ISSN: 2322 - 0902 (P)
ISSN: 2322 - 0910 (O)

International Journal of Ayurveda and Pharma Research

Research Article

PREPARATION AND EVALUATION OF CYCLODEXTRIN COMPLEXES OF ANTI-TUBERCULAR DRUG RIFAMPICIN FOR IMPROVED BIOAVAILABILITY

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Received on: 22/07/2015 Revised on: 09/08/2015 Accepted on: 14/08/2015

ABSTRACT

The aim of the study was to increase the aqueous solubility, dissolution rate, stability, in vitro anti-tubercular activity and bioavailability of rifampicin by the way of inclusion complexation. Methyl β-cyclodextrin in case of rifampicin were used. Based on phase solubility studies that stoichiometry of complex of with respect to β-cyclodextrin for rifampicin was found to be 1:1 molar ratio. Different methods of preparation such as kneading and common solvent were employed to prepare the complexes. Formation of complexes In case of rifampicin, interaction of 4-methyl piperazin-1-ylimino-methyl (side chain) of rifampicin with the cyclodextrin molecule was confirmed by FTIR and 1H-NMR. The complexes prepared by different methods were subjected to solubility and in vitro dissolution studies. In case of rifampicin, in vitro anti-tubercular activity was found to be enhanced for the complexes of rifampicin indicated by a reduction in MIC of rifampicin. The oral bioavailability of rifampicin-Mβ-CD complex prepared by common solvent method was improved significantly. The results of stability studies revealed that stability of the drugs in solution and solid state were improved significantly due to complexation. Photostability of rifampicin is enhanced significantly by the way of complexation. Thus inclusion complexation of rifampicin with β-cyclodextrin, β-cyclodextrin derivatives and γ-cyclodextrin improved its physical properties, bioavailability and in vitro activity.

KEYWORDS: Rifampicin, β-cyclodextrin derivatives, γ-cyclodextrin, Dissolution, Characterization, Bioavailability, Thermal Stability, Photostability, in vitro anti-tubercular activity.

INTRODUCTION

Pyrazinamide (Pyr) and rifampicin (Rif) are two of the first-line anti-tubercular drugs. They are poorly soluble in water. It is known to cause serious side effects such as hepatotoxicity, anorexia, nausea, vomiting and dysuria. The treatment for tuberculosis requires long time therapy, which may aggravate gastric adverse effects. Rif is prone to acid hydrolysis giving rise to 3-formylrifampicin SV where the side chain i.e. 4-methyl piperazin-1-ylimino-methyl is detached. Rifampicin is also prone to air oxidation of the paraphenolic groups in the naphthalene ring to give the p-quinone. It was reported that its oral absorption is decreased when side chain, 4-methyl piperazin-1-ylimino-methyl is detached. The mechanism of antibacterial action of rifampicin is inhibition of the activity of the enzyme DNA-directed RNA polymerase (DDRP). Although the main mechanism of resistance is the modification of the target enzyme, some mutagenic treatments yield resistant mutants in which the RNA polymerase is still highly sensitive to the drug, but the rate of rifampicin uptake is reduced. The mechanism of this permeability mutation is not yet clear. 6 Any attempt to increase the permeability would be advantageous in the treatment of resistant mutant. Cyclodextrin (CD) has been reported to increase permeability. Main adverse effects of rifampicin are flu-like syndrome and cutaneous syndrome, nausea vomiting, aneroxia, diarrhoea and epigastric distress. Thus it was felt that there is a need to reduce the gastric distress, increase the uptake of rifampicin in order to reduce the MIC, increase the stability and improve bioavailability.

Material and methods

β-CD (HiMedia Laboratories Pvt Limited, Mumbai, India) and all other chemicals used were

A.R.Grade (Merck, Limited, Mumbai, India).β-CD derivatives that is methyl-β-CD were obtained as gift sample. Rifampicin was obtained as gift sample from KAPL, Bangalore, India. All the chemicals (Sigma Aldrich Chemie Gmbh, Steinheim, Germany) were purchased for the preparation of Lowenstein-Jensen medium (L.J. medium). HPLC grade water (Merck Limited, Mumbai, India) and acetonitrile (Qualigens Fine chemicals, Mumbai) were purchased. Dichloromethane and diethyl ether are analytical grade were freshly distilled before use; hydrochloric acid; water was HPLC grade (Merck Limited, Mumbai, India), acetonitrile (Qualigens Fine chemicals, Mumbai) and paracetamol.

Phase Solubility: Phase solubility studies were carried by taking excess of rifampicin is added to 10 ml of an aqueous solution of β-CD ranging from 5 mM to 15 mM and different concentrations 100mM of β-CD derivatives in a series of 25 mL stoppered conical flasks and the mixtures were shaken for 48 hours at room temperature on a rotary flask shaker. After 48 hours shaking to achieve equilibrium, 2-mL aliquots were withdrawn at 12-hour intervals and filtered immediately using a 0.45 µl nylon disc filter. The filtered samples were diluted suitably and assayed for pyrazinamide by measuring absorbance at 475 nm by using Shimadzu double beam UV spectrophotometer. Shaking was continued until 3 consecutive estimations were the same (96 hours). The phase solubility experiments were conducted in triplicate. The blanks were performed on the same concentrations of CDs in water so as to cancel any absorbance that may be exhibited by the CD molecules.

$$K_{1:1} = \frac{\text{slope}}{S_o\,(1\text{-slope})}$$

Where S_o is the intrinsic solubility of rifampicin.

