Characterization and Solubility Studies of Mefloquinehydrochloride Inclusion Complexes with α -Cyclodextrin/ Hydroxypropyl α -Cyclodextrin.

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Abstract- The present study aimed to improve the solubility and ultimate bioavailability of poorly soluble Mefloquinehydrochloride, an antimalarial drug by encapsulating it in α-cyclodextrin and Hydroxy propyl α- cyclodextrin. Effect of these complexes was studied by UV-VIS spectroscopy, Fluorescence spectroscopy, Phase solubility study, SEM-EDS and FTIR spectroscopy. The association constant of the inclusion complexes were determined by the Benesi- hilde – brand relation and the inclusion ratio was found to be 1:1. The water solubility of Mefloquinehydrochloride was increased by inclusion with α-CD and HP-α-CD according to the phase solubility diagram.The results obtained from SEM-EDS and FTIR spectroscopy confirmed the inclusion of Mefloquinehydrochloride into cyclodextrins cavity.

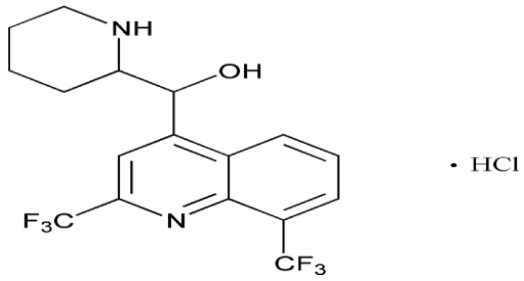
Index Terms- Alpha cyclodextrins, Hydroxypropyl alpha cyclodextrins, inclusion complex. Mefloquinehydrochloride.

I. INTRODUCTION

Malaria is the most life threatening disease among parasitic infections. Plasmodium falciparum, the human malaria parasite, is the overwhelming cause of serious disease and death(1). Mefloquinehydrochloride, a rapidly acting antimalarial drug is potent , efficient against acute and severe p.falciparum malaria. The efficiency of Mefloquinehydrochloride is greatly hampered due to its poor bioavailability and low aqueous solubility. The solubility of poorly soluble drug can be altered in many ways, such as modification of drug crystal forms, addition of co-solvents, addition of surfactants, complexation with cyclodextrins (CD) , etc. (2,3,4,5,6). Among the possibilities CD approach is of particular interest.

Cyclodextrins (CDs) are non–toxic cyclic oligosaccharides, consisting of (α-1,4) linked α–D–glucopyranose units with a hydrophilic interior. The most abundant natural cyclodextrins are α–Cyclodextrin (α–CD) , β-Cyclodextrin (β-CD), γ-Cyclodextrin (γ-CD) containing six, seven and eight glucopyranose units , respectively (7,8,9,10). Cyclodexrin complex has been successfully used to improve the solubility, chemical stability and bioavailability of a number of poorly soluble compounds (10,11,12). Recently, various hydrophilic, hydrophobic and ionic cyclodextrin derivatives have been utilized to extend the physicochemical properties of water insoluble drug through inclusion complexes formulations. (13,14,15). α-CD and HP-α-CD are water soluble molecules, which has been widely studied as a complexation agent for many pharmaceuticals.

In this study, an attempt was made to improve the solubility of Mefloquinehydrochloride by complexing with α-CD and HP-α-CD, thereby increasing its bioavailability and therapeutic efficiency. The characterization of drug with α-CD and HP-α-CD using UV-VIS spectroscopy , fluorescence spectroscopy,phase solubility study, SEM-EDS and FTIR spectroscopic studies were performed.



α-2-piperidinyl-2,8-bis (trifluoromethyl)-4- quinolinemethanol hydrochloride

II. EXPERIMENTAL SECTION

2.1.Materials and Methods

Mefloquinehydrochloride was obtained as gift sample from Ipca Laboratories ltd. Mumbai, India, α-CD and HP-α-CD were purchased from Sigma Aldrich; both were used as received with no further purification. All other reagents and chemicals were of analytical grade.

2.2.Instruments

The UV-VIS spectra were carried out with Systronic Double- beam spectrophotometer-2203. Fluorescence spectral measurements were carried out with JASCO spectrofluorometer FP-8200. Phase solubility studies were carried out using a rotary shaker. FTIR studies were carried out by FTIR-8400S type and SEM-EDS studies were examined by means of JEOL MODEL JSM – 6390 LV.

