ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Preparation and Characterization of Metronidazole-β-Cyclodextrin Inclusion Complex.

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ABSTRACT

Metronidazole (MTZ) is an anti protozoal drug with poor aqueous solubility and bioavailability. It was complexed with β - cyclodextrin (β - CD) by kneading method in 1:1 molar ratio in order to improve aqueous solubility of MTZ. The complex formation was determined by phase solubility measurements, obtaining A_L type of diagram. Prepared complexes were characterized by Fourier transform infrared spectroscopy (FTIR), differential scanning calorimetry (DSC), ^{1}H - Nuclear Magnetic Resonance (^{1}H-NMR), Results of *in - vitro* studies appraised of an increased solubility and dissolution rate of MTZ on complexation with β-CD as compared to MTZ alone.

Keywords: Metronidazole, β - cyclodextrin, FTIR, DSC, phase solubility, inclusion complex.

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ISSN: 0975-8585

INTRODUCTION

Cyclodextrin (CD) are cyclic oligosaccharides with six, seven or eight glucose units bonded by α (1, 4) linkage, named alpha (α), beta (β) and gamma (γ) CD. Out of these three CD, β - CD are most commonly used because its cavity fits common guest molecules and also because of its ready availability and reasonable price [1]. CD is having hydrophilic outer surface and hydrophobic internal cavity [2] into which various drug molecules can be incorporated. Thus non covalent inclusion complex were formed [3]. These inclusion complexes improve the solubility, dissolution rate, stability and bioavailability of drugs [4]. It has also been demonstrated that inclusion complex decrease the toxic effects of some therapeutic agents [5].

MTZ (1- [2 - hydroxyethyl] - 2 -methyl - nitroimidazole) is effective against protozoal and bacterial infections. It has been a drug of choice in the treatment of anaerobic infections and prophylactically in gynecological and colonic surgery [6, 7]. It has also been effectively used against *Entamoeba histolytica* and *Trichomonas vaginalis* infections [8]. It has been included in the essential drug list by WHO [9]. But this drug possesses low intrinsic solubility. So one of the promising approach was to encapsulate MTZ into the hydrophobic cavity of β-CD in an attempt to increase the solubility and thus better biological activity. Based on the above background, the present study was designed to prepare the inclusion complex of MTZ with β-CD by kneading method in 1:1 molar ratio and further the prepared complexes were characterized for physicochemical properties by phase solubility studies, DSC, FTIR, H^1NMR and dissolution studies.

MATERIALS AND METHODS

Materials

Metronidazole was received ex-gratia from La Pharma Pvt. Ltd. Ludhiana, Punjab, India, β-CD was purchased from Central Drug House Pvt. Ltd., New Delhi. All reagent used were of analytical grade.

Methods

Phase solubility studies

Phase solubility studies were carried out as per the method reported by Higuchi and Connors [10]. Excess amount of MTZ was added to phosphate buffer solution (PBS) pH 6.8 containing β-CD in different molar concentration (0 - 10 mM) and it was then shaken for 24 h at room temperature in shaking incubator (Daihan labtech., Korea). After equilibrium has been attained, the solutions were filtered through Whatman filter paper and analyzed spectro - photometrically using UV- Spectrophotometer (UV-1700 Pharmaspec, Shimadzu, Japan) at 277 nm. The procedure was repeated three times.

The apparent stability constant (Ka) of complex was calculated from phase solubility diagram according to the equation 1.

$$ka = \frac{\text{Slope}}{S^\circ (1-\text{Slope})} \quad \ldots\ldots\ldots\ldots 1$$

Where S° is the solubility of metronidazole at 30° C in the absence of β-CD and slope means corresponding slope of the phase solubility diagram i.e. slope of the molar concentration of drug verses β-CD molar concentration graph.

Preparation of inclusion complex by kneading method

MTZ and β-CD were blended for 30 min in 1:1 molar ratio by wetting with appropriate quantity of ethanol to form a paste. After that it was dried overnight at 40° C in oven (PERFIT, Gupta Scientific Corporation Pvt. Ltd, Ambala, India) crushed, sieved and stored at 25° C ± 2° C temperature and 40 - 50 % relative humidity till further use [11].

