has been reviewed by several authors [2,3,7–11]. In this study β-CD, γ-CD, HP-β-CD and RAMEB were tested for the separation of two fluoroquinolones and for demonstration of inclusion complex formation, in different experimental conditons. The

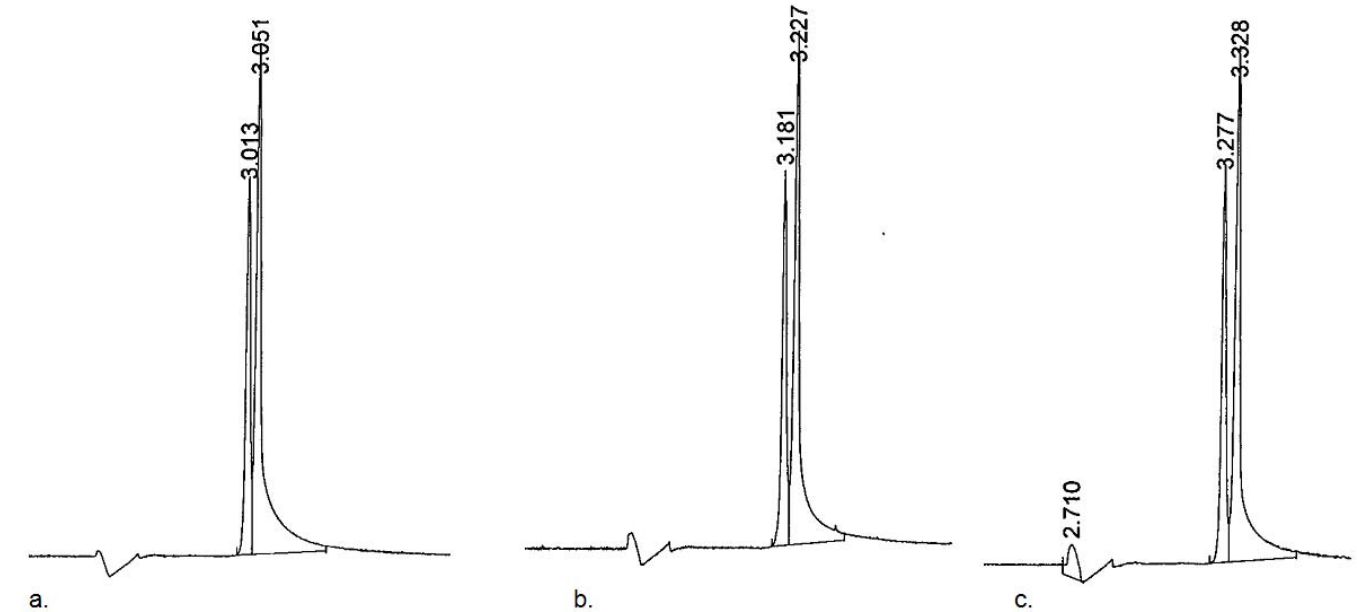

Fig. 2. Electropherogram of the mixture of norfloxacin and ciprofloxacin with addition of a.) 20 mM RAMEB, b.) 30 mM RAMEB, c.) 40 mM RAMEB, 20 kV, pH 9.3