2.3.Preparation of liquid inclusion Complex

The inclusion complex was prepared by adding constant volume of drug into 10ml volumetric flasks containing the absence and presence of increasing concentrations (2-10 mM) of

α-CD and HP-α-CD. The absorption and fluorescence spectra were recorded.

2.4.Preparation of solid inclusion complex

Solid dispersion / Co-evaporated dispersion method

The solid inclusion complex of Mefloquinehydrochloride with α-CD and HP-α-CD in 1:1 molar ratio were prepared by dissolving the drug in methanol and α-CD and HP-α-CD were dissolved in water separately (15,16). The α-CD and HP-α-CD solutions then added to the drug solution and stirred for about 48 hours at room temperature to attain equilibrium. The resulting solution was evaporated to dryness.

2.5.Phase solubility studies

Phase solubility studies were performed according to the method reported by Higuchi and Connors (17).Mefloquinehydrochloride , in amounts that exceeded its solubility, was taken into vials to which were added 15 ml of distilled water (pH 6.8) containing various concentration of α-CD and Hp-α-CD (2-10 mM). These flasks were sealed and shaken at 20^0 C for 5 days to reach equilibrium and the samples were filtered immediately through a 0.45 – μ nylon disc filter and appropriately diluted. A portion of the sample was analysed by UV spectrophotometer at 283 nm against blank prepared in the same concentration of α-CD and HP-α-CD in water so as to cancel any absorbance that may be exhibited by the α-CD and HP-α-CD.

III. RESULTS AND DISCUSSION

Table–1. Absorption maxima of Mefloquinehydrochloride at different concentration of α-CD and HP-α-CD

α-Cd / HP-α-CD concentration	λ_{max} (nm)		Absorbance	
	[α-CD]	[HP-α- CD]	[α-CD]	[HP-α-CD]
0	284.4	284.4	0.412	0.412
0.002	280.2	282.0	0.436	0.438
0.004	278.4	280.8	0.455	0.499
0.006	278.2	278.4	0.462	0.530
0.008	276.0	276.2	0.494	0.552
0.01	276.4	274.8	0.501	0.569

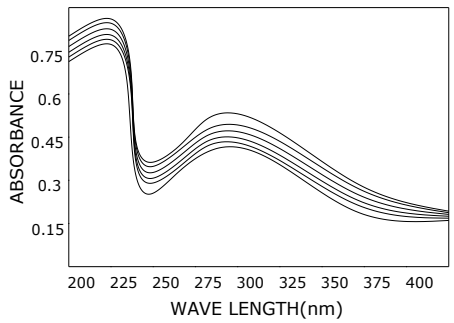


Figure1. Absorption spectra of mefloquinehydrochloride with α-CD

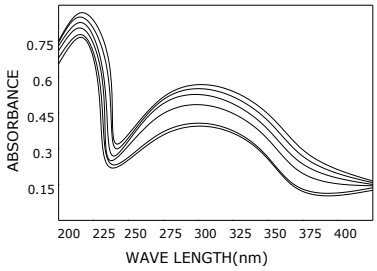


Figure 2.Absorption spectra of mefloquinehydrochloride with HP-α-CD

Table 2. Fluorescence maxima of Mefloquinehydrochloride at different concentration of α-CD and HP-α-CD

α-Cd/ HP-α-CD concentration	λ_{flu} (nm)		Intensity	
	[α-CD]	[HP-α-CD]	[α-CD]	[HP-α-CD]
0	387	387	282.65	282.65
0.002	384	385	344.46	347.24
0.004	382.5	383	347.63	348.05
0.006	381	382	349.34	350.79
0.008	380	380	350.24	351.37
0.01	378	380	351.79	353.82

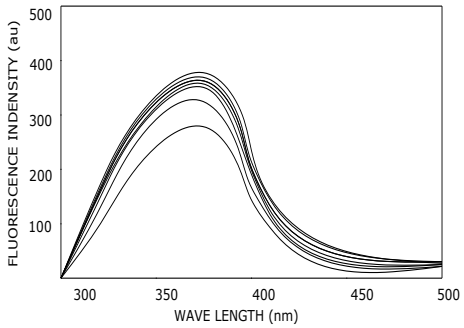


Figure 3. Fluorescence spectra of mefloquinehydrochloride with α-CD

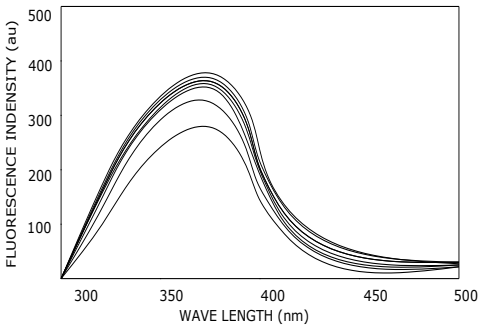


Figure 4 . Fluorescence spectra of mefloquinehydrochloride with HP-α-CD

Table-2 and fig (3) (4) shows the fluorescence maxima and spectra of Mefloquinehydrochloride containing various