ISSN: 0975-8585

Differential Scanning Calorimetry Studies (DSC)

DSC of MTZ and metronidazole inclusion complex were performed using Shimadzu differential scanning calorimetry (DSC TA - 60, Tokyo, Japan) in order to assess the thermotropic properties and thermal behavior of metronidazole and metronidazole inclusion complex and also to detect presence of interaction. The samples were weighed (2 - 3 mg), placed on aluminum pan, which was then crimped. On the other side, empty crimped aluminum pan was placed as a reference standard. The samples were heated between 30 - 450º C at the rate of 10º C / min. Nitrogen was introduced at 2 bars and flow rate of 50 ml/min. Data was analyzed using TA-60 Collector software.

Fourier Transform Infrared (FTIR) spectroscopy

IR spectrum (Alpha, Bruker, USA) of metronidazole, β-CD and metronidazole inclusion complex was obtained by crushing the samples into fine powder. The powder was dried under IR lamp (Micromax, Vansh surgical corporation, India) for 30 min. Then small quantities (1-2 mg) of samples were placed on FTIR crystal and were scanned over wave number region 4000 - 400 cm^{-1} using opus software. The characteristic peaks of IR were observed and were compared with the peaks of the standard for the identification of complex formation.

Nuclear magnetic resonance spectroscopy

Proton (^1H) Nuclear Magnetic Resonance (^1H-NMR) spectra were obtained using Bruker Advance 11 - 400 NMR, 400MHz spectrometer for metronidazole, β-CD and metronidazole inclusion complex. The chemical shifts were reported as parts per million (δ ppm) as shown in Figure 4 (a, b and c).

Dissolution study

The dissolution release profile of metronidazole and metronidazole inclusion complex was studied using dissolution test apparatus (Eight Station Tablet Dissolution Test Apparatus, DS-8000, Lab India) in phosphate buffer solution (PBS) pH 6.8 was used as dissolution medium [12]. Sample equivalent to 50 mg of metronidazole was weighed and put into 900 ml of PBS, maintained at 37º C ± 0.5º C in paddle type stirrer at 50 rpm. A sample of 5 ml (replaced with equal volume) was taken periodically, filtered, suitably diluted with PBS and analyzed spectrophotometrically using UV-Spectrophotometer ((UV-1700-Pharmaspec Shimadzu, Japan) at 277 nm. The procedure was repeated three times.

RESULTS AND DISCUSSION

Phase solubility studies

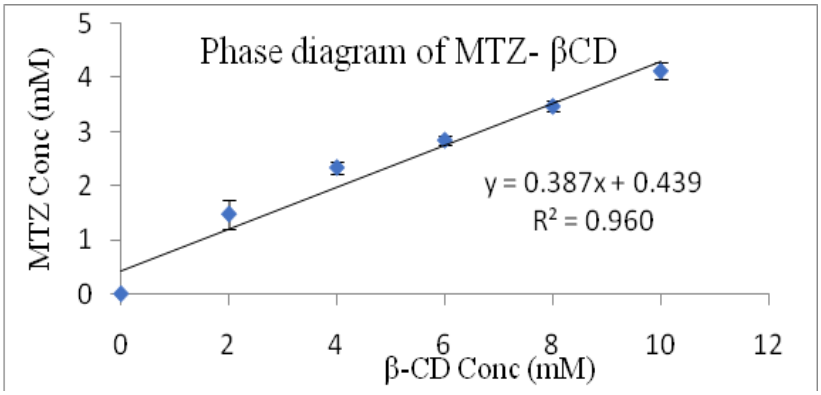

Figure 1: Phase solubility diagram for MTZ in β – CD

Phase solubility studies conducted at room temperature showed that the solubility of metronidazole increased linearly (R^2 = 0.960) as a function of β-CD as shown in Figure 1. The phase solubility diagram of

metronidazole with β-CD in selected concentration range displayed A_L type of diagram i.e. solubility of metronidazole was found to increase with increase in β-CD concentration under the concentration range that was tested. The complex exhibited higher solubility than the guest molecule [5]. The apparent stability of the complex was found to be 168 M^{-1} which was calculated from slope of the linear phase solubility equation 1.

