

Investigation of Enhancement of Solubility of Norfloxacin β -Cyclodextrin in Presence of Acidic Solubilizing Additives

Kamal Dua*, M.V. Ramana[#], U.V. Singh Sara, M. Himaja[#], Abhinav Agrawal, Vaibhav Garg and Kavita Pabreja

*D.J. College of Pharmacy, Niwari Road, Modinagar-201204, Uttar Pradesh, India; [#]N. G. S. M. Institute of Pharmaceutical Sciences, Mangalore-575005, Karnataka, India

Abstract: The present study is aimed at improving the solubility of a poorly water-soluble drug, norfloxacin by incorporating solubilizing additives such as ascorbic acid and citric acid into the β -cyclodextrin complexes. Norfloxacin, being amphoteric in nature, exhibits a higher solubility at pH below 4 and above 8. Addition of substances like ascorbic acid and citric acid in β -cyclodextrin complexes reduces the pH of the immediate microenvironment of the drug below pH 4. In the present work, β -cyclodextrin complexes of norfloxacin were prepared along with solubilizing additives such as citric acid and ascorbic acid in various proportion and the dissolution profile was performed in both HCl buffer, pH 1.2 and phosphate buffer, pH 7.4. The results have shown an enhanced dissolution rate in both media. DSC and IR spectral studies performed on the solid complexes have shown that there is no interaction of the drug with the additives and β -cyclodextrin. Disc diffusion studies have shown larger diameters of zone of inhibition indicating a greater diffusivity of the drug into the agar medium.

Keywords: Norfloxacin, dissolution rate, β -cyclodextrin, solubility, citric acid, ascorbic acid.

INTRODUCTION

Cyclodextrins and their derivatives play an important role in formulation development due to their effect on solubility, dissolution rate, chemical stability and absorption of drugs. Though cyclodextrins have been investigated widely during the last two decades, their commercial application in pharmaceutical formulation was started only in recent years with drugs such as piroxicam and nimesulide [1-4].

Norfloxacin is a fluoroquinolone, which has bactericidal activity. It is active on both actively dividing as well as dormant bacteria by inhibiting bacterial DNA gyrase. Norfloxacin is approved for urinary and genital tract infection. It is also good for bacterial diarrhoea and gonorrhoea [5]. Norfloxacin is very slightly soluble in water [6] and hence only 35-45% of orally administered drug is absorbed [5]. In order to enhance the extent of absorption, it is necessary to improve its solubility.

The objective of the present study was to investigate the possibility of improving the solubility and dissolution rate of norfloxacin in presence of solubilizing agents such as ascorbic acid and citric acid, which are incorporated into β -cyclodextrin complex (β -CD). β -CD was used for the study, as it has bigger cavity size of (7.5 Å) and it is the least toxic among all the other natural cyclodextrins [7].

MATERIALS AND METHODS

Norfloxacin (Pfiscar India Ltd., Murthal, Haryana), β -cyclodextrin (Cerestar, USA Inc.Hammond Indiana) of commercial purity grade were used. All other chemicals used were of analytical reagent grade.

*Address correspondence to this author at the D.J. College of Pharmacy, Niwari Road, Modinagar-201204, Uttar Pradesh, India; Tel: +91-9897814836; +91-1232325056; E-mail: kamalpharmacist@rediffmail.com

Phase Solubility Studies

The phase solubility studies were carried out according to the method reported by Higuchi and Connors [8]. Excess amount of Norfloxacin was added to the aqueous solution of β -CD at various concentrations (0.002-0.02M) and was shaken for 24 hrs at room temperature on rotary flask shaker. After equilibrium, the solutions were filtered and the portions of the solutions were analyzed for the drug content using JascoV-530 UV spectrophotometer at 277.6 nm.

Preparation of Solid Complexes

The solid complexes of norfloxacin and β -cyclodextrin were prepared in 1:1 and 1:2 molar ratios and also with various solubilizing additives such as citric acid or ascorbic acid in 1:1:0.5 molar ratio using kneading method [9] and solvent evaporation method.

In kneading method, accurately weighed quantity of β -cyclodextrin was mixed with sufficient quantity water to obtain a smooth and homogeneous paste. Weighed quantity of norfloxacin along with various solubilizing additives (Table 1) was added slowly by grinding. The mixture was ground for one hour. During this process appropriate quantity of water was added to maintain suitable consistency. Finally the paste was dried in oven at 40°C for 48 hours.

In solvent evaporation technique, Norfloxacin along with various solubilizing additives was dissolved in acetone at 25°C temperature to which the required moles of β -CD in hot distilled water were added drop wise with continuous stirring for 1 hr. Complexes formed were filtered and dried under vacuum.

