Original Article

Preparation and characterization of tinidazole-β-cyclodextrin inclusion complex

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How to cite this article: Kaur S, Arora D, Khurana B, Singh G. Preparation and characterization of tinidazole-β-cyclodextrin inclusion complex. Pharmaspire 2019;11(2):38-42.

Source of Support: Nil,
Conflicts of Interest: None declared.

ABSTRACT

Aim: Preparation and Characterization of Tinidazole-β-Cyclodextrin Inclusion Complex to increase the aqueous solubility and bioavailability of Tinidazole (TNZ) is an anti-protozoal drug. **Method:** TNZ was complexed with β-cyclodextrin (β-CD) by kneading method in 1:1 molar ratio in order to improve aqueous solubility and bioavailability of TNZ. The complex formation was determined by phase solubility measurements, obtaining AL type of diagram. Prepared complexes were characterized by Fourier transform infrared spectroscopy (FTIR), ^{1}H - Nuclear Magnetic Resonance (^{1}H-NMR). **Results:** The phase solubility diagram of tinidazole with β-CD in selected concentration range displayed AL type of diagram i.e. solubility of tinidazole was increased with increase in β-CD concentration under the concentration range that was tested. The *in-vitro* studies appraised of an increased solubility and dissolution rate of TNZ on complexation with β-CD as compared to TNZ alone. **Conclusion:** The complex prepared by kneading method in 1:1 molar ratio demonstrated notable increase in aqueous solubility and dissolution rate.

Keywords: Tinidazole, β-cyclodextrin, Fourier transform infrared, differential scanning calorimetry, phase solubility, inclusion complex

INTRODUCTION

Cyclodextrins (CDs) are cyclic oligosaccharides with six, seven, or eight glucose units bonded by α (1, 4) linkage, namely, alpha (α), beta (β), and gamma (γ) CD. Out of these three CDs, β-CDs 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 forming non-covalent inclusion complexes.[3] These inclusion complexes improve the solubility, dissolution rate, stability, and bioavailability of drugs.[4] It has also been demonstrated that inclusion complexes decrease the toxic effects of some therapeutic agents.[5]

Tinidazole (TNZ) and metronidazole are most 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 *Trichomona svaginalis* infections.[8] It has been included in the essential drug list by the WHO.[9] However, this drug possesses low intrinsic solubility. Hence, one of the promising approaches was to encapsulate mitoxantrone (MTZ) into the hydrophobic cavity of β-CD and with it try 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 characterized for physicochemical properties by phase solubility studies, Fourier transform infrared (FTIR), nuclear magnetic resonance (NMR), and dissolution studies.

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Website: www.isfcppharmaspire.com	**P-ISSN:** 2321-4732 **E-ISSN:** XXXX-XXXX

MATERIALS AND METHODS

Material

TNZ 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 solvents used were analytical grade reagent.