DSC studies

DSC of pure metronidazole exhibited sharp endothermic peak at 161° C, which is the melting point of pure metronidazole. The DSC of 1:1 inclusion complex prepared by kneading method showed the presence of peak at 284° C (Figure 2). It was remarkable indication that the drug melting point was shifted from its original position. This suggests the formation of inclusion complex.

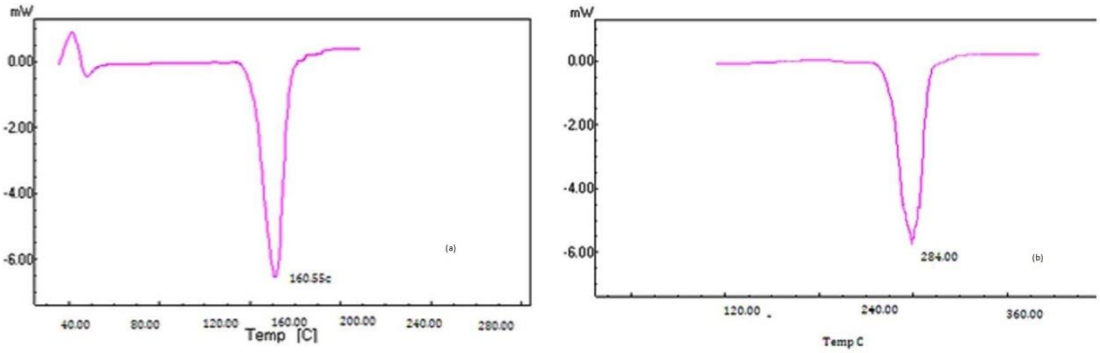

Figure 2: DSC of (a) MTZ and (b) inclusion complex

FTIR Spectroscopy

The FTIR Spectroscopy of (a) metronidazole (b) β- CD and (c) metronidazole Inclusion complex shown in Figure 3 and the interpretation was done using standard wave number ranges with observed wave number along with assignment of specific chemical group. Then comparison between the IR spectra in 1:1 molar ratio showed some significant changes in the shape and position of absorbance bands of metronidazole, β- CD and metronidazole Inclusion complex. The FTIR spectrum of metronidazole was characterized by principal absorption peaks at 1360.18 cm^{-1} (NO stretching vibration), this peak was completely disappeared in inclusion complex, 3453.77 cm^{-1} (N-H stretching vibration), 1080.54 cm^{-1} (C-OH stretching vibration). The FTIR spectrum of β- CD was characterized by principal absorption peaks at 3448.94 cm^{-1} (O-H stretching vibration) and 2925.37 cm^{-1} (C-H stretching vibration). There were obvious changes in the FTIR spectra after metronidazole Inclusion complex was formed. The band at 1151.05 cm^{-1} corresponding to C-O stretching was shifted to 1153.05 cm^{-1}, band at 3248.94 cm^{-1} corresponding to H bonding was shifted to 3213.86 cm^{-1} and band at 2925.37 cm^{-1} corresponding to C-H stretching was shifted to 2813.89 cm^{-1} and its intensity was decreased. The observed changes in the IR spectra of metronidazole complexed with β - CD were due to restriction of vibration of metronidazole molecule upon its encapsulation into β - CD cavity [13]. Therefore the FTIR spectroscopy results indicated that inclusion complex of metronidazole was successfully formed.

