



Molecular Inclusion of Sparfloxacin with Hydroxypropyl Beta Cyclodextrin

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The formation of an inclusion complex between hydroxypropyl- β -cyclodextrin and sparfloxacin is described. The 1:1 stoichiometry of the components is suggested by phase solubility studies and Benesi-Hildebrand plot showing the regression $y=0.0087x+32.273$ with the coefficient of correlation 0.7706 at slit width 1 nm. Stability constant evaluated by phase solubility analysis and Benesi-Hildebrand plot showed deviations. Thermodynamic parameters for the inclusion complex have been also estimated. The formation of the complex is detected by differential scanning calorimetry, potentiometry, UV, FTIR and fluorescence spectral methods. The complex displays enhanced aqueous solubility and dissolution.

Cyclodextrins (CDs) have been the subject of extensive, contemporary studies¹. The many practical applications of CDs include their ability to form inclusion complexes with pharmaceuticals with improved water-solubility in comparison to the drug itself². CDs are cyclic, non-reducing, hydrophilic oligosaccharides composed of 6-8 glucopyranose units joined by α -1,4-linkages³. The poor physicochemical properties of natural CDs have so far restricted their utility as an agent to improve solubilization. However, the availability of modified CDs has helped to overcome these lacunae⁴. Hydroxypropyl- β -cyclodextrin (HPCD) has gained wide acceptance in drug formulation by virtue of its ready water solubility, significant potential for solubilization and good safety profile⁵. The usual methods for preparation of such complexes of CDs with drug molecules include solvent evaporation⁶, lyophilization⁷, coprecipitation, slurry, paste or even dry mixing⁸. Characterization of the complexes has been achieved through techniques such as phase solubility⁹ and differential scanning calorimetry (DSC)¹⁰. A detailed review of all these methods is available¹¹.

In this study, sparfloxacin (SP) has been taken up for a study of its complexation behavior with HPCD. SP is among

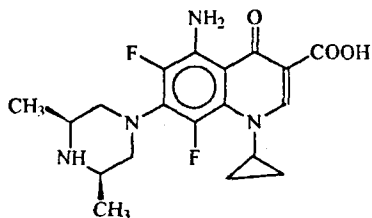
the frequently prescribed quinolone antibacterial used for the treatment of pneumonia, chronic bronchitis, acute sinusitis and lower respiratory tract infections. However, it possesses low aqueous solubility (less than 0.04 g/1 at pH 1 and less than 0.2 g/1 at pH 7.4)¹².

An interesting structural feature of SP is the presence of two polar groups, a piperazine ring at one end and carboxylic acid function at other. We have examined the inclusion complex formation of SP with HPCD (fig. 1). Here we were also interested to check the orientation of the two polar groups of the drug molecule in relation to the cavity of HPCD on complexation. To the best of our knowledge, study of such orientational behavior of drug molecule with more than one polar function has not been carried out so far. The present article describes our effort of solubilization of SP by formation of the inclusion complex with HPCD.

MATERIALS AND METHODS

The sample of HPCD used in this study was generously provided by Cerestar Inc., USA. SP was a gift sample from Blue-Cross Laboratories, Mumbai. It was recrystallized twice from methanol and its purity checked by HPLC. All chemicals and solvents used in this study were of analytical reagent grade. Freshly distilled water was used throughout the work.

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(cis)-5-Amino-1-cyclopropyl-7-(3,5-dimethyl-1-piperazinyl)-6,8-difluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid.

Fig. 1: Chemical structure of sparfloxacin.

Phase solubility studies:

SP, in quantity exceeding its solubility, was added to screw capped vials containing 5 ml aliquot of increasing concentration of HPCD in water (0.0 to 0.01 mol). Four sets of mixtures were prepared and the vials were agitated at 25, 40, 50, 60° for 72 h in a temperature-controlled bath to attain equilibrium. The supernatants were spectrophotometrically analyzed with a Shimadzu double beam double monochromator-250 1 PC instrument at 289.5 nm (slit width, 1 nm). The stability constants (K_c) of complex at different temperatures were determined¹³. The effect of added ethanol and dimethylformamide (DMF) on apparent K_c of the complex was also studied. The experiment was conducted as described above at 25° by using of mixtures of these solvents with water (20:80).