Preparation of the complexes (Solid Binary System): Different methods were used for the preparation of β-CD complexes of rifampicin. The methods include common solvent (cosolution) and kneading. In case of rifampicin 1:1 molar ratio of drug with methylβ-CD, were prepared. The optimum ratio was determined by phase solubility studies. Physical mixtures (PM) of drugs and cyclodextrin were prepared in the same ratios.

Physical Mixture (PM): 1:1 molar ratio in case of rifampicin to β-CD and its derivatives was taken in mortar in geometrical ratio and triturated for one hour. The dry powder obtained was passed through the sieve no. 85 and stored in a desiccator.

Kneading complex (KN):1:1 molar ratio of rifampicin to β-CD was taken in mortar and adequate distilled water was added to make paste-like consistency. The paste was kneaded for half an hour and dried in an oven at RT for one week. The obtained mass was then pulverized, passed through sieve no. 85 and stored in a desiccator.

Common Solvent (CS): Similarly drug and complexing agents were dissolved in suitable solvent (chloroform or DMF are used in case of rifampicin). The solutions were mixed in ratio as mentioned in physical, and kneading method and evaporated to dryness at ambient temperature. The dry mass obtained was pulverized, passed through the sieve no. 85 and stored in a desiccator.

Content uniformity

The percentage of rifampicin in each of complexes was determined by using the complex containing 100 mg of the drug. The sample that is rifampicin after suitable dilution with pH 7.4-phosphate buffer was determined at 475 nm by using Shimadzu double beam UV spectrophotometer model UV-1601 116.

CHARACTERIZATION STUDIES

β-CD complexes were investigated to establish formation of complex and the intactness of the drug in the complex by FAB mass spectrometry, FTIR, DSC, 1H-NMR, SEM and powder x-ray diffraction methods.

Fourier Transform Infrared (FTIR Studies)

IR spectroscopy was carried out for the following a) pure drugs, b) β-CD and derivatives, c) complexes using Shimadzu FTIR model 8700 by taking KBr disc.

Differential Scanning Calorimetry (DSC) Studies

Individual coils that are heated and cooled at the same rate heat DSC in which sample and reference containers are not contiguous and heated them separately. Platinum resistance thermometers monitor the temperature of the sample and reference holders and electronically maintain the temperature of the two holders constant. Differential scanning calorimetry of pure drug, its physical mixture of β-CD, all the complexes were carried out (Perkin Elmer Pyris) with a temperature increase of 5 o C /min. The scanning temperature range was from 50° C to 250°C. Temperature and heat flow calibration were performed using indium as a standard.

Powder X-ray diffraction Studies

X-ray diffraction study was carried out for pure drug, β-CD, and their complexes (Philips Diffractometer Model PW 17291 and Model PW 1050/37) with a vertical goniometric using a Nickel filter cu k α radiation operating at 30 KW and 20 milli amps in the range from 5°-40° angles. The scanning rate was 1 o/min.

Proton Nuclear Magnetic Resonance (1H-NMR) Spectrometry: 1H-nuclear magnetic resonance (1H-NMR) spectroscopic experiments were performed on a Varian 500 MHz with dual full-band channels and z-axis

gradients using a Varian. The spectra obtained were measured at 298 K with an operating frequency of 499.742 MHz. The 90 o pulse width for 1 H was 10.8 at a transmitter power of 50. Solutions were purged prior to data collection under a stream of argon for 1 hour to reduce the amount of dissolved oxygen.

Scanning Electron Microscopy (SEM)

The surface morphology of pure materials, their treated counterparts, and all binary systems were examined by scanning electron microscope. The samples were fixed on a brass stub using double-sided tape and then gold coated in vacuum by a sputter coater. The pictures were taken at an excitation voltage of 20 Kv. JSM-840A Scanning Microscope, Jeol-Japan with JFC-1100E Ion Sputtering Device was used.

Fast Atomic Bombard (FAB) Mass Spectra: were recorded on a JEOL SX 102/DA-Mass Spectrometer/Data system using argon/xenon (6kV, 10mA) as the FAB gas. The accelerating voltage was 10kV and the spectra were recorded at room temperature and m-nitrobenzyl was used as the matrix.

Solubility Studies

The solubility of pure rifampicin and the complexes was determined as reported. Excess of pure drug or the complex was added in 25 ml conical flask and the mixture was shaken for 24 hours in a thermostatic shaker water bath (REMI Model) at RT at the rate of 110-120 strokes/min, till a saturated solution in distilled water was obtained. The sample was filtered using Whatman filter paper No 44. The filtrate was suitably diluted and the concentration of drug was determined spectrophotometrically.

Bioavailability Studies

Eighteen male New Zealand rabbits weighing 1.5 to 2.5 kgs were used. The following formulation was tested (1) I.V. Injection of pure rifampicin, (2) rifampicin-M ethyl-β-cd inclusion complex (CS) suspension in 1% sodium carboxymethylcelulose, (10 mg rifampicin/kg) in 1%, (3) rifampicin suspension in 1% sodium carboxy methyl cellulose (low viscosity) given orally.