concentration of α-CD and HP-α-CD. The emission spectrum is blue shifted from 387nm to378nm and 387nm to 380nm for both α-CD and HP-α-CD. This results indicates that Mefloquine hydrochloride is entrapped in α-CD and HP-α-CD to form Mefloquinehydrochloride: α-CD and Mefloquinehydrochloride : HP-α-CD inclusion complexes. By the addition of α-CD and HP-α-CD the fluorescence maxima is blue shifted by the formation of hydrogen bonding.

The association constant (K) for the formation of an inclusion complex has been determined by analyzing the changes in the intensities of absorption and fluorescence maxima with the α-CD and HP-α-CD concentration. The association constant and stoichiometric ratios of the inclusion complex of Mefloquinehydrochloride with α-CD and HP-α-CD can be determined by using the Benesi - Hilde – brand relation(18). The equation for 1:1 complxes are given below.

Absorption

$1/A - A_0 = 1/A - A_0 + 1/k\,(A - A_0)\,[\alpha\text{-CD}]$

Fluorescence

$1/I - I_0 = 1/I - I_0 + 1/k\,(I - I_0)\,[\alpha\text{-CD}]$

In the above equation A_0/I_0 is the intensity of absorbance / fluorescence of Mefloquine hydrochloride without α-CD and HP-α-CD.

A / I is the absorbance / fluorescence intensity with a particular concentration of α-CD and HP-α-CD. Linearity is obtained in the plot of $1/A - A_0$ or $1/I - I_0$ verses 1/ [α-CD] and 1/ [HP-α-CD]. This confirms the formation of 1:1 inclusion complexes. The association constant K was calculated from the slope of Benesi – Hilde – Brand plot using the equation.

For absorption

K = 1 / slope (A – A_0) = 147.84 for Mefloquinehydrochloride : α-CD and 176.92 for Mefloquinehydrochloride : HP-α-CD inclusion complexes

3.1.Phase solubility

Phase solubility diagram of α-CD/Mefloquinehydrochloride system (fig.5) and HP-α-CD/Mefloquinehydrochloride system (fig.6) shows drug solubility increases linearly with increasing α-CD and HP-α-CD concentration.

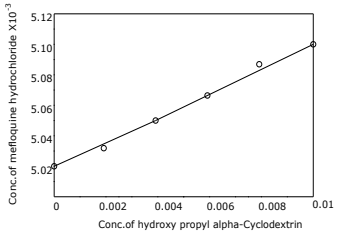


Figure :5Phase solubility diagram of α-CD/ Mefloquinehydrochloride

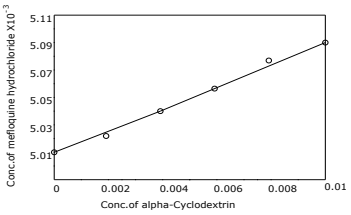


Figure :6 Phase solubility diagram of HP-α-CD/ Mefloquinehydrochloride

The diagram can be classified as A_L type according to the model proposed by Higuchi and Connors (19). It can be related to the formation of a soluble inclusion complex. The apparent stability constant (Ks) was calculated from the linear fit of the curve according to the following equation.

$$Ks = \frac{slope}{S_0(1 - slope)}$$

Where slope is the value found in the linear regression and S_0 is the aqueous solubility of the drug.Slope of less than one suggested the formation of 1:1 inclusion complex for both α-CD and HP-α-CD with Mefloquinehydrochloride. The apparent stability constant Ks obtained from the slope of the linear phase solubility diagram was found to be $110M^{-1}$ and $160M^{-1}$ which also indicates that the α-CD and HP-α-CD Mefloquinehydrochloride complexes at 1:1 ratios are adequately stable.

3.2.Scanning Electron Microscopy

It is clear from the SEM images that i) α-CD is present in plated form (fig.7), ii) HP-α-CD is present in spherical shape (fig.8) iii) Pure drug is present in irregular shaped crystal (fig.9).The SEM pictures of inclusion complexes are completely different from that of pure drugs and CDs(fig.10&11). The difference in morphological changes of these structures with EDS analysis can be taken as a proof of the formation of a new inclusion complex.