Benesi-Hildebrand plot:²⁰

One milliliter of SP solution containing 7.6×10^{-5} mol was added to aqueous solutions of HPCD of graded concentrations (8.0×10^{-4} mol to 1.52×10^{-2} mol). The solutions were agitated at 200 rpm for 8 h on a rotary shaker. The mixtures thus equilibrated at 25° were scanned for their absorbance at 289.5 nm and the stability constant (K_c) was computed as shown in Benesi-Hildebrand (B-H) Plot (fig. 2).

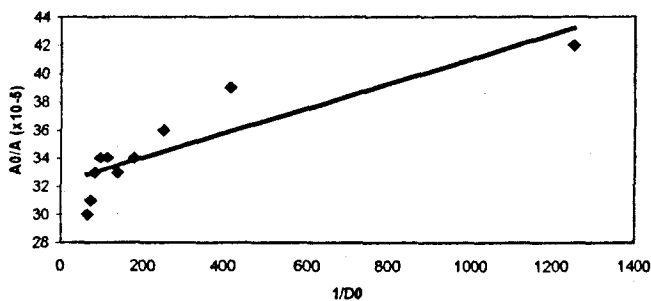


Fig. 2: Benesi-Hildebrand plot of SP-HP.

Preparation of inclusion complex and drug content determination:

The complex of SP with HPCD (SP-HP) was prepared by solution method¹¹. A solution of SP (0.40 g) in methanol was gradually added to equimolar quantity of HPCD (1.6 g) in water and agitated at 50° for 30 min and towards the end of this period, the solvent was stripped under vacuum at 70°. From the clear aqueous solution of the complex, water was distilled off under reduced pressure. The moist solid obtained was kept in an air oven for removal of last traces of solvent. A pale yellow solid was obtained.

Drug content of complex was determined by reverse phase HPLC method using an ICI isocratic HPLC instrument. The analysis was carried out at 20 μ l injection volume and 1 ml/min flow rate using a Nucleosil C18 column (4 mm ID X 25 cm). The UV detector was set at 289.5 nm and the mobile phase used was phosphate buffer (pH 2.4):acetonitrile (65:35). The method was validated. The solution was injected and the result compared with a blank.

Characterization of complex; spectral studies:

Formation of SP-HP was also studied using UV spectral shift method. UV spectra of SP (2.5×10^{-5} mol) and SP (2.5×10^{-5} mol) in presence of HPCD (1.5×10^{-2} mol) were recorded in the region of 200-600 nm. The Fluorescence spectra of SP and SP-HP were recorded using a Hitachi F-2500 FL Spectrophotometer. For SP the excitation wavelength was 250 nm and the emission spectrum was scanned in the range 300-800 nm. For SP-HP, the excitation wavelength was 226.0 nm and the emission in the range 300-800 nm. Both samples were scanned in the range of 300-800 nm. The FTIR spectrum of moisture free SP, HPCD and SP-HP were recorded using KBr pellets on Jasco FTIR Spectrophotometer in range of 4000-400 cm^{-1} .

Thermal studies:

Differential Scanning Calorimetry (DSC) of SP and SP-HP complex was carried out using a Mettler DSC 25 thermal analyzer with a TA 11 processor. The rate of heating was set to 10°/min. The thermograms were integrated by using Graphware® 4.01.

Physical studies:

Ionization constants of SP and SP-HP were separately determined by potentiometric titrations using Mettler DL-21 Autotitrator equipped with DG-111 SC combined glass electrode. The burette volume was set to 20 ml to gather all data

TABLE 1: THERMODYNAMIC PARAMETERS OF SP-HPCD COMPLEX.