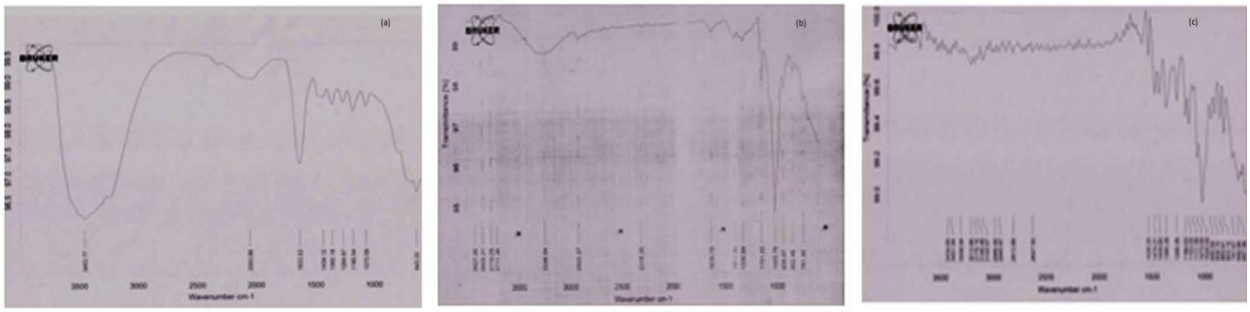

Figure 3: FTIR of (a) MTZ (b) β- CD and (c) inclusion complex

ISSN: 0975-8585

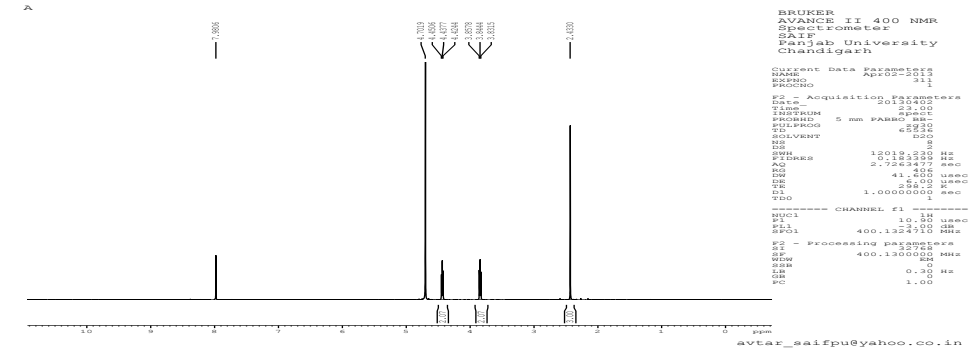

Figure 4a: ^{1}H NMR Spectra of MTZ

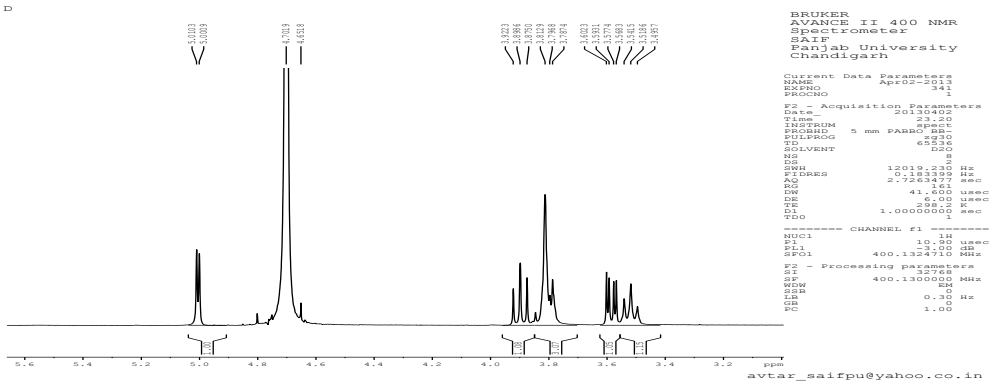

Figure 4b: ^{1}H NMR Spectra of -β CD

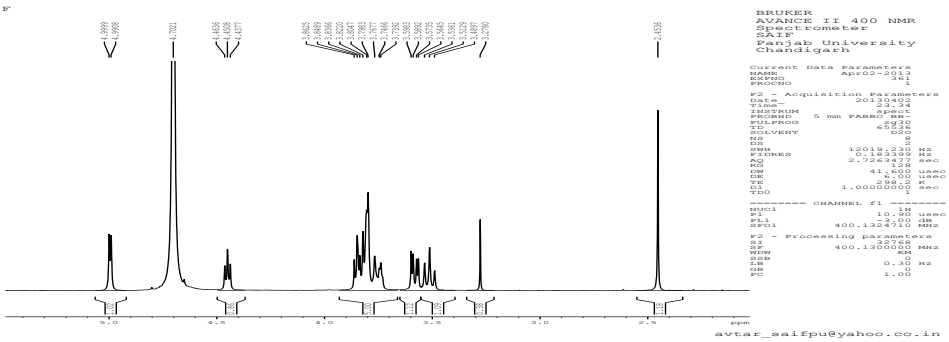

Figure 4c: ^{1}H NMR Spectra of MTZ-β – CD

Proton (^{1}H) Nuclear Magnetic Resonance Spectroscopy

In NMR spectra, there was a chemical shift of host and guest molecule when the guest molecule was inserted into the cyclodextrin hydrophobic cavity. The chemical shift is defined as the difference in chemical shift, where positive sign denotes downfield shift and the negative sign denotes up field shift. Generally there is a large chemical shift observed in the inner cavity of β - CD at H3 and H5 due to inclusion phenomena [14].

Figure 4a, 4b and 4c showed the protons ^{1}H-NMR spectra of MTZ, β - CD and inclusion complex. Complex formation can be proved from changes in the chemical shifts of β - CD and inclusion complex in ^{1}H - NMR spectra because of overlapping of many peaks in 3.9223-3.2790 ppm region indicating aromatic ring of β - CD [15] (Lee et al., 2010). Table 1 showed some chemical shifts observed for H1, H2, H3, H4 and H5. Though, the chemical shifts was somewhat higher located within the β - CD cavity for protons such as H3 were compare to the protons, which were located at the exterior side of the cavity. Therefore, the change in shift between β-CD and MTZ inclusion complex further confirms the formation of the inclusion complex [16, 17].

ISSN: 0975-8585

Table 1: β - CD and inclusion complex protons chemical shift

