



# Determination of the structure of quinolone- $\gamma$ -cyclodextrin complexes and their binding constants by means of UV–Vis and $^1\text{H}$ NMR

Elisa Leyva<sup>1</sup> · Edgar Moctezuma<sup>1</sup> · Silvia E. Loredo-Carrillo<sup>1</sup> · Claudia G. Espinosa-González<sup>1</sup> · Agobardo Cárdenas-Chaparro<sup>1</sup>

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## Abstract

The host–guest inclusion complex structure and binding ability of two different quinolones with  $\gamma$ -cyclodextrin ( $\gamma$ -CD) were investigated in solution by means of UV–Vis and  $^1\text{H}$  NMR spectroscopy. Competition of oxolinic and nalidixic acid molecules for the  $\gamma$ -CD cavity was evaluated by determination of association constants. Both quinolones form 1:1 inclusion complexes, their binding constants at room temperature (25 °C) under acidic and basic conditions were calculated using Benesi–Hildebrand equation. The stability of the complexes was dependent on the structure of the quinolone. In general, the weaker binding constants were observed for oxolinic acid- $\gamma$ -CD complexes (1616 and 1765  $\text{M}^{-1}$ ) and the larger binding constants were obtained for nalidixic acid- $\gamma$ -CD complexes (3760 and 3840  $\text{M}^{-1}$ ).  $^1\text{H}$  NMR studies in  $\text{D}_2\text{O}$  were performed to elucidate the structure of each inclusion complex, nalidixic acid molecule penetrates more deeply into the  $\gamma$ -CD cavity and an intermolecular hydrogen bond is formed. Knowledge about structure and relative stability of quinolone- $\gamma$ -CD complexes will be useful for future applications of these antimicrobial agents in medicinal chemistry.

**Keywords** Nalidixic acid · Oxolinic acid · Cyclodextrin · Inclusion complex · Quinolones

## Introduction

Two organic acids, nalidixic (NA) and oxolinic (OA), were the first analogues of a large class of antimicrobial agents with a quinolone structure (Fig. 1). Latter derivatives with broader biological activity contained fluorine atoms and were called fluoroquinolones [1]. NA was the first synthetic quinolone derived from 1,8-naphthyridine and it was used in the treatment of urinary tract infections in 1963 because of its biological activity against infections caused by *E. coli*, *Klebsiella aerogenes*, *Proteus mirabilis*, and *Shigella sonnei* [2]. Recent studies have evidenced several side-effects that induce gastroenteritis due to the abuse of this quinolone [3]. OA is commonly used in urinary tract infections and in several veterinary applications [4]. It has been demonstrated to be a potent, and well-tolerated antibiotic but upon exposure to light it undergoes photodecomposition, leading to several

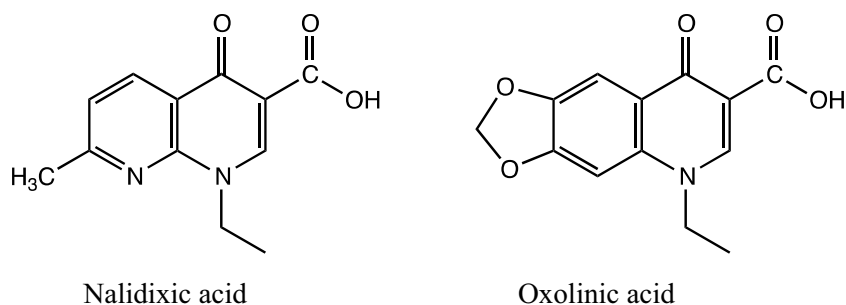
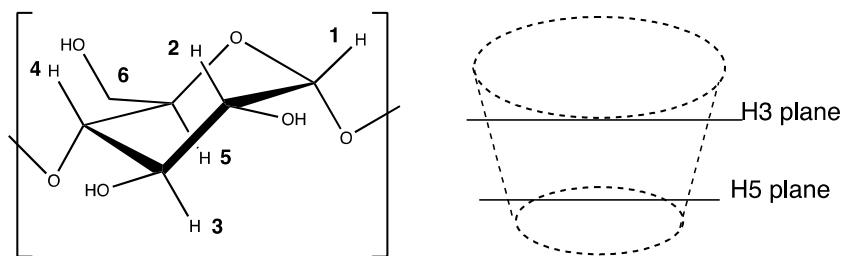
psychopharmacological side effects such as nervous excitation and insomnia [5].