High Performance Liquid Chromatography (HPLC):

The liquid chromatography consisted of a Waters 6000 A pump, a U6K injector with a 25 loop (Waters Assoc., Milford, MA, U.S.A.) and a variable-wavelength Hitachi 220-S UV detector with a chart recorder (Hitachi, Tokyo, Japan). Analyses were performed on a reversed-phase C 8 Column (Hibar, LiChroCart RP-8, 250 mm x 4.6 mm I.D., Merck).

The operating conditions for the HPLC system were: a mobile phase of a freshly prepared mixture of acetonitrile and 10 mM phosphate buffer at pH 3.5 (1:9 v/v); flow-rate 1.5 ml/min; temperature, ambient (25 ±1o C); UV detector wavelength, 340.

Design: The rabbits were divided into three groups of six rabbits (n = 6). The order of administration was randomly selected. All rabbits were fasted over night with ad libitum access to water. All rabbits received rifampicin solution by intravenous administration into marginal vein at a dose of 30 mg/kg. Rabbit's in-group 1 received pure rifampicin plain powder suspension in 1% sodium carboxy methyl cellulose while rabbit's in-group 2 received rifampicin complex powder suspension in 1% sodium carboxy methyl cellulose by oral feeding tube at a dose of 30 mg/kg, followed by 1 ml of deionized water. Rabbit's in-group 3 received pure rifampicin intravenous injection. Blood samples were collected from the marginal vein at 0, 0.25, 0.5, 1, 2, 3, 5, 7 and 24 h. Immediately the samples were precipitated the plasma proteins by adding methanol (200 µL), followed by 1 M potassium dihydrogen phosphate in 0.2% ascorbic acid (1mL) to adjust the pH to 4.2.

Extraction of Rifampicin: To 2 ml of plasma in a 15-ml test-tube 0.4 ml of 10 % (v/v) aqueous acetic acid was added to adjust the pH to 4.2. Rifampicin was extracted by shaking with 7 ml of diethyl ether:dichloromethane (2:1 v/v) that, after centrifugation for 10 min at 2059 g, was transferred to a tapered test-tube and evaporated under nitrogen at 40° C. The residue was dissolved in 200 µl of methanol of which 20 µl aliquot was injected into the HPLC column and eluted with system. The elute was detected at 248 nm. A simple, specific and sensitive high-performance liquid chromatography (HPLC) method was developed for the determination of rifampicin in human plasma. Rifampicin and sulindac (internal standard) are extracted from human plasma using a C 2 Bond Elutextraction column. A 100-µl volume of 0.1 M HCl is added to the plasma before extraction to increase the retention of the compound on the extraction column. Methanol (1ml) is used to elute the compounds and 0.5 ml of 3-mg/ml ascorbic acid in water is added to the final elute to reduce the oxidation of rifampicin. Separation is achieved by reversed-phase chromatography on a Zorbax Rx C 8 column with a mobile phase composed of 0.05 M potassium dihydrogen phosphate-acetonitrile (55: 45, v/v). Detection is by ultraviolet absorbance at 340 nm. The retention times of rifampicin and internal standard are approximately 4.4 and 7.8 min, respectively. The assay is linear in concentration ranges of 50 to 35 000 mg/ml. The quantitation limit is 50ng/ml. Both intra-day and inter-day accuracy and precision data showed good reproducibility.

STABILITY STUDIES

Solid-state Stability studies: Accelerated Thermal Stability studies (ICH): For rifampicin the 25° C ± 2° C /60% RH ± 5% are used as it is stored at cool temperature.

Photostability Studies: Photostability studies are carried out in Newtronic chamber. Studies are carried in UV light and tube light 1.2 million lux hours and an integrated near ultraviolet energy of not less than 200-watt hours/square meter to allow direct comparisons

to be made between the drug substance and drug product.

HPLC System: The liquid chromatography consisted of a Waters 6000 A pump, a U6K injector with a 25 loop (Waters Assoc., Milford, MA, U.S.A.) and a variable-wavelength W 2487 UV detector. Analyses were performed on reversed-phase Symmetry C 18 5 µm W032815 4.6 x 150 mm.

The operating conditions for the HPLC system were: Composition of mobile phase is methanol and 0.015 disodium hydrogen phosphate (75+25, v/v), pH adjusted to 4.5 with 85% phosphoric acid. Flow rate is (isocratic) 1 ml/min, temperature, ambient (25 ±1o C); UV detector wavelength 254 nm; sensitivity scale, 0-0.01 a.u.f.s.

PROTOCOL OF HPLC

Sample preparation: Suitable quantity of composite sample was extracted with methanol and filter. The filtrate was suitably diluted with MP to get concentrations as described under standard solution to establish linearity range. **HPLC System:** Waters LC systems equipped with dual piston. 510 reciprocating pump and 7125 Rheodyne injector with 20 µl fixed loop.