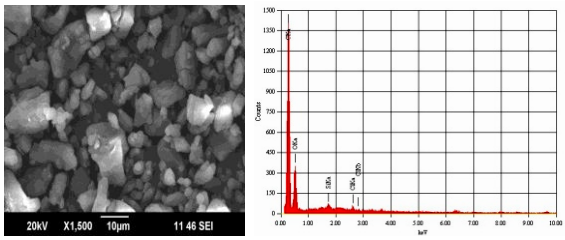


Figure 7: SEM-EDS image of α-CD

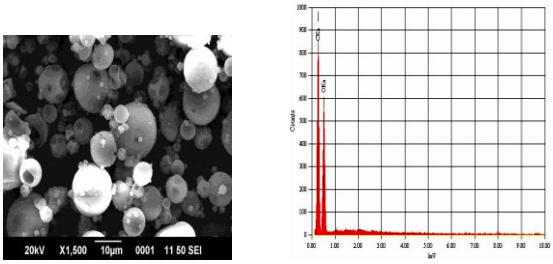


Figure 8: SEM-EDS image of HP-α-CD

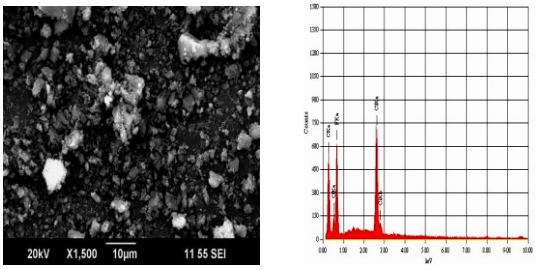


Figure 9: SEM-EDS image of Mefloquine hydrochloride

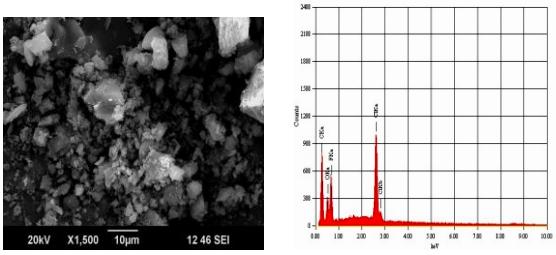


Figure 10: SEM-EDS image of inclusion complex of α-CD and Mefloquinehydrochloride

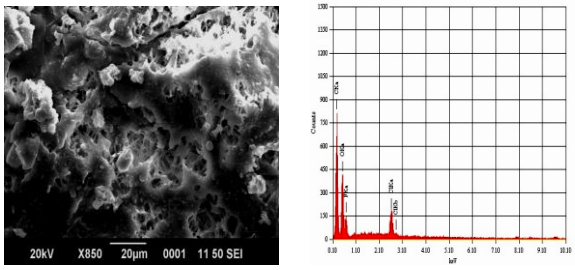


Figure 11: SEM-EDS image of inclusion complex of HP-α-CD and Mefloquine hydrochloride

3.3.Fourier Transform Infra red (FTIR) Spectroscopy:

The FTIR spectra of pure Mefloquinehydrochloride (fig.12) showed a characteristic peak at 3240.19 cm^{-1} (N-H stetching vibration), 2850.59cm^{-1} (C-H bridge), 2947.03cm^{-1} (CH2), 1583.45cm^{-1}(C=N / C=C) ,1267.14cm^{-1}(C-N), 1049.20cm^{-1}(Piperidine ring),1166.85cm^{-1}(C-C / N-H Stretching vibration),1311.5cm^{-1} and1135.99 cm^{-1} (CF3 Stretching vibration), 1110.92cm^{-1},1380.94cm^{-1} and 1517.87cm^{-1} (Quinine ring stretching).The spectrum of pure α-CD (fig.13) showed characteristic peak at 3382.91cm^{-1} (O-H Stretching vibration), 2925.81cm^{-1} (C-H), 1641.31 cm^{-1} (H-O-H bending), 1155.28cm^{-1} (C-O) and 1029.92cm^{-1} (C-O-C).The spectrum of pure HP-α-CD(fig.14) showed a characteristic peak at 3388.70cm^{-1} (O-H Stretching vibration), 2927.74cm^{-1} (C-H), 1643.24cm^{-1} (H-O-H bending),1155.28cm^{-1} (C-O) and 1033.77 (C-O-C). In the IR spectra of HP-α-CD inclusion complex(fig.15) , the peaks at 1380.94cm^{-1} and 1110.92 cm^{-1} of Mefloquinehydrochloride appears at same frequency with high intensity where as in α-CD inclusion complex(fig.16) they appear with low intensity.The absorption peak of α-CD (C-H) at 2925.81cm^{-1} is shifted to 2931.80cm^{-1} in complex and there is no change in the (C-H) stretching vibration in HP-α-CD inclusion complex. Absorption peaks at 1311.5cm^{-1} (CF3 stretching vibration) appears with high intensity at 1315cm^{-1} in HP-α-CD inclusion complex. Some peaks at1583.45cm^{-1}(C=N/C=C),2850cm-1(C-H bridge) and 1049.20cm^{-1} (piperidine ring) are missing in the inclusion complexes.These changes occurred in FTIR spectra of samples indicating the formation of an inclusion complex in solid state.