Cyclodextrins (CDs) are water soluble macrocyclic oligosaccharides containing six ( $\alpha$ -CD), seven ( $\beta$ -CD) and eight ( $\gamma$ -CD) glucosepyranose units which are arranged to give a truncated cone structure (Fig. 2) with a hydrophobic inside and a hydrophilic outside [6, 7]. Due to their structure and physicochemical properties, CDs could be considered biochemical templates to form stable supramolecular structures with several pharmaceutical compounds, resulting in an improvement in the solubility, stability and controlled releasing of these compounds [8].

Complexation behavior, characterization and binding ability of natural and synthetic drugs with several CDs have been investigated during the last decades, and it has been reported that CDs indeed enhance the water solubility and stability of several pharmaceutical compounds [9–12]. CDs complexes have found diverse applications in the pharmaceutical industry as excipients of drugs, in protein formulation, and in crystallization of pharmaceutical compounds [6–8]. Recently, specific properties of diverse CDs complexes have been studied. Anti-inflammatory and antioxidant activity of ellagic acid in rats was improved when this

✉ Elisa Leyva  
elisa@uaslp.mx

<sup>1</sup> Facultad de Ciencias Químicas, Universidad Autónoma de San Luis Potosí, Manuel Nava No. 6, Zona Universitaria, C.P. 78210 San Luis Potosí, SLP, Mexico

**Fig. 1** Structure of quinolones**Fig. 2** Truncated cone structure of cyclodextrin with all the hydrogens

well-known compound was associated to form a  $\beta$ -CD complex [13]. Bioavailability of different compounds has been evaluated in nanoscaled CDs derivatives to accelerate their pharmaceutical effect [14].

In the literature, there are several studies about the formation of CDs complexes with quinolones [15–23]. In the case of NA the reports present contradictory results. It has been claimed that  $\alpha$ -CD forms complexes with NA and enhances its solubility [19]. However, latter studies indicated that there is no complex formation between NA and  $\beta$ -CD due to guest molecular size or steric hindrance [20, 21]. In some cases, a different binding constant has been found for a given quinolone-CD complex and this is most likely due to the limitations of the analytical technique used in the measurement [20–22]. In a more recent investigation, the formation of NA complex with  $\alpha$ -CD and  $\beta$ -CD was demonstrated by a combination of UV–Vis spectroscopy and voltammetry methods [23]. Most of the studies on pharmaceutical-CD complexes have been limited to investigate their supramolecular interactions with  $\alpha$ -CD or  $\beta$ -CD. However,  $\gamma$ -CD is more soluble in water and has a bigger cavity that can accommodate larger organic molecules [7]. In this study, we investigated the complex formation between a quinolone like NA or OA with  $\gamma$ -CD. Supramolecular CD structures will improve some physicochemical properties of these quinolones such as solubility, release into solution and undesirable photodegradation. We evaluated the binding constants of the NA- $\gamma$ -CD and OA- $\gamma$ -CD complexes by UV–Vis spectroscopy and investigated their structure by  $^1\text{H}$  NMR.

## Experimental

### Materials

All reagents used were of the highest purity and commercially available. Oxolinic acid, nalidixic acid, phenolphthalein, and methyl orange were purchased from Aldrich and  $\gamma$ -CD was donated by Cerestar. Deionized water was used as solvent for all UV–Vis spectra and data were acquired with a Shimadzu UV-2401 UV–Vis spectrophotometer.  $^1\text{H}$  NMR spectra and data were acquired with a BRUKER AC 200 using deuterated water  $\text{D}_2\text{O}$  as solvent.