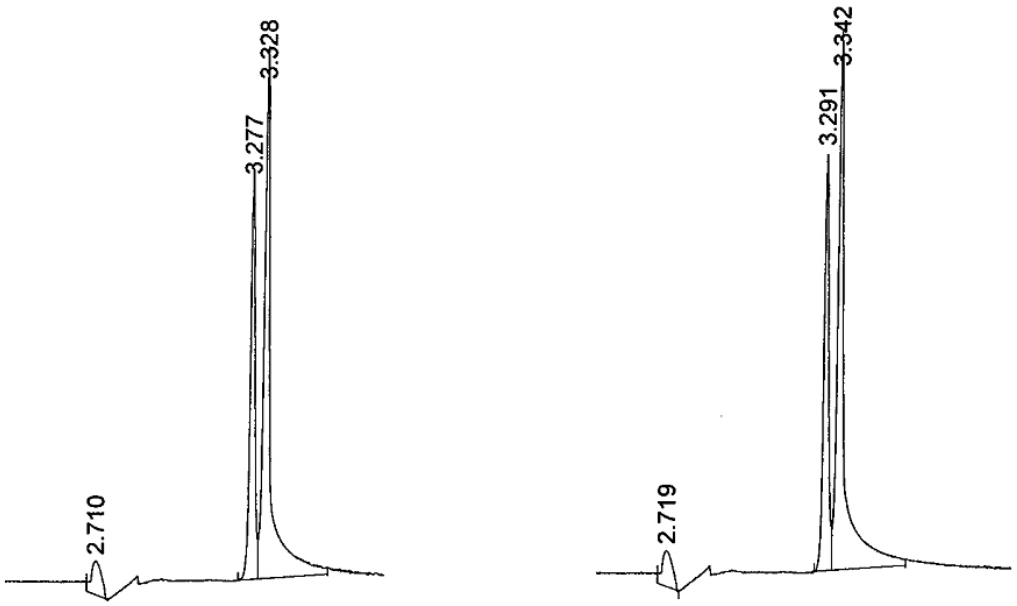

Fig. 3. Electropherogram of the mixture of norfloxacin and ciprofloxacin with addition of 40 mM RAMEB, pH 9.3 and pH 11

experimental parameters for a better resolution are buffer type and concentration, pH, organic modifier and voltage [11–14]. Norfloxacin and ciprofloxacin both have two relevant ionizable functional groups, the 3-carboxyl group and N-4 of the piperazine ring, which determine their acid-base properties [5]. According to Barbosa et al [15] who have studied the effect of pH on electrophoretic behaviour of various quinolones, when pH values are comprised between pK1 and pK2 the major form is the zwitterionic one, so quinolones migrate with electroosmotic flow and electrophoretic mobility values are nil. At pH values greater than the pK2, quinolones have a negative net charge and are detected after the electroosmotic flow marker, mobility values are negative at these pH values. Working with borate running buffer in pH range 7.2–12 we were led to the same conclusion, no electrophoretic mobility was observed under pH 8.8. Buffer pH may affect mobility and EOF not only by changing dissociation constant of analyte but even by affecting ionization of silanol groups on the capillary wall. The chemical composition and the concentration of the buffer can affect the baseline stability, peak shape and separation selectivity [11]. In this study sodium tetraborate buffer was used in the range of 25–100 mM and the effect of buffer concentration upon migration times was investigated. There was observed a slight increase in migration times due to a decrease in the EOF with increasing buffer concentration. Moderately high ionic strenght buffers are desirable for the suppression of ionic interactions between charged analyte ions and ionized silanol groups on the capillary wall. However, high concentrations of buffers may overcome the capillary thermostatting capability of system, so relatively low concentration, 25 mM was chosen [15]. The addition of an organic modifier such as methanol in background electrolyte can influence several parameters like stability constants of the inclusion complexes, analysis time, solubility of the analytes or CD, viscosity of the BGE, and EOF [9]. Increasing methanol concentration from 0 to 15 % an increase in migration time was observed for both solutes. C. Fierens et al. applied capillary zone electrophoresis (CZE) in the study of ten quinolones using a phosphate buffer (pH 7,0. 125 mM), but norfloxacin and ciprofloxacin could not be separated in this way [16]. Since these two FQs are moving together, a difference in their migration times can be achieved by exploiting the differences of their affinity to charged or neutral molecules such as CDs. In our study β-CD, γ-CD, HP-β-CD and RAMEB were used in concentrations of 20–40 mM. CD type and concentration are essential parameters for the optimization of chiral and achiral separations. Separation was achieved with each of the four CDs used, concentration of about 40 mM was found to give a better resolution.

Conclusions

Our study demonstrates the formation of inclusion complexes between fluoroquinolones and cyclodextrins. Differences in their affinity to cyclodextrins resulted in different migration times and separation of the two fluoroquinolones by capillary electrophoresis.

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