The prepared solid mass was stored in dessicator and dried under vacuum to a constant weight. The dried products

Table 1. Quantity of Substances taken for Preparation of β -CD Complexes

Molar ratio	Norfloxacin (g)	β -CD (g)	Solubility enhancing additive (g)
1:1	1	3.55	–
1:2	1	7.10	–
1:1:0.5	1	3.55	0.60 (citric acid).
1:1:0.5	1	3.55	0.27(ascorbic acid).

Table 2. Physical Characteristics of the β -CD-Norfloxacin Complexes Prepared by Kneading Method

Composition	Ratio	Drug content (Theoretical value) in $\mu\text{g/ml}$	Average particle size (μm)	% Moisture absorption	
				70 \pm 5% RH, 30 \pm 2 $^{\circ}$ C	99 \pm 1%RH, 30 $^{\circ}$ C \pm 2 $^{\circ}$ C
NF : β -CD	1:1M	9.74 \pm 0.28 (10)	64.20	3.0	10.
	1:2M	9.80 \pm 0.37 (10)	58.32	1.0	4.2
NF : β -CD: AA	1:1:0.5M	9.97 \pm 0.21 (10)	55.20	6.3	13.5
NF: β -CD: CA	1:1:0.5M	9.53 \pm 0.43 (10)	59.21	2.2	5.2

Values given in the parenthesis indicate theoretical values.

NF = norfloxacin, β -CD = β -cyclodextrin, AA= ascorbic acid, CA = citric acid, M = Molar

Table 3. Physical Characteristics of the β -CD-Norfloxacin Complexes Prepared by Solvent Evaporation Method

Composition	Ratio	Drug content (Theoretical value) in $\mu\text{g/ml}$	Average particle size (μm)	% Moisture absorption	
				70 \pm 5% RH, 30 \pm 2 $^{\circ}$ C	99 \pm 1%RH, 30 $^{\circ}$ C \pm 2 $^{\circ}$ C
NF: β -CD	1:1M	9.37 \pm 0.18 (10)	61.21	3.3	10.9
	1:2M	9.37 \pm 0.42 (10)	62.67	0.8	3.9
NF: β -CD: AA	1:1:0.5M	9.53 \pm 0.14 (10)	56.34	6.7	12.9
NF: β -CD: CA	1:1:0.5M	9.75 \pm 0.40 (10)	55.11	2.7	5.8

Values given in the parenthesis indicate theoretical values.

NF = norfloxacin, β -CD = β -cyclodextrin, AA= ascorbic acid, CA = citric acid, M = Molar

Table 4. Dissolution of β -CD-Norfloxacin Complexes Prepared by Kneading Method

Composition	Ratio	% Release at 120 min.			
		0.1N HCl buffer pH 1.2		Phosphate buffer pH 7.4	
Norfloxacin	1:0	32.4		25.2	
		Physical mixture	β -CD complex	Physical mixture	β -CD complex
NF: β -CD	1:1M	33.6	40.5	27.3	32.4
	1:2M	38.7	50.4	30.1	43.2
NF: β -CD: AA	1:1:0.5M	45.4	74.3	47.9	67.5
NF: β -CD: CA	1:1:0.5M	39.3	72.1	41.4	63.9

NF = norfloxacin, β -CD = β -cyclodextrin, AA= ascorbic acid, CA = citric acid, M = Molar

Table 5. Dissolution of β -CD-Norfloxacin Complexes Prepared by Solvent Evaporation Method

Composition	Ratio	% Release at 120 min.			
		0.1N HCl buffer pH 1.2		Phosphate buffer pH 7.4	
Norfloxacin	1:0	32.4		25.2	
		Physical mixture	β -CD complex	Physical mixture	β -CD complex
NF: β -CD	1:1M	34.7	42.1	30.1	35.4
	1:2M	40.2	56.3	33.3	47.4
NF: β -CD: AA	1:1:0.5M	47.5	79.2	50.2	69.9
NF: β -CD: CA	1:1:0.5M	43.3	73.3	45.4	66.4

NF = norfloxacin, β -CD = β -cyclodextrin, AA = ascorbic acid, CA = citric acid, M = Molar

Table 6. Antimicrobial Activity of the β -CD-Norfloxacin Complexes Prepared by Solvent Evaporation Method

Formulation↓	Inhibition Zone Diameter (mm) \pm S.D.*			
	Bacterial culture→ <i>B. subtilis</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>
NF	24.63 \pm 0.71	22.43 \pm 0.39	24.31 \pm 0.85	16.02 \pm 0.13
NF: β -CD	27.72 \pm 0.91	24.37 \pm 0.77	26.21 \pm 0.92	19.55 \pm 0.99
NF: β -CD: AA	39.32 \pm 1.5	33.33 \pm 0.57	31.17 \pm 0.76	21.39 \pm 0.83
NF: β -CD: CA	31.73 \pm 0.32	29.21 \pm 1.3	28.31 \pm 0.99	22.17 \pm 0.97

*Average of three determinations.

were removed, pulverized and passed through sieve no. 100 and finally stored in closed airtight container.