### General procedures for UV–Vis spectroscopic studies

#### Direct UV–Vis procedure to measure binding constants in solution

UV–Vis spectroscopy is the experimental technique mostly used to determine binding constants of CD inclusion complexes because it is simple and cheap [24]. The direct spectroscopic method relies in the spectral differences between the free guest and the complex. Changes in the absorption intensity of methyl orange (ME) at  $\lambda_{\text{max}} = 507$  nm in acidic solution (HCl 0.03 M) were monitored upon changing  $\gamma$ -CD concentration. The binding constants were calculated at room temperature (25 °C)

using the Benesi–Hildebrand equation or a modified version of it [25]. In this experimental procedure, a 1:1 stoichiometry is verified by the presence of two isosbestic points at 460 and 564 nm. This observation indicates that only two species induce changes in the observed UV–Vis spectrum of the solution, the free guest (colorant) and the complex (colorant- $\gamma$ -CD). In addition, a binding isotherm curve is obtained by plotting  $\Delta A$  against [Cyclodextrin]. A double reciprocal plot gives a straight line for a case in which a 1:1 complex is formed [25]. To perform these experiments, a volume (1 mL) of a standard solution of ME ( $1 \times 10^{-4}$  M) and a volume (0, 1, 2, 4 and 6 mL) of a standard solution of  $\gamma$ -CD ( $2 \times 10^{-2}$  M) were placed in a volumetric flask (10 mL). The volume was completed with deionized water and the mixtures were stirred for 30 min to allow the formation of the complex. After this time, the UV–Vis spectrum of each solution was obtained.

In the case of phenolphthalein (PHE), changes in the absorption at  $\lambda_{\max} = 552$  nm in basic solution (NaOH 0.03 M) were monitored as a function of  $\gamma$ -CD concentration to measure the binding constants by a direct method. The curves presented two isosbestic points at 445 and 600 nm. A volume (2 mL) of a standard solution of PHE ( $1 \times 10^{-4}$  M) and a volume (0, 0.2, 0.4, 0.6, 0.8 and 1.0 mL) of standard solution of  $\gamma$ -CD ( $1 \times 10^{-3}$  M) were placed in a volumetric flask (10 mL). The volume was completed with deionized water and the mixtures were stirred for 30 min to allow the formation of the complex. After this time, the UV–Vis spectrum of each solution was obtained.

#### Indirect UV–Vis procedure to measure binding constants in acidic solution

The binding constants of  $\gamma$ -CD complexes with oxolinic and nalidixic acids (in an acidic solution) were determined by means of a competitive method previously reported [16, 25]. A volume (1 mL) of a standard solution of ME ( $1 \times 10^{-4}$  M in HCl 0.03 M) and a volume (3 mL) of a standard solution of  $\gamma$ -CD ( $2 \times 10^{-2}$  M) were placed in a volumetric (10 mL) flask. Each solution was added a volume (0.0, 0.2, 0.4, 0.6, 0.8 and 1 mL) of a standard solution of oxolinic ( $1 \times 10^{-4}$  M in HCl 0.03 M) or nalidixic acid ( $5 \times 10^{-4}$  M in HCl 0.03 M). The volume was completed with deionized water and the mixtures were stirred for 30 min. The UV–Vis spectrum of each solution was obtained. The binding constants were calculated utilizing the method previously reported in the literature [25].

#### Indirect UV–Vis procedure to measure binding constants in basic solution

The binding constants of  $\gamma$ -CD complexes with both quinolones in basic solution were determined by means of a

competitive method [16]. A volume (2 mL) of a standard solution of PHE ( $1 \times 10^{-4}$  M in NaOH 0.03 M) and a volume (1 mL) of a standard solution of  $\gamma$ -CD ( $1 \times 10^{-3}$  M) were placed in a volumetric (10 mL) flask. Each solution was added a volume (0.0, 0.2, 0.4, 0.6, 0.8 and 1 mL) of a standard solution of OA ( $5 \times 10^{-4}$  M in NaOH 0.03 M). The volume was completed with deionized water and the mixtures were stirred for 30 min. UV–Vis spectrum of each solution was obtained.

In the case of NA, a volume (2 mL) of a standard solution of PHE ( $1 \times 10^{-4}$  M in NaOH 0.03 M) and a volume (2 mL) of a standard solution of  $\gamma$ -CD ( $1 \times 10^{-3}$  M) were placed in a volumetric (10 mL) flask. Each solution was added a volume (0.0, 1.0, 2.0, 3.0, and 4.0 mL) of a standard solution of NA ( $1 \times 10^{-3}$  M in NaOH 0.03 M). The volume was completed with deionized water and the mixtures were stirred for 30 min. UV–Vis spectrum of each solution was obtained. The binding constants were calculated utilizing the method previously reported in the literature [25].

