

## Effect of Organic Solvents on the Inclusion of Sparfloxacin and Norfloxacin with $\beta$ -Cyclodextrin

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The complexation of sparfloxacin (SPR) and norfloxacin (NOR) by  $\beta$ -cyclodextrin ( $\beta$ -CD) in water and in mixed organic/aqueous media has been studied by absorption spectroscopy. Three organic solvents [dimethyl-sulphoxide, ethanol and 2-methyl-1-propanol have been adopted which differ in polarity and hydrophobicity. It has been shown that the presence of organic solvent causes the degradation of the 1 : 1 SPR :  $\beta$ -CD and NOR :  $\beta$ -CD complexes. The binding constants of the inclusion complexes of SPR and NOR with  $\beta$ -CD in water are greater than those with mixed organic-water media. The higher the hydrophobicity of the co-solvent, the weaker the complexation of SPR and NOR by  $\beta$ -CD.

**Key Words:** Sparfloxacin, Norfloxacin,  $\beta$ -Cyclodextrin, 2-Methyl-1-propanol.

### INTRODUCTION

$\beta$ -Cyclodextrin ( $\beta$ -CD), a cyclic oligosaccharide composed of seven glucose units linked together by  $\alpha$ -1,4-glucosidic bond, giving an overall shape of truncated cone, has been widely applied for the inclusion of a variety of organic guest molecules of appropriate size and hydrophobicity from the bulk aqueous solution within its cavity<sup>1-3</sup>.

Sparfloxacin (SPR), 5-amino-1-cyclopropyl-7-(*cis*-3,5-dimethyl-1-piperazinyl)-6,8-difluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid and norfloxacin (NOR), 1-ethyl-6-fluoro-4-oxo-7-(1-piperazinyl)-1,4-dihydroquinoline-3-carboxylic acid are synthetic fluoroquinolone, usually used as antimicrobial agent, practically insoluble in water<sup>4</sup>. The chemical structure of SPR and NOR is shown in Fig. 1.

An organic solvent, when added to an aqueous solution containing CD and aromatic molecules may interact with both hosts and guests. Since the aromatic

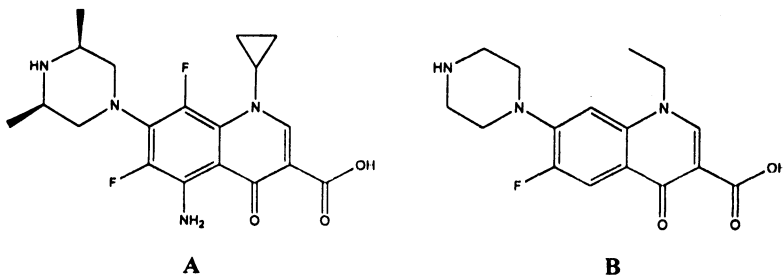


Fig. 1. Structures of sparfloxacin (A) and norfloxacin (B)

molecules are believed to be preferentially solvated by the organic component in a water-rich mixed solvent system<sup>5</sup>, the concurrent process to cyclodextrin complexation may occur, although the inclusion of the organic solvent into the CD cavity cannot be ruled out.

Water-rich mixed solvent systems are very often used in CD complexation studies as well as in many other applications. As an example, they are used as an eluent in high-performance liquid chromatography with a CD-bound column or a CD-containing mobile phase<sup>6</sup>; this method is also used for estimation of equilibrium constants of CD complexes.

Inclusion processes between cyclodextrins and any guest were done usually in aqueous media; addition of an organic solvent to the inclusion media is not desirable. Organic solvent in aqueous media behave as a molecular system rather than continuous media and are subjected to the same intermolecular forces as guest molecule in the inclusion process. The inclusion of organic solvents, such as alcohol, DMSO, with  $\alpha$  and  $\beta$ -cyclodextrins has been studied in literature<sup>7-9</sup>. It has been found that the complexation in non-aqueous and water/organic solvent mixtures is weaker than in pure aqueous solutions. This new environment leads to a competition between the guest and the organic solvent molecules toward inclusion with cyclodextrin; on this basis, it will affect the binding constant.

The aim of the present study is to systematically examine how the addition of an organic solvent to an aqueous solution containing the SPR and NOR:  $\beta$ -CD complexes influences the stability and stoichiometry of the CD complex. We have chosen for this study SPR and NOR as model guest compounds. These molecules have an aromatic moiety and water-soluble functional groups. Therefore, these seem ideal for CD complexation studies. When these compounds form a 1 : 1 complex with  $\beta$ -CD, the absorption spectrum in UV region shows a change in absorbance and in some cases a shift in the absorption band maximum ( $\lambda_{\max}$ ).