Chemical shift	β - CD	β - CD inclusion complex	Δδ
H1	5.0103	4.9995	0.0108
H2	4.7019	4.7021	-0.0002
H3	3.9223	3.8625	**0.0598**
H4	3.6023	3.5735	0.0288
H5	3.5931	3.5645	0.0286

Dissolution study

The dissolution profiles of MTZ and its 1:1 inclusion complex with β – CD is shown in Figure 5. The pure drug showed solubility up to 5 % while its inclusion complex showed 62 % dissolution in first 5 min. The total drug dissolved in 1 h from metronidazole was about 18 % compared to 91 % from inclusion complex. Thus there was five times increase in solubility which may help in increasing the bioavailability of metronidazole. This could be due to aggregate formation by CD in aqueous solution and these aggregates act as solublizers in micelles like fashion [18].

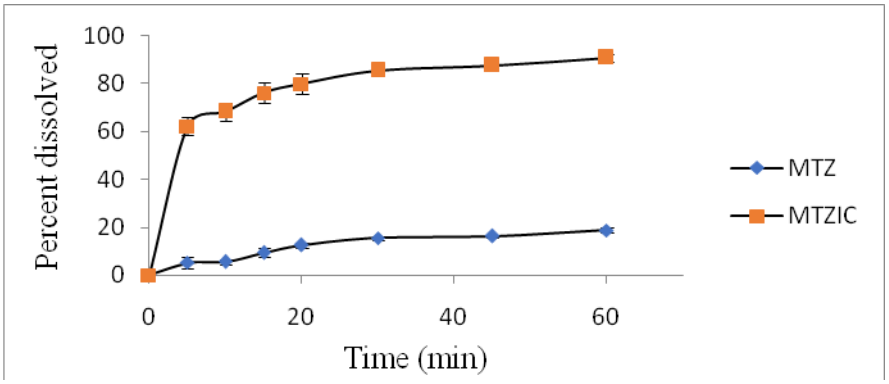

Figure 5: Dissolution profile of MTZ and inclusion complex (MTZIC)

CONCLUSION

β - CD can be used to prepare inclusion complex of metronidazole. The complex prepared by kneading method in 1:1 molar ratio demonstrated notable increase in aqueous solubility and dissolution rate. The results obtained by different characterization techniques clearly point out that kneading method leads to formation of inclusion complex between metronidazole and β - CD. Thus the technique can be used to increase the aqueous solubility and bioavailability of poorly soluble drugs.

ACKNOWLEDGEMENT

The authors are grateful to the Chairman and Department of Pharmaceutics, ISF College of Pharmacy, Moga, Punjab, India for providing necessary facilities to complete the research work.

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