Column: Novapak RP C – 18 (5 µm) column. 150 x 3.9 mm

Temperature: Ambient

Chromatographic conditions:

1. Composition of mobile phase and its pH: Methanol-0.01 to 0.02 M disodium hydrogen phosphate (75+ 25, v/v) or (70+30, v/v), pH adjusted to 4.5 with 85% phosphoric acid.
2. Flow rate isocratic: 1 ml/min
3. Volume injected: 20 µl
4. Type of detector, detector mode and wavelength: UV-254 nm
5. Retention time for Rifampicin- 2.91 and
Degradation product of Rifampicin- 5.95
6. Linearity range: Rifampicin – 10- 100 µg/ml
7. Recoveries (%): 97.50- 100.45 for drugs.

RESULTS

Rifampicin with β-cyclodextrin derivatives

Phase Solubility

Phase solubility studies of rifampicin with β-CD derivatives showed that it followed A L- type of phase solubility (Fig. 33 and Fig 34). 41 The slope of the line of phase solubility is less than unity indicating that stoichiometry is 1:1 for drug to complexing agent. The apparent 1:1 stability constants of all complexes were calculated according the equation given below.

$$\frac{\text{slope}}{S_o\,(1\text{-slope})} = K_{1:1}$$

Where S_o is the intrinsic solubility of rifampicin. Slope of the phase solubility line.

Content Uniformity The actual drug content in each binary mixture was determined. The results are reported (Table 22). The physical mixture, kneaded and common solvent products showed a good agreement between theoretical and actual drug content.

Solubility

The aqueous solubility of rifampicin was found to be maximum in Mβ-CD complexes, (Table 3, Fig.2) while the physical mixture showed a 2.4 fold increase in solubility. The increase in solubility was 2 fold when compared to that of pure drug in case of CS complex of Mβ-CD. In case of β-CD complexes, CS complex showed 2 fold increases in solubility while KN complex showed the least solubility, less than that of the pure drug.

Fourier Transform Infrared Spectroscopy (FTIR Spectroscopy): Considerable information can be obtained from FTIR spectral interpretation, regarding the involvement of various functional groups in hydrogen bonding. This generally shifts the absorbance bands to the lower frequency increases the intensity and widens the band caused by stretching vibration of the group involved in the formation of the hydrogen bond. In case of FTIR of M β-CD complexes (Fig. 3), the stretch of >CH=N of rifampicin from 1647.1 cm -1 to 1625.9 cm -1, the stretch of -C-H, from 2883.4 cm -1 to 2844.8 cm -1 were shifted, and 1436.9 cm -1 of C-H was disappeared in the KN further confirmed the formation of complex with part of molecule i.e. 4-methyl piperazin-1-ylimino-methyl side chain was included in the complex. Breakage of hydrogen bond at 1023 cm -1 frequency of secondary alcoholic group was observed in all the cases. But no changes were observed in case of PM. The reduced intensities of peaks of rifampicin were observed in CS complex. Thus complexation of rifampicin could be obtained in M β-CD complexes where a part of drug molecule might have included within the cyclodextrin molecule.

Differential Scanning Calorimetry (DSC)

M-β-CD Complexes

In DSC thermograms of M β-CD complexes (Fig. 4), endothermic melting peaks of pure drug at 185.020° C and 229.110° C were reduced in area significantly or absent in the complexes obtained by different methods. New exothermic peak was observed at 214.230°C in kneading complex and endothermic peaks of rifampicin were almost disappeared, which indicated maximum interaction. Similarly in case of CS complex the endothermic peaks of rifampicin were absent, which indicated complete interaction.

Powder X-ray diffraction

X-ray diffraction reveals that the crystalline nature and change in the crystalline structure. There

were 47 peaks and 29 peaks in powder X-ray diffraction patterns of β-CD and pure rifampicin respectively that showed their crystalline nature. One peak at 12 o of 2 θ angles was observed in pure M β-CD and 12 peaks were observed in the range of 2 θ angles from 11 o to 27.5 o in pure rifampicin. As expected 13 peaks is their mixture, provided there is no interaction. However X-ray diffraction pattern showed 4 peaks in the physical mixture and single peak each in the complexes prepared by KN and CS.

Proton Nuclear Magnetic Resonance (1H-NMR)

1H-NMR studies are used to investigate whether the type of interaction is hydrogen bond formation, van der Waals forces or dipole-dipole interaction. It also helps to interpret the geometry, stoichiometry and alignment of the guest molecule in the CDs cavity. 1H-NMR study is used to investigate the type of interaction. The changes in the chemical shift were positive (desheilding) and negative (shielding). In this study the changes in chemical shifts to second decimal were taken. The 1H-NMR studies revealed that changes in chemical shift of group, –CH=N of rifampicin molecule in the complexes are given in Table 23. Another group, >NH group ppm of pure rifampicin has been changed to second decimal (Table 24). The 1H-NMR spectra of rifampicin, cyclodextrin complexes prepared by different methods are given in Fig. 6, Fig. 7, Fig.8

Scanning Electron Microscopy Studies

SEM studies reveal change in the morphology of particles and homogeneity of the product. Even if there is a clear difference in crystallization state of the raw material and the products, this study is inadequate to confirm inclusion complexation, but helps to assess the existence of homogeneity of single component in the preparations obtained. SEM observed that SEM of the M β-CD (Fig. 9) had shown ball shaped in pure compound and these ball shaped particles are disappeared in the complexes. Both the drug and pure rifampicin particles were observed in the PM. KN complex has shown small crystals while irregular shaped crystals were seen in the CS. It was observed that complex is single component indicating the interaction in KN and CS.