In the present studies three solvents (dimethyl-sulphoxide, DMSO; ethanol, EtOH; and 2-methyl-1-propanol, 2MP) that differ considerably in their properties such as polarity, acidity-basicity and hydrophobicity have been chosen.

## EXPERIMENTAL

Sparfloxacin and norfloxacin were a gift from Jordanian Pharmaceutical Manufacturing (JPM).  $\beta$ -Cyclodextrin (99%) was purchased from Lancaster Chemical Company and used without further purification. The solvents have been used as 10% (v/v) using doubly distilled water. 2-Methyl-1-propanol (Aldrich, 99.9%), ethanol (Koch-Light Laboratories Ltd., 99.7%) and dimethyl sulfoxide (DMSO) (MERK-Schuchardt, 99%) were used without further purification.

Absorption electronic spectra were recorded on a double-beam Unicam-Herios- $\alpha$ -spectrophotometer. The stock solutions of the drug were made using ultrasonic LC 20H-Elma, and 0.45  $\mu\text{m}$  filter syringes (Organic Scientific-Gyro Disc) have been used for filtration. The direct spectroscopy method was used for obtaining the binding constants.

### Determination of binding constant in aqueous and mixed organic/aqueous solutions

The binding constants were determined in terms of spectral absorption changes of SPR and NOR due to the addition of  $\beta$ -cyclodextrin. Stock solutions of drugs

were prepared by dissolving a suitable amount of sparfloxacin and norfloxacin, then diluted to 100 mL by doubly distilled water. The solution were sonicated for 3 h, and kept overnight; the solutions were filtered using 0.45  $\mu\text{m}$  cellulose acetate filter syringe, then sonicated for 1 h. Stock solution of  $\beta$ -cyclodextrin was prepared in 0.012 M. Then, equal volumes of drugs solutions were withdrawn into 100 mL volumetric flasks and mixed with different volumes of  $\beta$ -cyclodextrin and diluted to the mark with doubly distilled water. The solution has equal concentrations of the drugs (0.15 mM for sparfloxacin, 0.16 mM for norfloxacin). Sample measurements were made within 24 h of being prepared.

## RESULTS AND DISCUSSION

Inclusion of drugs with  $\beta$ -cyclodextrin results in altering and modification of its physical and chemical properties; there is generally a small shift in  $\lambda_{\text{max}}$  to a higher or lower value and an increase or decrease in intensity with slight broadening of the absorption band as the concentration of cyclodextrin increases<sup>10</sup>. Fig. 2 shows the absorption spectra of SPR in aqueous solutions as a function of  $\beta$ -cyclodextrin.

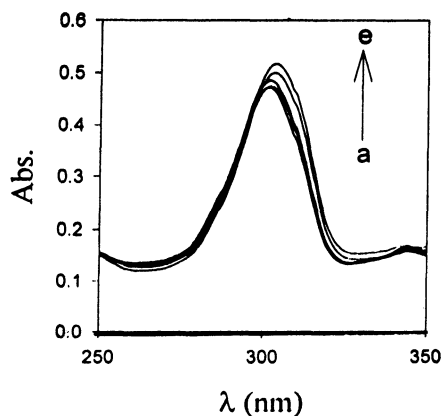


Fig. 2. Absorption spectra of SPR in aqueous solutions with several  $\beta$ -cyclodextrin's concentration at 25°C: (a) 0 M, (b)  $1 \times 10^{-3}$  M, (c)  $2.5 \times 10^{-3}$  M, (d)  $5 \times 10^{-3}$  M, (e)  $1 \times 10^{-2}$  M

For a long time it was believed that cyclodextrin inclusion complex formation occurred exclusively in aqueous solution. After some research, it was concluded that the complexation in nonaqueous and water-organic solvent mixtures is weaker than in pure aqueous solutions<sup>11</sup>; however, Nelson *et al.*<sup>12</sup> reported the opposite behaviour for pyrene complexes in water/*t*-butanol mixture. On this basis, one can conclude that the solvent plays a key role in the complexation process.