#### General procedure for $^1\text{H}$ NMR spectroscopic studies

Complexes formed with  $\gamma$ -CD and the corresponding quinolone Q were studied by  $^1\text{H}$  NMR spectroscopy. The intermolecular interactions presented between  $\gamma$ -CD and each quinolone structure led to a complex formation and such interactions were evidenced [26]. In order to determine the arrangement within the cavity of each quinolone molecule, changes in the chemical shift ( $\delta$ ) of the principal signals in the  $\gamma$ -CD and quinolone spectra were evaluated.

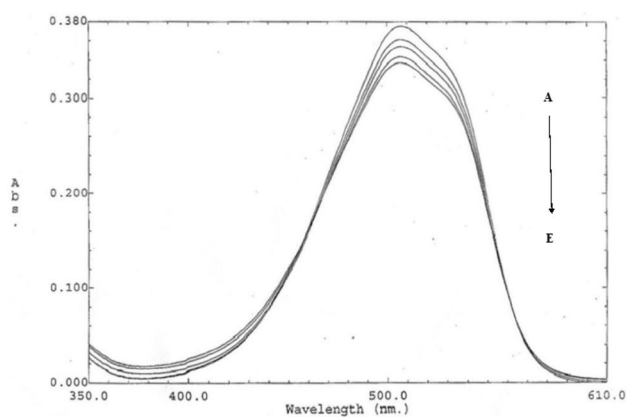
#### Q- $\gamma$ -CD complex solutions for $^1\text{H}$ NMR

Solutions of Q- $\gamma$ -CD complex were prepared in 2 mL volumetric flasks. In each flask, 0.2 mL of  $\gamma$ -CD solution (0.1 M) in  $\text{D}_2\text{O}$  were added and then, aliquots of 0.0, 0.2, 0.4, and 0.8 mL of a NA or OA standard solution (0.1 M) were added, respectively. The flask volume was completed with  $\text{D}_2\text{O}$ . The mixture was stirred for 30 min to induce formation of complex.

### Results and discussion

#### UV–Vis spectroscopy studies

The formation of inclusion complexes has been investigated by different spectroscopy methods [24–26]. The evaluation of binding constants by direct UV–Vis method relies on spectral differences between the free guest and the complex. However, in the case of quinolones OA and NA, very small changes in their UV–Vis spectra were observed upon formation of the Q- $\gamma$ -CD complexes. The binding constants were

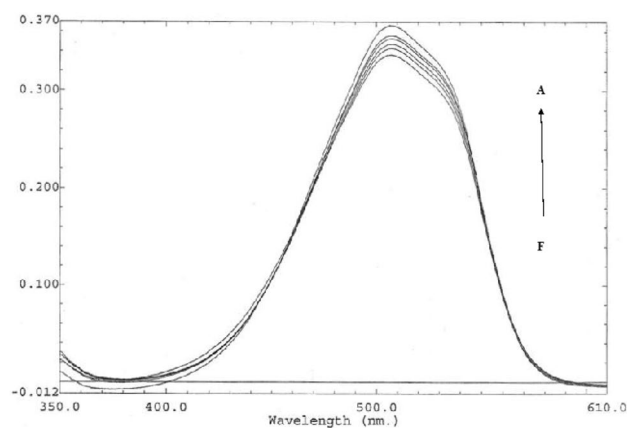


**Fig. 3** UV–Vis spectra of a ME solution ( $1 \times 10^{-5}$  M) with different concentrations of  $\gamma$ -CD (A: 0.0 M; B:  $1 \times 10^{-3}$  M; C:  $2 \times 10^{-3}$  M; D:  $4 \times 10^{-3}$  M; E:  $6 \times 10^{-3}$  M)

determined by spectrophotometric examination of the inhibitory effect of the quinolone on the association of  $\gamma$ -CD with a colorant (ME or PHE). First, the binding constant of  $\gamma$ -CD with the corresponding colorant was measured by direct spectrophotometric method (Fig. 3). In order to do this, the changes in intensity of the maximal absorption of the colorant were analyzed as a function of quinolone concentration.