Mass Spectroscopy

Mass spectrometry of CS complex of M β-CD and rifampicin reveals that mass of theadduct formed out of complexation and also supports that the stoichiometry of the complexes. The FAB mass spectroscopy showed that adduct, with molecular weight 2126 +, which is sum of M β-CD, 1303 +and, rifampicin, 822.95 +. This supports the stoichiometry of rifampicin and M β-CD for the formation of complex is 1:1.

In vitro Anti-tubercular Activity

The MIC for laboratory strain (H 37 RV) was found to be identical that is 4μg/ml for pure rifampicin as well as complexes of rifampicin, but, in case of wild strain No.10934, MIC was found to be 64μg/ml for pure rifampicin. However, a reduction in MIC from 64μg/ml to32 μg/ml was observed in all the complexes of rifampicin. 28 M β-CD complexes were showing no growth at all concentration thus exhibiting maximum effectiveness.

BIOAVAILABILITY

Plasma profiles are given the in Table 8. AUC IV, 0-24 of rifampicin (Fig. was found to be 90.34 ± 1.54 hr μg/ml (Kinetica Software was used. The AUC 0-24 of rifampicin CS complex was 85.86 ± 2.98 hr μg/ml (prepared by M β-CD (common solvent) and AUC 0-24 for pure rifampicin (oral) was 64.52 ± 3.6 hr μg/ml. The C max found to be 25 ± 0.73 μg/ml, 14.4± 0.13μg/ml, and 15.7 ± 0.35 μg/mlfor IV, oral pure drug, and CS respectively. The T max was found to be 1.15 hrs in case pure rifampicin and CS complex. The half-life was found to 6.2 ± 1.2 hrs, 6.5 ± 1.3 hrs, and 6.96 ± 1.32 hrs for IV, oral pure drug, and CS respectively.

Stability Studies in Solution

Thermal Stability Studies: The thermal degradation constant for pure rifampicin on the basis of first order kinetics was found out to be 8.9535 ± 0.2795 x 10 -5 day -1, the KN complex of M β-CD showed the least value, 6.101 ± 0.190 x 10 -5 day -1. The reduction in degradation constant found to be statistically significant ($p < 0.05$).

Photostability Studies: The photostability studies revealed that there is no physical changes were observed. There is no observable colour change in samples. The reduction or decrease in purity for rifampicin and the least of all rifampicin-CD in 6 months were observed in 6 months is 1.69%, 1.50% respectively.

CONCLUSION

Thus it can be concluded that rifampicin complex rifampicin could be successfully prepared by the kneading and common solvent methods. The complexes were found to have enhanced solubility, and dissolution rate when compared to the pure drug. FAB mass spectrometry, FTIR, and 1H-NMR methods confirmed the intactness of drug in the complexes. In case of rifampicin a part of rifampicin molecule i.e. 4-methyl piperazin-1-ylimino-methyl chain was interacted with cyclodextrin molecules as indicated by FTIR and 1H-NMR. Powder x-ray diffraction, DSC and SEM studies confirmed the change in crystallinity of both drugs. The M β-CD complexes had shown maximum stability constant, solubility, and anti mycobarium activity. In case of DSC of M β-CD complexes, the endothermic peaks of rifampicin were almost disappeared. The thermal and photostability of rifampicin was improved significantly. Increased i n vitro anti-tubercular activity of the rifampicin was observed and it is concluded that these complexes have a potential of increased anti-tubercular activity.

Similarly the dose of rifampicin can be reduced as MIC was reduced. Thus inclusion complexation of pyrazinamide with β-CD and rifampicin with β-cyclodextrin, β-cyclodextrin derivatives and γ-cyclodextrin improved its physical properties, bioavailability and in vitro activity.

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Cite this article as:
B. Prakash Rao, Sarasija Suresh, Suresh Sah, Narendra C, Manuka. Preparation and Evaluation of Cyclodextrin Complexes of Anti-Tubercular Drug Rifampicin for Improved Bioavailability. International Journal of Ayurveda and Pharma Research. 2015;3(8):65-76.

Source of support: Nil, Conflict of interest: None Declared

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TABLES

Table: 1 Comparison of the stability constants (K s) of complexing agents with the rifampicin

Types of cyclodextrin	Stability constant(K_a) By solubility method(m^{-1})
M β-CD	294.27±21.4

Each value is an average of three determinations with SE

Table: 2 Drug content in complexes of rifampicin (Binary Mixtures) (% molar ratio ± SE)

Complexing agent	Methods of preparation	Theoretical (%)	Actual (%)
Mβ-CD	PM	50	49.78 ± 0.18
	KN	50	48.89 ± 0.12
	CS	50	49.70 ± 0.14

Each value is an average of three determinations with SE

Table: 3 Solubility of rifampicin and its complexes in water

S.N.	Complexing agent	Methods of preparation	Solubility (mg/ml)
1	Methyl-β-cyclodextrin	Physical mixture	3.200 ± 0.058
2		kneading	2.760 ± 0.058
3		Common solvent	2.960 ± 0.048