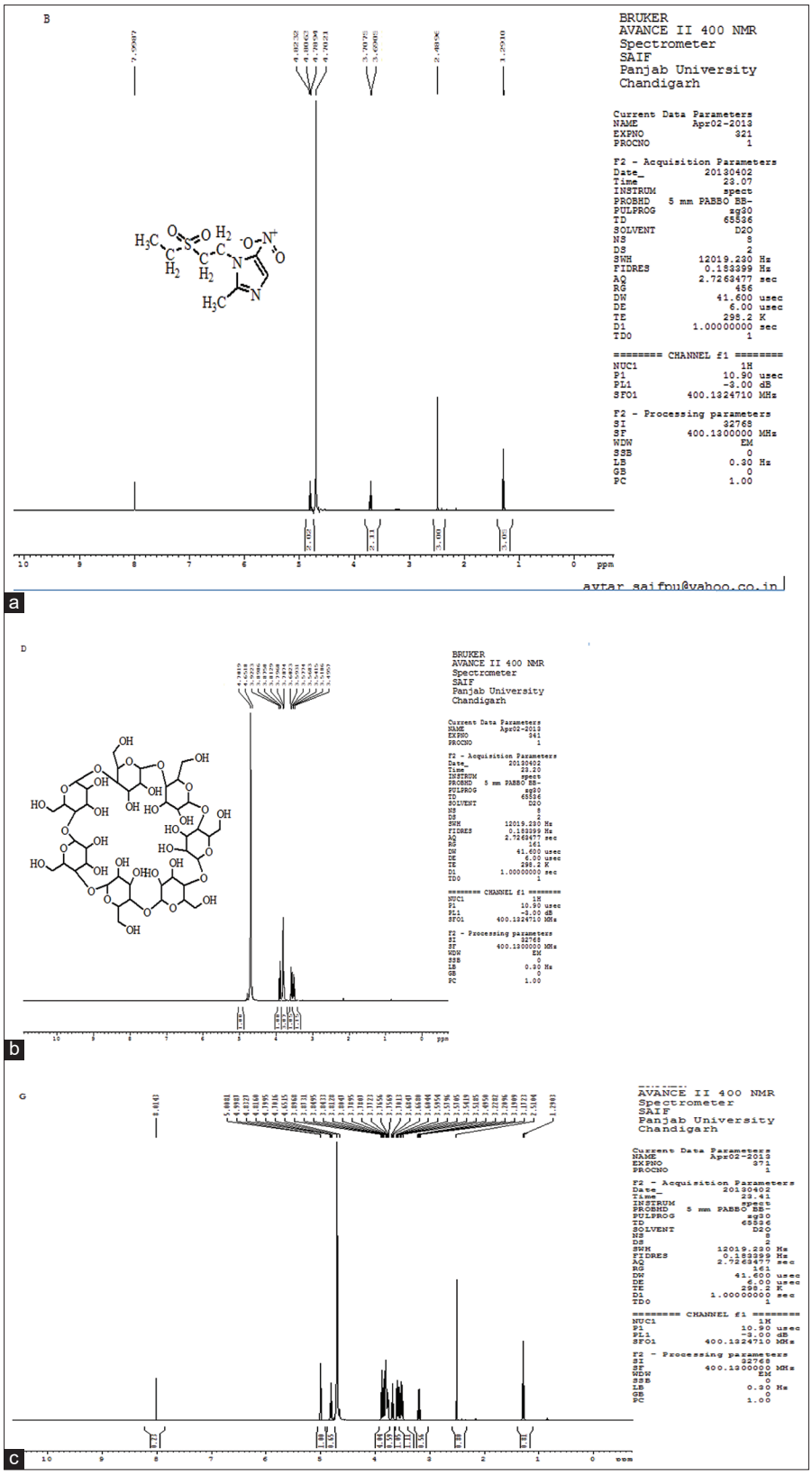

Figure 1: (a) [1]H-nuclear magnetic resonance ([1]H-NMR) spectroscopy of tinidazole. (b) [1]H-NMR spectroscopy of β-cyclodextrin. (c) [1]H-NMR spectroscopy of tinidazole-β-CD inclusion complex

Methods

Phase solubility studies

Phase solubility studies were carried out as per the method reported by Higuchi and Connors.[10] Excess amount of TNZ was added phosphate buffer solution (PBS) pH 6.8 containing β-CD in different molar concentrations (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 spectrophotometrically using ultraviolet (UV) spectrophotometer (UV-1700 Pharmaspec, Shimadzu, Japan) at 277 nm. The procedure was repeated 3 times.

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

$$ka = \frac{\text{Slope}}{\text{Slope}} \quad (1)$$

Where, Sh is the solubility of TNZ 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 versus β-CD molar concentration graph.

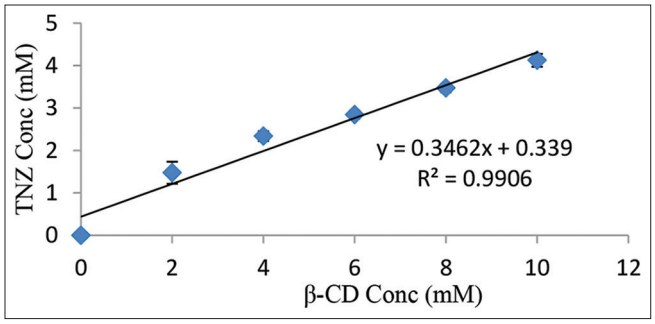

Figure 2: Phase solubility diagram for tinidazole in β-cyclodextrin

Preparation of inclusion complex by kneading method

TNZ 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 used.[11]

FTIR spectroscopy

IR spectrum (Alpha, Bruker, USA) of TNZ, β-CD, and TNZ 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 wavenumber 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.