We consider the organic solvents influence on the inclusion complexes stability due to the great importance of the solvophobic effect on the inclusion phenomena. We have chosen DMSO, EtOH and 2MP due to their poor inclusion into the cyclodextrin cavities. They show small binding constants, as for example, for DMSO the binding constants are  $0.41 \text{ M}^{-1}$  with  $\alpha$ -CD<sup>7</sup> and  $1.8 \text{ M}^{-1}$  with  $\beta$ -CD<sup>8</sup>, for EtOH the binding constant is  $0.93 \text{ M}^{-1}$  with  $\beta$ -CD<sup>9</sup>. Therefore, the presence of these solvents at high concentrations is competing with SPR and NOR in the reaction of complexation with  $\beta$ -CD. On the other hand, the presence of these solvents in the

medium changes the polarity of the media diminishing the tendency of SPR and NOR to be included inside the cyclodextrin cavity. Thus, a percentage of organic solvents of 10% in the medium were selected. Besides, it allows us to maintain the cyclodextrin concentration used in aqueous solutions.

The addition of organic solvents to an aqueous solution containing the SPR :  $\beta$ -CD and NOR :  $\beta$ -CD complexes scarcely affects the absorption spectra maxima but dramatically alters the intensities. For both complexes, SPR :  $\beta$ -CD and NOR :  $\beta$ -CD, the presence of EtOH causes a shift in  $\lambda_{\max}$  to a higher value and an increase in intensity of the absorption band as the concentration of  $\beta$ -CD increases. The absorption spectra of SPR in EtOH/water mixture are shown in Fig. 3.

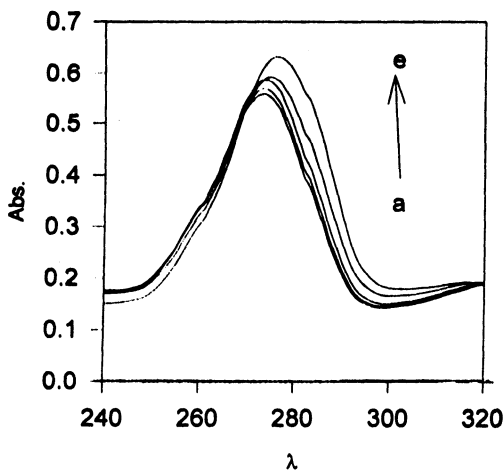


Fig. 3. Absorption spectra of NOR in ethanol/water mixture (10%) with several  $\beta$ -cyclodextrin's concentrations at 25°C: (a) 0 M, (b)  $7.7 \times 10^{-4}$  M, (c)  $1.7 \times 10^{-3}$  M, (d)  $2.1 \times 10^{-3}$  M, (e)  $2.7 \times 10^{-3}$  M

In case of the NOR :  $\beta$ -CD complexes, the presence of DMSO and 2MP causes an increase in intensity of the absorption band and no shift in  $\lambda_{\max}$  as the concentration of  $\beta$ -CD increases. In contrast, in the case of the SPR :  $\beta$ -CD complexes, the presence of DMSO and 2MP causes an increase in intensity of the absorption band and a red shift in  $\lambda_{\max}$  as the concentration of  $\beta$ -CD increases, the absorption spectra of SPR :  $\beta$ -CD complexes in 10% 2MP is shown in Fig. 4.

The calculation of binding constants has been made using the spectrophotometric method. The absorption intensity measurements were determined, keeping the concentration of SPR and NOR at the same constant values of 0.15 mM and 0.16 mM, respectively, while the concentrations of the  $\beta$ -CD varied from  $1 \times 10^{-4}$  to  $1 \times 10^{-2}$  M. Each measurement is an average of two experimental values. The binding constants were determined from the absorption data assuming a 1 : 1 stoichiometry, by using the modified Benesi-Heldbrand equation<sup>13</sup>:

$$\frac{1}{A - A_0} = \frac{1}{[G]_0 K_{11} \Delta \epsilon_{11} [CD]} + \frac{1}{[G]_0 \Delta \epsilon_{11}} \quad (1)$$

where A and  $A_0$  are the absorption intensities (*i.e.*, absorbance) in the presence and in absence of  $\beta$ -cyclodextrin, respectively;  $[G]_0$  is the initial concentration of