All the binding constants (Table 1) of the inclusion complexes were measured at room temperature (25 °C) and were calculated using Benesi–Hildebrand equation [24, 25].

The binding constant for the Q- $\gamma$ -CD complexes were calculated utilizing the method previously reported [25], investigating the effect of the addition of the quinolone on the UV–Vis spectrum of a colorant- $\gamma$ -CD complex (Fig. 4). In both cases, the addition of quinolone resulted



**Fig. 4** UV–Vis spectra of ME solutions. A: ME ( $1 \times 10^{-5}$  M); F: with  $\gamma$ -CD ( $6 \times 10^{-3}$  M) and with different concentrations of OA; B: OA ( $10 \times 10^{-6}$  M); C: OA ( $5 \times 10^{-6}$  M); D: OA ( $4 \times 10^{-6}$  M); E: OA ( $2 \times 10^{-6}$  M)

in a progressive increment of the absorbance indicating that each quinolone forms a complex with  $\gamma$ -CD and expels the colorant to the solution. Based on the experimental results, the two colorants and the two quinolones investigated, OA and NA, form stable 1:1 complexes with  $\gamma$ -CD. In addition, several previous spectrofluorometric studies with a similar Q/ $\gamma$ -CD concentration range have demonstrated the formation of a 1:1 complex in solution [15, 20, 21]. The fact that a comparable binding constant is obtained for each quinolone in acidic and basic environment, clearly indicates that the carboxylic COOH functional group remains in the aqueous environment.

## <sup>1</sup>H NMR studies

Since NMR was introduced to investigate cyclodextrin complex formation in aqueous solution [26], there have been a large number of studies involving aromatic compounds [27, 28]. This methodology relies on changes on chemical shifts caused by the proximity of the host and guest in the complex. When the guest is an aromatic compound such as a quinolone Q, the spectral changes that occur upon formation of the Q- $\gamma$ -CD complex come from diamagnetic shielding of the guest on the nearby spins of the host. In a  $\gamma$ -CD truncated cone structure (Fig. 2) only hydrogens H3 and H5 are located inside the cavity. All H3 are located near the wider rim while all the H5 are located near the narrower rim. In contrast, all the other  $\gamma$ -CD hydrogens (H1, H2, H4, and H6,6') are located outside the cavity.

To investigate the effect on chemical shift of  $\gamma$ -CD hydrogens upon formation of a complex with OA we obtained the <sup>1</sup>H NMR of  $\gamma$ -CD as a function of OA concentration (Fig. 5). In spite of its macromolecular structure  $\gamma$ -CD presents a simple <sup>1</sup>H NMR spectra. H1 gives a doublet signal at 4.97 ppm,

**Table 1** Binding constants for cyclodextrin complexes

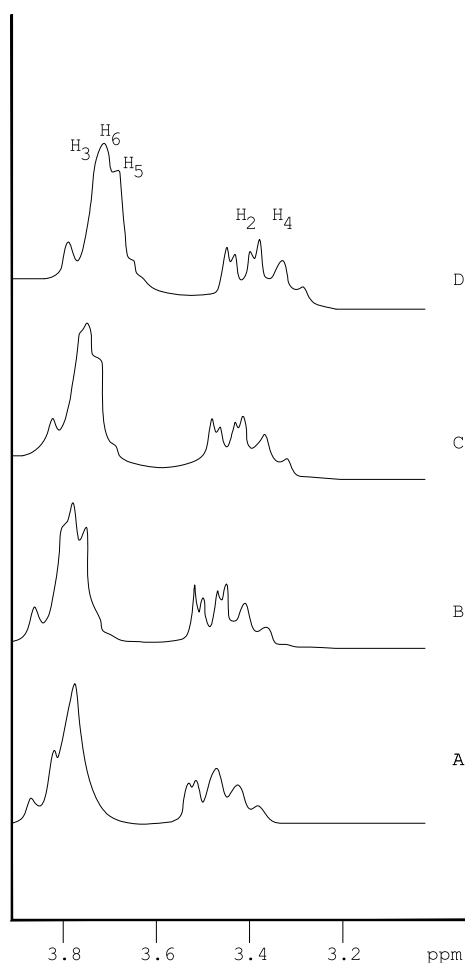
Guest	Kb ( $M^{-1}$ ) at 25 °C
ME	$400 \pm 10^a$
PHE	$1227 \pm 12^b$
OA	$1765 \pm 13^c$
OA	$1616 \pm 12^d$
NA	$3840 \pm 25^c$
NA	$3760 \pm 22^d$