NMR spectroscopy

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

Dissolution study

The dissolution release profile of TNZ and TNZ inclusion complex was studied using dissolution test apparatus (Eight Station Tablet Dissolution Test Apparatus, DS-8000, Lab India) in PBS pH 6.8 dissolution medium.[12] Sample equivalent to 50 mg of TNZ was weighed and put into 900 ml of PBS, maintained at 37°C ± 0.5°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

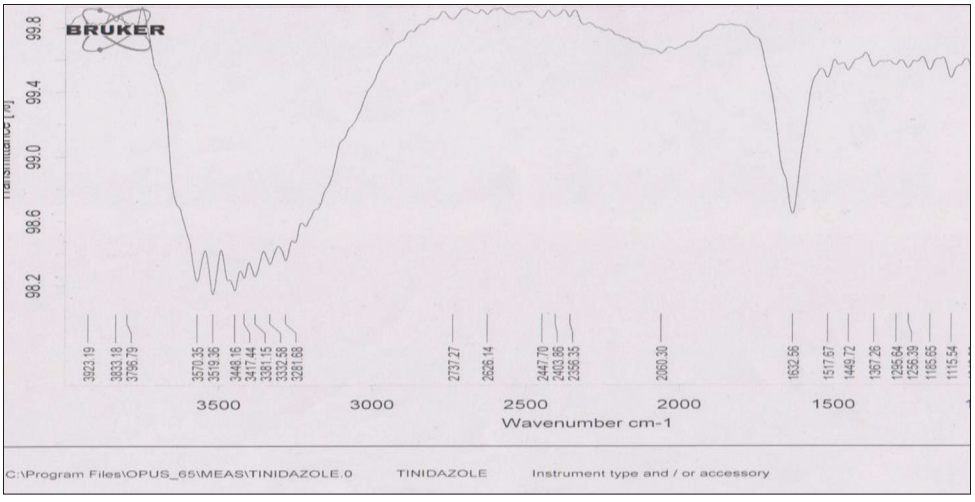

Figure 3: Fourier-transform infrared spectroscopy of tinidazole

Table 1: Wavenumber for tinidazole and tinidazole and inclusion complex

S. No.	Wavenumber of complex (cm^{-1})	Wavenumber of tinidazole (cm^{-1})
1	3922.51	3923.19
2	3822.97	3833.18
3	3739.79	3796.79
4	3363.69	3381.15

Table 2: 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	4.9019	4.8702	0.0317
H3	4.9223	4.8927	0.0296
H4	4.6023	3.9735	0.6288
H5	4.5931	3.9964	0.5967

β-CD: β-cyclodextrin

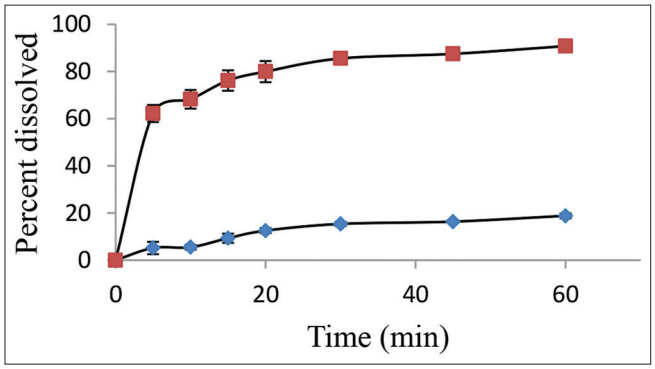

Figure 4: Dissolution profile of tinidazole and inclusion complex (TNZIC)

using UV spectrophotometer (UV-1700-Pharmaspec Shimadzu, Japan) at 277 nm. The procedure was repeated 3 times.

RESULTS AND DISCUSSION

Phase solubility studies

Phase solubility studies conducted at room temperature showed that the solubility of TNZ increased linearly ($R^2 = 0.960$) as a function of β-CD, as shown in Figure 2. The phase solubility diagram of TNZ with β-CD in selected concentration range displayed A_L type of diagram, i.e., solubility of TNZ was increased 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.

FTIR spectroscopy

The FTIR spectroscopy of (a) TNZ, (b) β-CD, and (c) tinidazole inclusion complex shown in Figure 3 and the interpretation was done using standard wavenumber ranges with observed wavenumber 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 TNZ, β-CD, and TNZ inclusion complex. The FTIR spectrum of TNZ 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) and 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 TNZ 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 TNZ complexed with β-CD were due to restriction of vibration of TNZ molecule upon its encapsulation into β-CD cavity.[13] Therefore, the FTIR spectroscopy results indicated that inclusion complex of TNZ was obtained.

Proton ^{1}H-NMR spectroscopy

In NMR spectra, there was a chemical shift of host and guest molecule when the guest molecule was inserted into the CD 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. In general, there is a large chemical shift observed in the inner cavity of β-CD at H3 and H5 due to inclusion phenomena.[14]

Figure 1a-c showed the protons ^{1}H-NMR spectra of TNZ, 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 4.6525–4.9983 ppm region indicating aromatic ring of β-CD.[15] Table 2 shows some chemical shifts observed for H1, H2, H3, H4, and H5. Although, the chemical shifts were somewhat higher located within the β-CD cavity for protons such as H3 that were compared to the protons, which were located at the exterior side of the cavity. Therefore, the change in shift between β-CD and TNZ inclusion complex confirms the formation of the inclusion complex.[16,17]

Dissolution study

The dissolution profiles of TNZ and its 1:1 inclusion complex with β-CD are shown in Figure 4. The pure drug showed solubility up to 5% while its inclusion complex showed 64% dissolution in the first 5 min. The total drug dissolved in 1 h from TNZ was about 19% compared to 89% from inclusion complex. Thus, there was 5 times increase in solubility which may help in increasing the bioavailability of TNZ.