Each value is an average of three determinations with SE

Table 4: 1H-Chemical Shifts corresponding to CH=N group of rifampicin (δ_{free} = 8.826) in the Presence and Absence of complexing agents

Complexing agent	Cmplex	$\delta_{complex}$	$\Delta\delta^a$
M-β-CD	PM	8.834	+0.008
	KN	8.862	+0.036
	CS	8.866	+0.040

Table 5: ^{1}H-Chemical Shifts corresponding to >NH group of rifampicin group (δ_{free} = 12.533) in the presence and absence of complexing agents

Complexing agent	Complex	$\delta_{complex}$	$\Delta\delta^a$
M-β-CD	PM	12.529	-0.010
	KN	12.516	-0.017
	CS	12.519	-0.014

Table: 6 Anti-tubercular activities of rifampicin and its cyclodextrin complexes Strain: H 37 RV

Complexing agent	Methods of preparation	Drug c. 1µg/ml	Drug c. 2µg/ml	Drug c. 4µg/ml
Pure rifampicin		**2+**	**1+**	**NG**
M-β-CD	**KN**	**3+**	**1+**	**NG**
	CN	**3+**	**5C**	**NG**

Note: 1+ = >100 colonies &<200 **2+** = >200 colonies &<300 **3+** = >300 colonies NG = No growth C = Colonies

Table-7 Anti-tubercular activities of rifampicin and its cyclodextrin complexes Strain 10934

Complexing agent	Prep^n method	Drug conc^n 16µg/ml	Drug conc^n 32µg/ml	Drug conc^n 64µg/ml
Pure rifampicin		1+	25C	NG
M-β-CD	KN	20C	NG	NG
	CS	NG	NG	NG

Note: 1+ = >100 colonies &<200 C = Colonies NG = No growth

Table: 8 Bioavailability rifampicin of IV, oral, and CS of M- β-CD

S No.	Time (hrs)	Plasma concentration (µg/ml)		
		I.V.	Oral Pure drug	Oral CS
1	0	25.00±2.00	0	0
2	0.5	18.00±1.26	5.8±0.79	7.00±0.57
3	1	13.60±1.77	11.3±0.88	12.10±1.51
4	1.5	10.30±0.96	14.4±0.23	15.70±0.61
5	2	8.8±0.17	9.3±1.07	12.10±1.03
6	3	6.6±1.51	8.6±0.99	8.60±0.44
7	4	4.1±0.84	5.4±1.32	6.90±0.35

8	6	3.8±0.62	3.1±1.10	3.60±0.44
9	8	2.4±0.62	2.2±0.30	1.98±0.18
10	24	0.8±0.11	0.8±0.14	0.80±0.18

*Each value is an average of three determinations with SD

Table 9: Degradation constants complexes of rifampicin, and β-CD derivatives in aqueous solutions at different temperatures

Complexing agent	Methods of prep^{n}	100^{o}C -k x 10^{-2} (Min^{-1})	45^{0}c -k x 10^{-2} (Min^{-1})	RT -k x 10^{-2} (Min^{-1})
Pure drug		1.126±0.016	0.136±0.021	0.0005±0.00002
M-β-CD	PM	0.8370±0.120	0.0721±0.002	0.0004±0.00002
	KN	0.5087±0.050	0.0858±0.001	0.0003±0.00002
	CS	0.5409±0.061	0.0328±0.002	0.0004±0.00002

*Each value is an average of three determinations with SE

k = first order rate constant,

- ve sign indicate the decrease in the value

Table10: Accelerated stability study of rifampicin and M β-CD complex

S.No.	Time in days	Concentration in mg			
		R	PM	KN	CS
1	0	9.997±0.0058	9.997±0.0033	9.996±0.0033	9.983±0.0033
2	30	9.975±0.0022	9.979±0.0010	9.977±0.0033	9.968±0.0017
3	60	9.947±0.0025	9.965±0.0050	9.957±0.0033	9.950±0.000
4	90	9.921±0.0047	9.942±0.0072	9.936±0.0033	9.931±0.0007
5	120	9.889±0.0003	9.916±0.0088	9.924±0.0031	9.910±0.0000
6	150	9.870±0.0058	9.892±0.0017	9.903±0.0033	9.893±0.0033
7	180	9.841±0.0058	9.873±0.0012	9.886±0.0033	9.860±0.0058

*Each value is an average of three determinations with SE

Table 11: Degradation constants of rifampicin and its cyclodextrin complexes (Thermal)

S.No.	Complexing agent	Methods of prep^{n}	Degredatation constant -k x 10^{-5}
1	Pure drug		8.954 ± 0.280
2	M-β-CD	PM	7.088 ± 0.924
3		KN	6.101 ± 0.190
4		CS	7.719 ± 0.209

*Each value is an average of three determinations with SE

Table 12: Photo stability study of rifampicin and M β-CD complexes

S.No.	Time in days	Concentration in mg			
		R	PM	KN	CS
1	0	9.997±0.0033	9.990±0.0058	9.983±0.0033	9.985±0.0029
2	30	9.971±0.0058	9.972±0.0061	9.963±0.0033	9.967±0.0015
3	60	9.942±0.0015	9.960±0.0029	9.942±0.0043	9.944±0.0031
4	90	9.917±0.0015	9.938±0.0017	9.921±0.0017	9.920±0.0006
5	120	9.897±0.0033	9.921±0.0010	9.987±0.0033	9.908±0.0044
6	150	9.869±0.0021	9.903±0.0017	9.875±0.0050	9.888±0.0044
7	180	9.841±0.0067	9.891±0.0017	9.853±0.0067	9.985±0.0029