<sup>a</sup>Measured by direct spectrophotometric method in acid solution (HCl 0.03 M)

<sup>b</sup>Measured by direct spectrophotometric method in basic solution (NaOH 0.03 M)

<sup>c</sup>Measured by competitive spectrophotometric method in acid solution (HCl 0.03 M)

<sup>d</sup>Measured by competitive spectrophotometric method in basic solution (NaOH 0.03 M)



**Fig. 5**  $^1\text{H}$  NMR (200 MHz) spectra of  $\gamma\text{-CD}$  ( $1 \times 10^{-2}$  M) and OA in different molar ratios: (A) 0.0, (B) 1.0, (C) 2.0 and (D) 4.0

for simplicity H1 is not shown in Fig. 5. H2 gives a characteristic doublet of doublet signal centered at 3.50 ppm and H4 gives a triplet at 3.42 ppm. Three hydrogens appear in the region from 3.7 to 3.9 ppm. H3 gives a triplet centered at 3.83 ppm (this triplet signal overlaps with the neighboring broad signal), while H6 and H5 give a broad signal at 3.78 ppm (although H6 should give a doublet of doublet and H5 should give a doublet of triplets).

Upon addition of OA and subsequent formation of the complex (Fig. 5) only two protons, namely H3 and H5, experiment significant ( $> 0.02$ ) chemical upfield shift

(Table 2). This change indicates that OA forms complexes with  $\gamma\text{-CD}$ .

The  $^1\text{H}$  NMR of  $\gamma\text{-CD}$  in the presence of NA was also investigated (Fig. 6). The H1 doublet signal that appears at 4.97 ppm is not shown for simplicity. In this complex, the chemical upfield shift observed for H3 and H5 was larger than the one observed for OA- $\gamma\text{-CD}$  complex. This observation indicates that NA is more deeply inserted into the  $\gamma\text{-CD}$  cavity (Table 3). In addition to this, there is line broadening and a significant ( $> 0.02$ ) upfield shift for the H2 that is located outside  $\gamma\text{-CD}$  cavity. This H2 in the complex actually overlaps with the H4 signal. This latter observation was unique for NA- $\gamma\text{-CD}$  complex indicating that H2 in particular experiences some changes upon complex formation. In this case, the carbonyl group (C=O) present in the quinolone forms a hydrogen bond with the peripheral  $\gamma\text{-CD}$  hydroxyl group (O-H) at C2 thus modifying H2 chemical shift. This hydrogen bonding interaction explains the larger binding constant (Table 1) observed for this complex. Similarly, in the complex of hydroxycinnamate with  $\beta\text{-CD}$  a chemical shift for H2 was also observed due to the formation of hydrogen bonding between C=O and O-H at C2 [29].

The proposed average structures of quinolone- $\gamma\text{-CD}$  complexes are given in Fig. 7. The position of a given quinolone in the CD cavity reflects a balance between repulsive and attractive intermolecular forces. The former would be due to the relative sizes of the host and the guest and the latter to hydrophobicity of aromatic structure in quinolone and hydrophilicity of a carboxylic COOH group. In both quinolones, it is expected that the highly hydrophilic COOH group would prefer to remain exposed to the bulk of the aqueous solution. A larger binding is observed for NA- $\gamma\text{-CD}$  complex (Table 1) in basic and acidic media. This must be due not only to a better fitting between quinolone and  $\gamma\text{-CD}$  which allows for deeper penetration in CD cavity, but also to the more favorable hydrophobic interactions. Furthermore, a hydrogen bond between C=O in NA and peripheral O-H in CD will enhance the value of the binding constant.