CONCLUSION

β-CD can be used to prepare inclusion complex of TNZ. 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 the formation of inclusion complex between TNZ

and β-CD. Thus, the technique can be used to increase the aqueous solubility and bioavailability of poorly soluble drugs.

ACKNOWLEDGMENT

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.

REFERENCES

1. Mangolim CS, Cristiane M, Ana CN, Francielle S, Mauro LB, Antônio MN, *et al*. Curcumin-b-cyclodextrin inclusion complex: Stability, solubility, characterisation by FT-IR, FT-raman, X-ray diffraction and photoacoustic spectroscopy, and food application. Food Chem 2014;153:361-70.
2. Bekers O, Uijtendaal EV, Beijnen JH, Butt A, Underberg WJ. Cyclodextrins in the pharmaceutical field. Drug Dev Ind Pharm 1991;17:1503-9.
3. Hedges AR. Industrial applications of cyclodextrins. Chem Rev 1998;98:2035-44.
4. Fromming KH, Szejtli J. Cyclodextrin in Pharmacy. Dordrecht: Kluwer Academic Publishers; 1994.
5. Lopes MS, Policarpo AS, Amanda GF, Maria IY, Horta AS, Alvaro JR, *et al*. The activity of a metronidazole analogue and its β-cyclodextrin complex against *Trypanosoma cruzi*. Mem Inst Oswaldo Cruz 2011;106:1055-7.
6. Tally FP, Sutter VL, Flnegold SM. Treatment of anaerobic infections with metronidazole. Antimicrob Agents Chemother 1975;7:672-5.
7. Goldring J, McNaught W, Scott A, Gillespie G. Prophylactic oral antimicrobial agents in elective colonic surgery. A controlled trial. Lancet 1975;2:997-1000.
8. Upcroft P, Upcroft JA. Drug targets and mechanisms of resistance in the anaerobic protozoa. Clin Microbiol Rev 2001;14:150-64.
9. World Health Organization. WHO Model List of Essential Medicines. 16th ed. Geneva: World Health Organization; 2015. Available from: http://www.who.int/medicines/publications/essmedlist15.pdf. [Last accessed on 2019 Jun].
10. Higuchi T, Connors KA. Phase solubility techniques. Adv Anal Chem Instrum 1965;4:117-212.
11. Sapkal NP, Kilor VA, Bhusari KP, Daud AS. Evaluation of some methods for preparing gliclazide-β-cyclodextrin inclusion complexes. Trop J Pharm Res 2007;6:833-40.
12. Juni E, Retno W, Dewi I. Preparation of an inclusion complex system of ethyl P-methoxycinnamate-hydroxypropyl-β-cyclodextrin: Characterization and solubility evaluation. Res J Pharm Biol Chem Sci 2017;8:1486-94.
13. Yapeng LU, Siyuan L, Yu ZL, Shuqin Y. Complexation of Z-ligustilide with hydroxypropyl-β-cyclodextrin to improve stability and oral bioavailability. Acta Pharm 2014;64:211-22.
14. Jullian C, Montecinos JM, Torres GZ, Aguilera B, Rodriguez J, Aranc V, *et al*. Characterization, phase-solubility, and molecular modeling of inclusion complex of 5-nitroindazole derivative with cyclodextrins. Bioorg Med Chem 2008;16:5078-84.
15. Lee CW, Kim SJ, Youn YS, Widjojokusumo E, Lee YH, Kim J, *et al*. Preparation of bitter taste masked cetirizine dihydrochloride/β-cyclodextrin inclusion complex inclusion complex by supercritical antisolvent (SAS) process. J Supercrit Fluids 2010;55:348-57.
16. Youming Z, Xinrong D, Liangcheng W, Taibao W. Synthesis and characterization of inclusion complexes of aliphatic-aromatic poly (Schiff base) s with β-cyclodextrin (highlight). J Incl Phenom Macrocycl Chem 2008;60:313-9.
17. Chen M, Diao G, Zhang E. Study of inclusion complex of β-cyclodextrin and nitrobenzene. Chemosphere 2006;63:522-9.