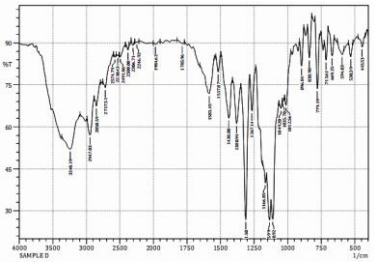


Figure 12.FTIR spectra of Mefloquine hydrochloride

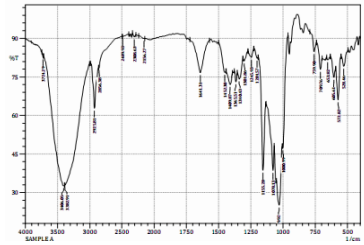


Figure 13.FTIR spectra of α-CD

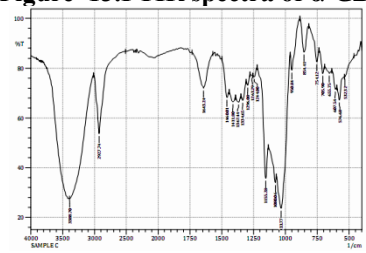


Figure 14.FTIR spectra of HP-α-CD

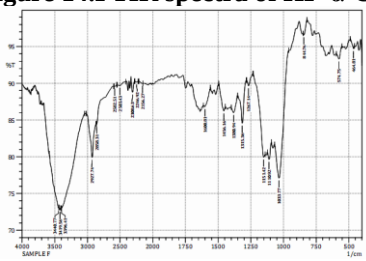


Figure 15: FTIR spectra of HP-α-CD - Mefloquine hydrochloride inclusion complex

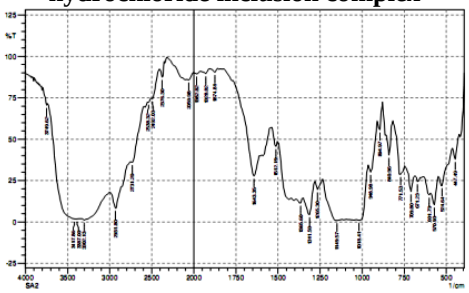


Figure 16. FTIR spectra of α-CD - Mefloquine hydrochloride inclusion complex

3.4.Comparative study

Inclusion of Mefloquinehydrochloride an anti malarial drug with both α-CD and HP-α-CD shows the same features. Due to the presence of propyl group in HP-α-CD, the absorption and emission wave lengths slightly increased than α-CD. The association constant value is higher for HP-α-CD complex than

α-CD complex. The stability constant value is also higher for HP-α-CD complex. From these observations , it can be concluded that HP-α-CD is the best host for the guest Mefloquinehydrochloride .

IV. CONCLUSION

In the present research work the results obtained from UV-Visible spectroscopy, phase solubility studies , FTIR spectroscopy and SEM analysis showed a 1:1 complex of Mefloquinehydrochloride with α-CD and HP-α-CD.The aqueous solubility and apparent stability of Mefloquinehydrochloride can be increased by inclusion complex with HP-α-CD. The FTIR data provided information about the functional groups involved in the complexation. According to the present observation HP-α-CD seems to be the best host for the Mefloquinehydrochloride. A complete inclusion can be detected in a complex with HP-α-CD This solid state structure should be benefit to improve solubility and stability of Mefloquinehydrochloride. Consequently the bioavailability of Mefloquinehydrochloride in human body can be increased.

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