*Each value is an average of three determinations with SE

Table 13: Degradation constants of rifampicin and its cyclodextrin complexes in photostability studies

S.No.	Complexing agent	Methods of prep^{n}	Degratation constant -K X 10^{5}
1	Pure drug		8.734 ± 0.107
2	M-β-CD	PM	5.646 ± 0.296
3		KN	7.351 ± 0.327
4		CS	0.222 ± 0.050

*Each value is an average of three determinations with SE

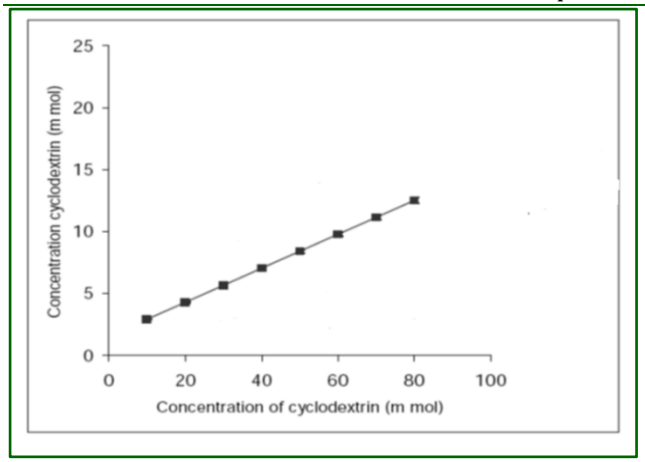

Fig: 1 Phase solubility studies of rifampicin and M β-cyclodextrin.

mcd is M –β-CD, y = 0.2216 x + 1.5573, R ² = 1

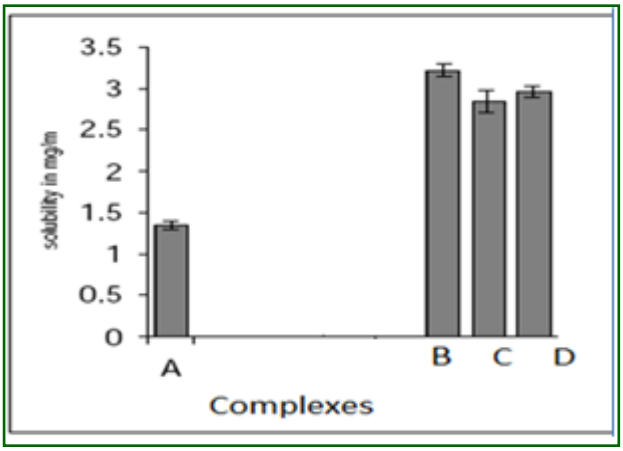

Fig: 2 Solubility of complexes of rifampicin and β-CD derivatives in water.

Solubility of (A) pure rifampicin, (B) Mβ-CD-rifampicin prepared physical mixture, (c) M β-CD-rifampicin prepared by kneading, (d) M β-CD-rifampicin prepared by common solvent method.

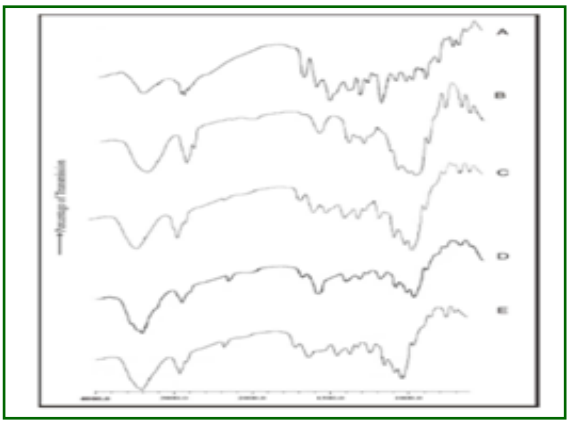

Fig: 3 FTIR Spectra of rifampicin, M- β-CD and their inclusion complexes.

FTIR Spectra of rifampicin (A) pure rifampicin; (B) M-β-CD; (C) M- β-CD-rifampicin physical mixture; (D) M-β-CD-rifampicin prepared by kneading (E) M-β-CD-rifampicin prepared by common solvent.

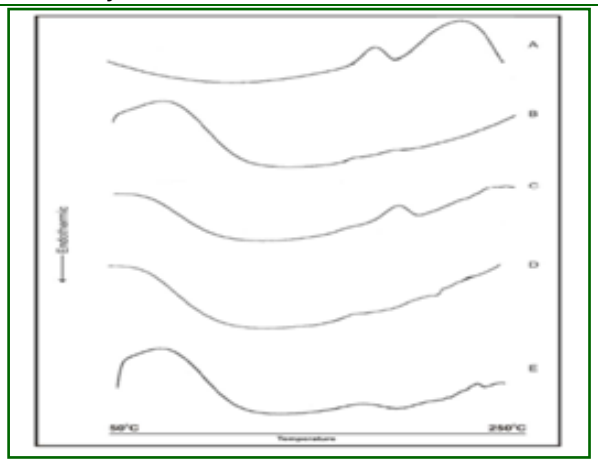

Fig. 4: DSC Thermograms of rifampicin, M- β-CD their inclusion complexes.