Temperature (°)	Log Kc	ΔH° (KJ/mol)	ΔG° (KJ/mol)	ΔS° (J/mol.deg)
25	2.3957		-13.6234	-89.6601
40	2.2806		-13.6671	-85.2163
50	2.2301		-13.7918	-82.9641
60	2.1409	-13.0056	-13.6500	-80.0470

Where Kc is the reaction rate constant, ΔH , change in enthalpy, ΔG , change in Gibb's free energy and ΔS , change in entropy.

points at different volumes of added titrant. The predisensing volume was 0.2 ml and drift was kept at 0.25 mV/s for 3 s. Stirring was continued throughout the titration. Aqueous sodium hydroxide (0.0092 N) and aqueous HCl (0.0117 N) were used as titrants and pH at half neutralization was considered as pKa of sample.

Single surface dissolution studies¹⁴ of SP and SP-HP were performed using Disotest™ USP type II dissolution apparatus. Fifty milligrams of SP was taken for dissolution studies and dissolution medium used was 0.1 M HCl. Pellets of SP and SP-HP were prepared on a hydraulic press at 10-ton pressure. The pellets (average area, 1.35 cm²) were mounted on paddles of the apparatus and subjected to single surface dissolution in 900 ml, 0.1 M HCl as dissolution medium at 50 rpm (37±0.5°). Sampling and replacement (10 ml) were carried out initially at interval of 5 min up to 20 min and then at interval of 10 min up to 90 min for SP. For SP-HP, sampling and replacement (1 ml) were carried out at 1-min intervals and up to 20 min. The reason of this discrimination in sampling time of SP and SP-HP is higher water solubility of complex. The filtered samples were analyzed spectrophotometrically for SP content.

RESULTS AND DISCUSSION

Increasing amounts of HPCD increased the amount of SP going into water, improving aqueous solubility of SP. The PSA diagram (fig. 3) was found to be A_L type. This type of diagram suggests 1:1 stoichiometry of the inclusion complex¹⁵. Slopes and intercepts were determined by performing linear regression analysis of data. From above figure the regression equations for calculation of Kc at various temperatures were found to be $y(25^\circ)=0.0657x+0.0003$, $R^2=0.993$, $y(40^\circ)=0.0726x+0.0004$, $R^2=0.981$, $y(50^\circ)=0.0924x+0.0006$, $R^2=0.98$, $y(60^\circ)=0.0933x+0.0007$, $R^2=0.994$. Stability constants at different temperatures were computed using the relationship.

The values of Kc were found to be 248.76, 190.81,

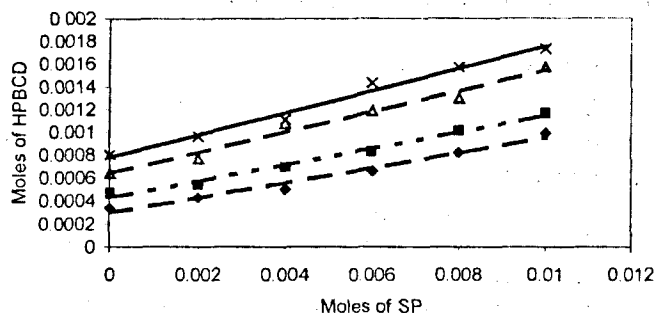


Fig. 3: Phase solubility diagram at various temperatures. Phase solubility diagram of sparfloxacin at temperatures 25° (■), 40° (◆), 50° (Δ) and 60° (x).

169.86 and 138.34 mol⁻¹ at 25, 40, 50 and 60°, respectively. The complex stability decreases with increase of temperature. Similar observations have been reported concerning the stability of inclusion complexes of HPCD with several other drug molecules^{16,17}. Van't Hoff plot was constructed to estimate thermodynamic parameters of SP-HP (Table 1).