Preparation of Physical Mixtures

The physical mixtures were prepared by mixing mesh. No 100-sieve fractions of norfloxacin and β -cyclodextrin along with citric acid or ascorbic acid in the same proportions that were used in solid complexes.

Characterization

% Yield and Drug Content

Norfloxacin: β -cyclodextrin complexes in different molar ratios were prepared under similar set of conditions. The percentage yield and drug content were estimated. The results were subjected to statistical analysis (Standard deviation and Variance) to assess the reproducibility and uniformity of drug content in the β -cyclodextrin complexes.

Content of norfloxacin in β -cyclodextrin complexes was estimated by UV spectrophotometric method using JascoV-530 UV spectrophotometer at 277.6nm. β -cyclodextrin complexes equivalent to 10mg of pure drug was accurately weighed and dissolved in 100ml of 1% v/v acetic acid, from that 1ml was diluted to 10ml and absorbance was measured. Drug content was determined from the regression equation generated from the standard plot for norfloxacin.

Particle Size Analysis

Norfloxacin- β -cyclodextrin complexes were evaluated for particle size distribution and average particle diameter using microscopic method.

Hygroscopic Studies

β CD-norfloxacin complexes were dried in a dessicator under anhydrous CaCl_2 for two days. 100mg each of the dried samples were exposed to ambient atmospheric conditions (70 \pm 5% RH, 30 \pm 2 $^\circ\text{C}$) and accelerated humidity condition (99 \pm 1%RH, 30 $^\circ\text{C}$ \pm 2 $^\circ\text{C}$) for 2 days. The gain in their weight was determined and the percentage moisture absorbed was calculated [10].

Compatibility Studies

Compatibility of the norfloxacin with β -cyclodextrin and the solubilizing additives was confirmed by comparing the IR spectra and DSC thermograms taken for the drug, β -cyclodextrin complexes with solubilizing agents.

In Vitro Dissolution Study

Dissolution studies were performed separately in 900ml HCl buffer (pH 1.2) and phosphate buffer (pH 7.4), maintained at 37 $^\circ\pm$ 0.5 $^\circ\text{C}$ using USP XXII type 2 dissolution rate test apparatus at a stirring speed of 50 rpm. Samples equivalent to 100mg of norfloxacin were taken for dissolution

studies. 5 ml aliquot was withdrawn at different time intervals up to 2 hours, filtered and replaced with same volume of fresh dissolution medium. The samples were estimated for amount of norfloxacin dissolved by measuring the absorbance at 277.6nm. The dissolution experiments were done in triplicate.

Microbiological Studies

Anti-microbial activity of β CD complexes of norfloxacin was compared with norfloxacin pure drug against various strains of aerobic and anaerobic bacteria by cup-plate method. Muller-Hinton agar medium was used and the bacterial cultures were incubated at $37 \pm 0.2^\circ\text{C}$ for 24 hrs. Inhibition zone diameters were measured with the help of zone reader.

RESULTS AND DISCUSSION

Phase solubility studies of Norfloxacin β -CD systems in water at 25°C revealed that the solubility of Norfloxacin increased linearly with the increase in the concentration of β -CD, showing a typical A_L -type phase solubility curve. This curve may be ascribed to the formation of a stoichiometric 1:1 complex of norfloxacin and β -CD. The apparent 1:1 stability constant (K_C) was calculated from the straight line of the phase solubility diagram by using the following equation.

$$K_C = \text{slope/intercept (1-slope)}$$

The constant value was found to be 22.39M^{-1} .

Solid inclusion complexes of norfloxacin were prepared by kneading method and solvent evaporation method in two molar ratios (1:1M and 1:2M) along with addition of solubilizing additives such as citric acid or ascorbic acid (1:1:0.5M). The presence of solubilizing additives appears to modify the dissolution behaviour of the drug by altering its surrounding environment.

Low values of standard deviation in drug content of β -cyclodextrin complexes in respect of drug content indicated uniform drug distribution in all the complexes.

The particle size of the β -cyclodextrin complexes ranged from 3.27μ - 163.5μ and the average diameter was found to be in the range of 55.2μ - 64.2μ . Smaller particle size of the complexes is also responsible for the enhanced solubility of the drug.