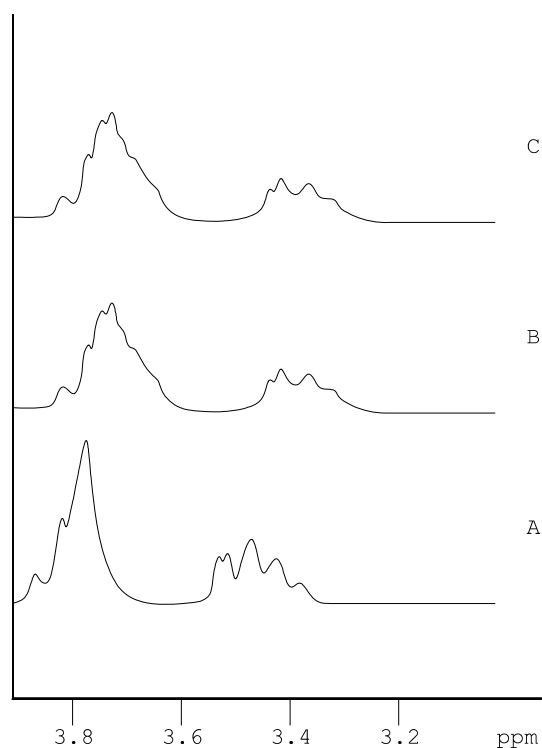
NMR spectroscopy is an adequate technique to study molecular complexes and their stereochemistry. Compared to other techniques, NMR offers the advantage of allowing the observation of both, the host and guest hydrogens to certify complex formation. In this context, the effect on the quinolone hydrogens environment upon formation of  $\gamma\text{-CD}$

**Table 2** Hydrogen chemical shift of  $\gamma\text{-CD}$  and OA- $\gamma\text{-CD}$  complex

	H1	H3	H6,6'	H5	H2	H4
$\gamma\text{-CD}^a$	4.97	3.83	3.78	3.78	3.50	3.42
OA- $\gamma\text{-CD}^b$	4.95	3.76	3.76	3.71	3.49	3.40
$\Delta\delta$ (ppm)	0.02	0.07	0.02	0.07	0.01	0.02

<sup>a</sup> $\gamma\text{-CD}$  (0.01 M) in  $\text{D}_2\text{O}$

<sup>b</sup> $\gamma\text{-CD}$  (0.01 M) and OA (0.04 M) in  $\text{D}_2\text{O}$



**Fig. 6**  $^1\text{H}$  NMR (200 MHz) spectra of  $\gamma\text{-CD}$  ( $1 \times 10^{-2}$  M) and NA in different molar ratios: (A) 0.0, (B) 2.0 and (C) 4.0

complex was also investigated (Table 4). In the case of OA, all the hydrogens experienced a small chemical upfield shift upon formation of the complex.

Due to an encapsulation of NA by  $\gamma\text{-CD}$ , the formation of a NA- $\gamma\text{-CD}$  complex will certainly have an effect on the chemical shift of the hydrogens on NA. Upon the addition of  $\gamma\text{-CD}$ , all the NA hydrogens experience a large ( $> 0.21$ ) upfield chemical shift as a consequence of a complex formation (Table 5). Thus, it is clear that all the NA hydrogens get inside the complex. From the size of chemical shifts, it is clear that a NA- $\gamma\text{-CD}$  complex is stronger than OA- $\gamma\text{-CD}$  complex. In the case of NA the hydrophobic methyl-pyridine structure accommodates inside the  $\gamma\text{-CD}$ . In fact, there have been previous reports on the formation of this type of complex with  $\alpha\text{-CD}$  and  $\beta\text{-CD}$  that contain a smaller cavity [23].

## Conclusions

$\gamma\text{-CD}$  forms inclusion complexes with oxolinic and nalidixic acid. Their binding constants at room temperature ( $25^\circ\text{C}$ ) under acidic and basic solution were calculated using Benesi–Hildebrand equation. Results from UV–Vis and previous spectrofluorometric studies are consistent with a simple 1:1 stoichiometry and the stability of the complexes is dependent on the structure of the quinolone. Experimentally, weaker binding constants are obtained for oxolinic acid- $\gamma\text{-CD}$  complexes ( $1616$  and  $1765\text{ M}^{-1}$ ) and larger binding constants are obtained for nalidixic acid- $\gamma\text{-CD}$