TABLE 2: EFFECT OF SOLVENTS ON Kc OF SP-HP COMPLEXATION.

M (HPBCD)	20% Alcohol (M SP)	20% DMF (M SP)	Water M (SP)
0	5.4x10 ⁻⁴	7.8x10 ⁻⁴	2.9x10 ⁻⁴
2x10 ⁻³	6.6x10 ⁻⁴	7.7x10 ⁻⁴	3.9x10 ⁻⁴
4x10 ⁻³	8.5x10 ⁻⁴	9.8x10 ⁻⁴	5.4x10 ⁻⁴
6x10 ⁻³	1.0x10 ⁻⁴	1.0x10 ⁻³	6.8x10 ⁻⁴
8x10 ⁻³	1.3x10 ⁻⁴	1.1x10 ⁻³	7.6x10 ⁻⁴
Kc	186.9	39.54	248.8

Where M (HPBCD) is the molar concentration of hydroxypropyl-β-cyclodextrin and M (SP) is the molar concentration of sparfloxacin.

Large negative ΔH suggests that SP-HP inclusion is an enthalpy driven process. The negative values obtained for both ΔH and ΔS would seem to suggest that the stabilizing interactions in the complex are predominantly hydrophobic¹⁸. Any contribution to the stability of the complex through hydrogen bonding appears to be minor indicated in our molecular modeling studies.

We have also examined the influence of water-miscible organic solvents in affecting the stability of the complex. The PSA data suggest their 1:1 stoichiometry. It may be noted that the Kc values decrease from 248.76 mol⁻¹ in water to 186.89 mol⁻¹ in ethanol to 39.54 mol⁻¹ in DMF (Table 2).

This approach initially developed for determination of stoichiometry and stability constants determination of charge transfer complexes and has since been routinely used by several workers for cyclodextrin complexation¹⁹. The double reciprocal B-H plot was used to determine Kc using following relationship²⁰.

$$\frac{A_0}{A} = \frac{1}{\epsilon} + \frac{1}{K\epsilon} + \frac{1}{D_0}$$

Where, A_0 and D_0 are molar concentrations of drug and cyclodextrin respectively, K is the stability constant, E is the molecular extinction coefficient, and A is absorbance of sparfloxacin.

We have obtained a value of Kc 3886 mol⁻¹, which is quite high compared to the value obtained from PSA (248.76 mol⁻¹). We have noticed parallel observations with other PSA-(B-H) studies on a few other molecules and found that the deviations are significant. Hence, comparison of Kc from PSA and B-H plot method should be used with caution. The reason for such deviations in results of these two methods deserves thorough investigations.

HPLC determination of SP content in the complex was found to be 20.17% w/w, which was in good agreement with the expected value of 20.30% w/w for 1:1 inclusion complex formation. PSA and B-H plot exhibited linearity, thereby conforming the 1:1 stoichiometry²¹.

UV spectrum of an aqueous SP-HP solution exhibited a 25% hyperchromic shift indicating solubilization of SP in presence of HPCD. It was associated with a 6.5 nm bathochromic shift from SP to SP-HP. The marginal spectral shift ($\Delta\lambda_{max}$) is to be noted in contrast to the much higher $\Delta\lambda_{max}$ values noticed for true charge transfer bands.

Fluorescence spectrum of SP-HP exhibited peaks at 332, 493, 620 and 736 nm, the corresponding spectrum of SP in water and methanol manifested negligible fluorescence. Provision of an apolar environment due to organic solvent enhances the normal fluorescence of many compounds. The cyclodextrin cavity behaves similarly as organic solvents and affords apolar surrounding^{22,23}.

The C-F stretch bands at 1084.76 cm⁻¹ and 1027.87 cm⁻¹ in SP appear to be shifted to 1081.87 cm⁻¹ and 1030.72 cm⁻¹ in the SP-HP. The spectrum could not reveal the finer structural aspects of the complex significantly due to the overlapping of hydrogen stretch region by hydroxyl groups of HP.