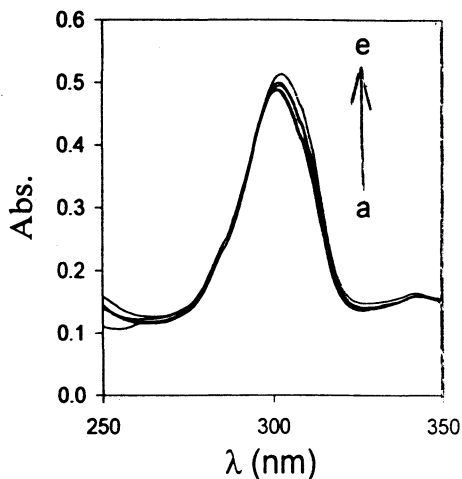


Fig. 4. Absorption spectra of SPR :  $\beta$ -CD complexes in 10% 2MP with several  $\beta$ -cyclodextrin's concentration at 25°C: (a) 0 M, (b)  $1 \times 10^{-3}$  M, (c)  $2.5 \times 10^{-3}$  M, (d)  $5.5 \times 10^{-3}$  M, (e)  $1 \times 10^{-2}$  M

drugs (SPR and NOR);  $\Delta\epsilon_{11}$  is the change in molar absorptivity, which is given by a specific equation<sup>13</sup>;  $K_{11}$  is the binding constant, which was calculated from the intercept and the slope of the straight line obtained by plotting  $1/(A - A_0)$  vs.  $1/[\text{CD}]$ .

The plots of  $1/(A - A_0)$  vs.  $1/[\text{CD}]$  for SPR and NOR with all types of solvents are linear, according to Eq. (1). Fig. 5 shows this type of plot for NOR :  $\beta$ -CD complex in 10% EtOH. The linearity of the plots confirms the fact that the stoichiometry of the complex (SPR :  $\beta$ -CD and NOR :  $\beta$ -CD) is 1 : 1. The binding constant has been calculated from the intercept and the slope of the straight lines according to Eq. (1).

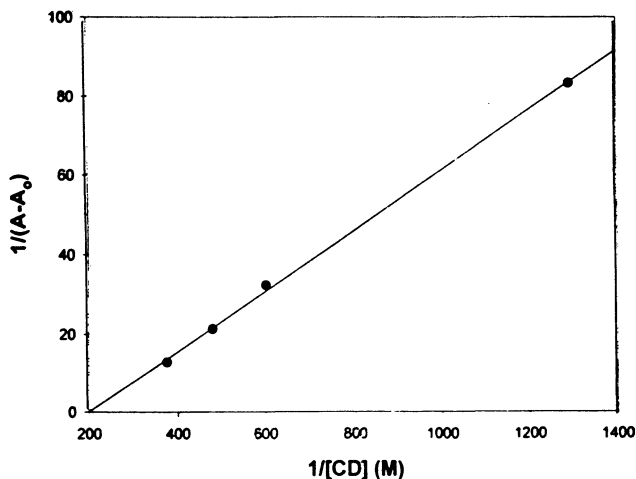


Fig. 5. Typical plot of  $1/(A - A_0)$  vs.  $1/[\text{CD}]$  of NOR :  $\beta$ -CD complex in 10% EtOH

The binding constants calculated by the spectrophotometric method described above are summarized in Table-1. It is clearly visible that the binding constants of both SPR :  $\beta$ -CD and NOR :  $\beta$ -CD complexes are decreased as the hydrophobicity of the co-solvent increases, which is more pronounced in the following order:  $K_{11}$  (10% 2MP) <  $K_{11}$  (10% DMSO) <  $K_{11}$  (10% Ethanol) <  $K_{11}$  (aqueous solution).

TABLE-1  
THE BINDING CONSTANTS OF SPARFLOXACIN AND  
NORFLOXACIN WITH  $\beta$ -CYCLODEXTRIN

Solvent	$10^{-2} K_{11}(\text{SPR}), \text{M}^{-1}$	$10^{-2} K_{11}(\text{NOR}), \text{M}^{-1}$
Aqueous solution	17.90 $\pm$ 0.10	19.28 $\pm$ 0.10
10% Ethanol	5.80 $\pm$ 0.08	1.48 $\pm$ 0.08
10% DMSO	5.45 $\pm$ 0.08	1.20 $\pm$ 0.08
10% 2MP	5.19 $\pm$ 0.08	1.00 $\pm$ 0.08

### Conclusion

It has been shown that the organic solvents influence the inclusion process by competing the guest molecule to form an inclusion complexation with  $\beta$ -cyclodextrin. Thus, the binding constants of the sparfloxacin :  $\beta$ -CD and norfloxacin :  $\beta$ -CD complexes in mixed organic/aqueous media are less than those obtained in aqueous media. The higher the hydrophobicity of the co-solvent, the weaker the complexation of SPR and NOR by  $\beta$ -CD. These results show how the medium composition may affect the stability of cyclodextrin complexes, but it has no effect on the stoichiometry.

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