The hygroscopic studies revealed that the maximum moisture absorption was observed in complexes containing ascorbic acid as a solubilizing additive where as complexes prepared with citric acid are less hygroscopic.

DSC thermograms of norfloxacin β -cyclodextrin complexes containing solubilizing agents showed peaks at 221°C , 260°C , 190°C and 153°C which indicate the melting point of norfloxacin, β -cyclodextrin, ascorbic acid and citric acid respectively. Absence of additional peaks indicated that there was no interaction between the drug and carriers (Tables 2 and 3).

IR spectra for the drug and complexes have shown no additional peaks, which also gave evidence that there is no interaction between drug and carriers.

In vitro dissolution studies exhibited that the solubility of norfloxacin was found better in acidic media. It is also ob-

served that the β -cyclodextrin complexes exhibited higher dissolution rate than the pure drug and their corresponding physical mixtures but physical mixtures also exhibited higher dissolution rate than pure drug.

Increase in dissolution from physical mixtures and solid complexes is exhibited due to the surface tension lowering effect of the β -cyclodextrin, resulting in wetting of hydrophobic drug surface. The increase in dissolution rate is also due to the formation of water-soluble inclusion complexes with the β -cyclodextrin. Dissolution rate of the β CD norfloxacin inclusion complexes containing ascorbic acid in 1:1:0.5 M ratio was found to be superior than all other formulations in both the dissolution media and the solvent evaporation technique was found to be more efficient in enhancing the drug dissolution in comparison to kneading method for preparing the β -CD complexes of Norfloxacin Tables (4 and 5).

As the drug is amphoteric in nature, it exhibits enhanced solubility at pH below 4 and above 8 [11,12]. In β -cyclodextrin inclusion complex addition of ascorbic acid or citric acid as solubilizing agent, reduces the pH of immediate microenvironment of the drug, probably pH below 4 and enhance the solubility of norfloxacin. Addition of ascorbic acid enhanced the dissolution rate to greater extent than citric acid. The mechanism that could be suggested is the complex formation. Two molecules of norfloxacin might have aligned themselves with a molecule of ascorbic acid in such a way that the orientation of the electron donor/acceptor groups becomes ideal to allow charge interaction (Fig. 1). In charge transfer complex, one molecule polarizes the other by virtue of the group or atoms it has. The polar hydroxyl groups of ascorbic acid induce a dipole in the readily polarizable quinolone molecule. The shape of such a complex may presumably be the sandwich type with the molecule of ascorbic acid being sandwiched between the two molecules of norfloxacin. This particular complex may fit into β -cyclodextrin cavity, which improves its further dissolution. The possibility of hydrogen bonding and effect of solvation energy may also play a significant role and could be a vital part of this hypothesis.

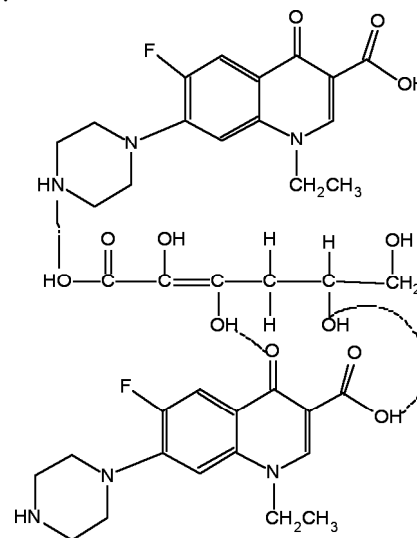


Fig. (1). Probable spatial arrangement of norfloxacin ascorbic acid complex: Depicting the points of attachment between ascorbic acid and norfloxacin molecules.

The results obtained for the *in vitro* antibacterial activity showed that the prepared inclusion β -CD complexes of Norfloxacin have better antibacterial activity as compared to pure drug, Norfloxacin (Table 6). The enhanced activity of complexes is due to its increase in water soluble nature and because of which they diffuses to a greater extent in the aqueous agar medium and hence the diameter of zone of inhibition is greater for complexes than the pure drug, Norfloxacin. Moreover the complexes containing ascorbic acid as a solubilizing additive have shown maximum zone of inhibition, which is in good correlation with the results obtained for *in vitro* dissolution studies.

CONCLUSION

The above studies conclude that complexation of norfloxacin with β -cyclodextrin can enhance the dissolution rate of the norfloxacin. Dissolution rate of norfloxacin can be enhanced further by incorporating acidic substances like ascorbic acid and citric acid into the formulations and thereby improve its bioavailability and have the potential to produce a faster onset of action.

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