DSC thermogram of SP displayed a characteristic endotherm at 267.3° and showed no evidence of exothermic degradation up to 300°. In the case of the complex, no endothermic peak was noticed up to 300°, thereby providing a sound evidence of inclusion of SP in HPCD cavity.

The pKa of SP-HP (9.103) is 0.071 unit more than that of SP (9.032) when titrated with 0.0093 N aqueous sodium hydroxide solution. The relatively higher value of pKa for SP-HP than SP indicated that in the complex, the carboxylic group in SP becomes relatively weaker than in SP. This may be due to the established hydrophobic environment of HPCD cavity.

The large decrease of 0.431 unit in pKa of SP-HP (5.64) compared to SP (6.071) when titrated with 0.0117 N HCl indicated that the piperazine 4-nitrogen in SP-HP is less basic than in SP. This may be due to the difficulty in protonation of the piperazine 4-nitrogen when it is engulfed inside the HPCD cavity. In consequence, the measurement appears to indicate that the piperazine end of the SP creeps into the HPCD cavity while the carboxylic end remains outside the cavity. The results are in agreement with published reports²⁴.

It was observed that at 20 min SP and SP-HP showed 15.41% and 81.71% release of drug respectively indicating the efficacy of complexation in improving drug dissolution. The amount of drug dissolved versus square root time plots were constructed to compare the efficiency of the complex in improving dissolution. The slopes of dissolution plots were 0.162 and 2.3919, demonstrating nearly many fold improvement in release rate of drug (Figs. 4a and 4b). Sparfloxacin forms a 1:1 inclusion complex with HPCD. The physical, thermal and spectral properties of the complex substantiate this conclusion. HPCD complex of SP has significantly improved

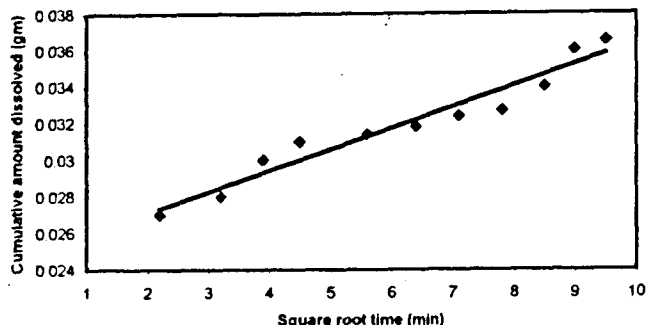


Fig. 4a: Higuchi square root time plot of SP.

Higuchi square root time plot of sparfloxacin showing improvement in release. The correlation coefficient was found to be 0.9407.

the dissolution property of the sparfloxacin.

ACKNOWLEDGEMENTS

We are grateful to All India Council for Technical Education, India for financial assistance. We would also like to thank Cerestar, Inc., USA, for donating hydroxypropyl- β -cyclodextrin.

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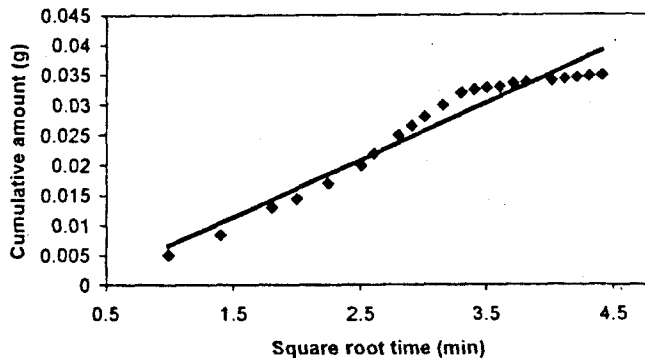


Fig. 4b: Higuchi square root time plot of complexed sparfloxacin.

Higuchi square root time plot of SP-HP complex showing many fold improvement in release as compared with plain SP. The coefficient of correlation was found to be 0.9447.

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