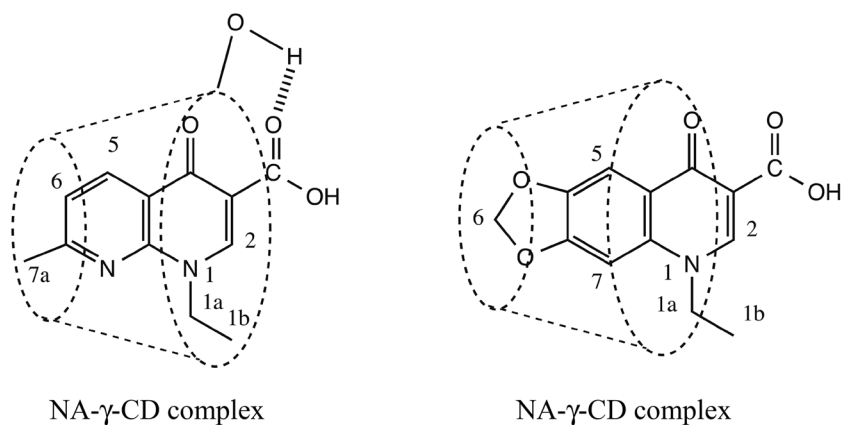
**Table 3** Hydrogen chemical shift of  $\gamma\text{-CD}$  and NA- $\gamma\text{-CD}$  complex

	H1	H3	H6,6'	H5	H2	H4
$\gamma\text{-CD}^{\text{a}}$	4.97	3.83	3.78	3.78	3.50	3.42
NA- $\gamma\text{-CD}^{\text{b}}$	4.96	3.75	3.75	3.50	3.75	3.35
$\Delta\delta$ (ppm)	0.01	0.08	0.03	0.28	0.25	0.07

<sup>a</sup> $\gamma\text{-CD}$  (0.01 M) in  $\text{D}_2\text{O}$

<sup>b</sup> $\gamma\text{-CD}$  (0.01 M) with NA (0.04 M) in  $\text{D}_2\text{O}$

**Fig. 7** Structure of quinolone- $\gamma\text{-CD}$  complexes





**Table 4** Hydrogen chemical shift for OA and OA- $\gamma$ -CD complex

	H1b	H1a	H6	H5	H7	H2
OA <sup>a</sup>	1.30 (t, 3H)	4.11 (q, 2H)	5.99 (s, 2H)	6.86 (s, 1H)	7.31 (s, 1H)	8.19 (s, 1H)
OA- $\gamma$ -CD <sup>b</sup>	1.27	3.99	5.93	6.80	7.21	8.15
$\Delta\delta$ (ppm)	0.04	0.02	0.06	0.06	0.10	0.04

<sup>a</sup>OA (0.1M) in D<sub>2</sub>O<sup>b</sup> $\gamma$ -CD (0.01M) and OA (0.04M) in D<sub>2</sub>O**Table 5** Hydrogen chemical shift for NA and NA- $\gamma$ -CD complex

	H1b	H7a	H1a	H6	H5	H2
NA <sup>a</sup>	1.40 (t, 3H)	2.64 (s, 3H)	4.48 (q, 2H)	7.36 (d, 1H)	8.46 (d, 1H)	8.52 (s, 1H)
NA- $\gamma$ -CD <sup>b</sup>	1.18	2.30	4.04	6.88	7.94	8.28
$\Delta\delta$ (ppm)	0.22	0.34	0.44	0.48	0.52	0.24

<sup>a</sup>NA (0.1M) in D<sub>2</sub>O<sup>b</sup> $\gamma$ -CD (0.01M) and NA (0.04M) in D<sub>2</sub>O

complexes (3760 and 3840 M<sup>-1</sup>). NMR studies were performed to elucidate complexes structures and indicated that nalidixic acid forms a stronger complex. These results could be explained in terms of several physicochemical properties of the NA- $\gamma$ -CD complex. The hydrophobic methylpyridine structure in NA accommodates better into the  $\gamma$ -CD cavity. In addition, a hydrogen bond is formed between the C=O of the quinolone and the peripheral O–H present at C2 in  $\gamma$ -CD.

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