DSC thermogram of (A) pure rifampicin; (B) M β-CD; (C) M β-CD-rifampicin physical mixture; (D) M β-CD-rifampicin prepared by kneading; (E) M β-CD-rifampicin prepared by common solvent method.

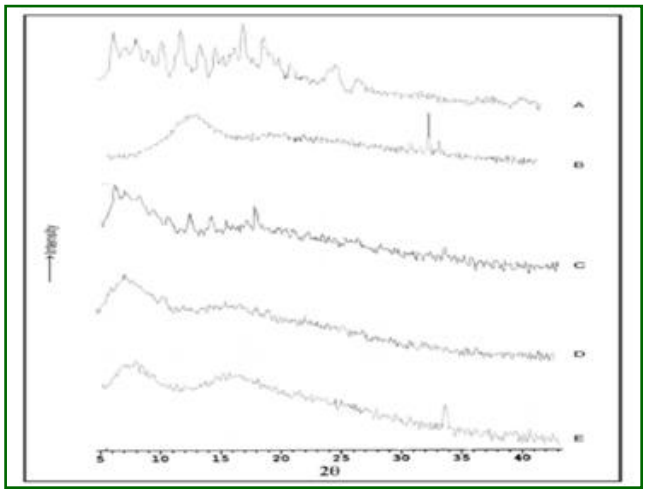

Fig. 5: Powder x-ray diffraction pattern of rifampicin, M-β-CD and their inclusion complexes.

Powder X-ray diffraction pattern of (A) pure rifampicin; (B) Mβ-CD; (C) M β-CD- rifampicin physical mixture; (D) M β-CD-rifampicin prepared by kneading and (E) M β-CD-rifampicin prepared by common solvent.

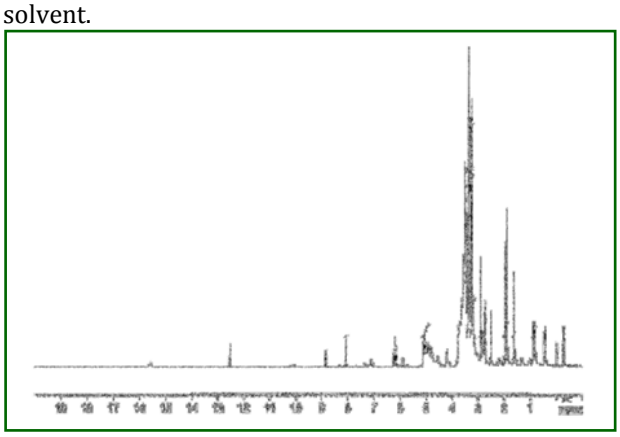

Fig. 6: 1H-NMR spectrum of rifampicin-M- β-CD (PM) in DMSO (D 6)

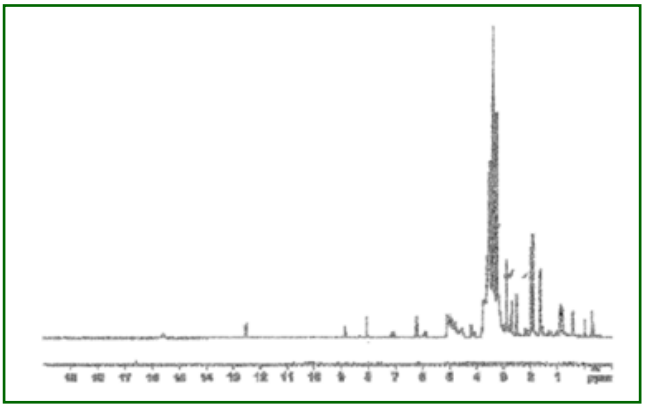

Fig. 7: 1H-NMR spectrum of rifampicin-M- β-CD (KN) in DMSO (D 6)

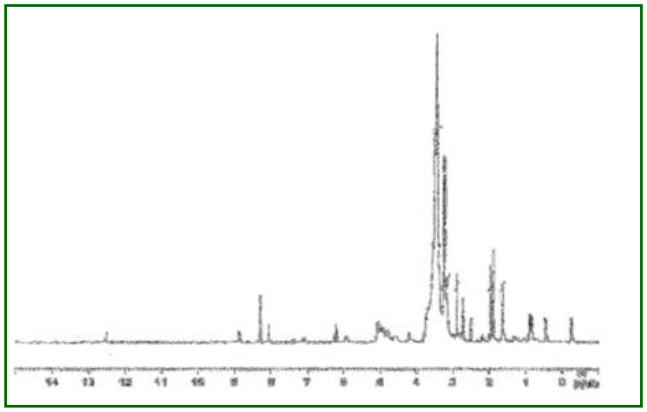

Fig. 8: 1H-NMR spectrum of rifampicin-M-β-CD (CS) in DMSO (D 6)

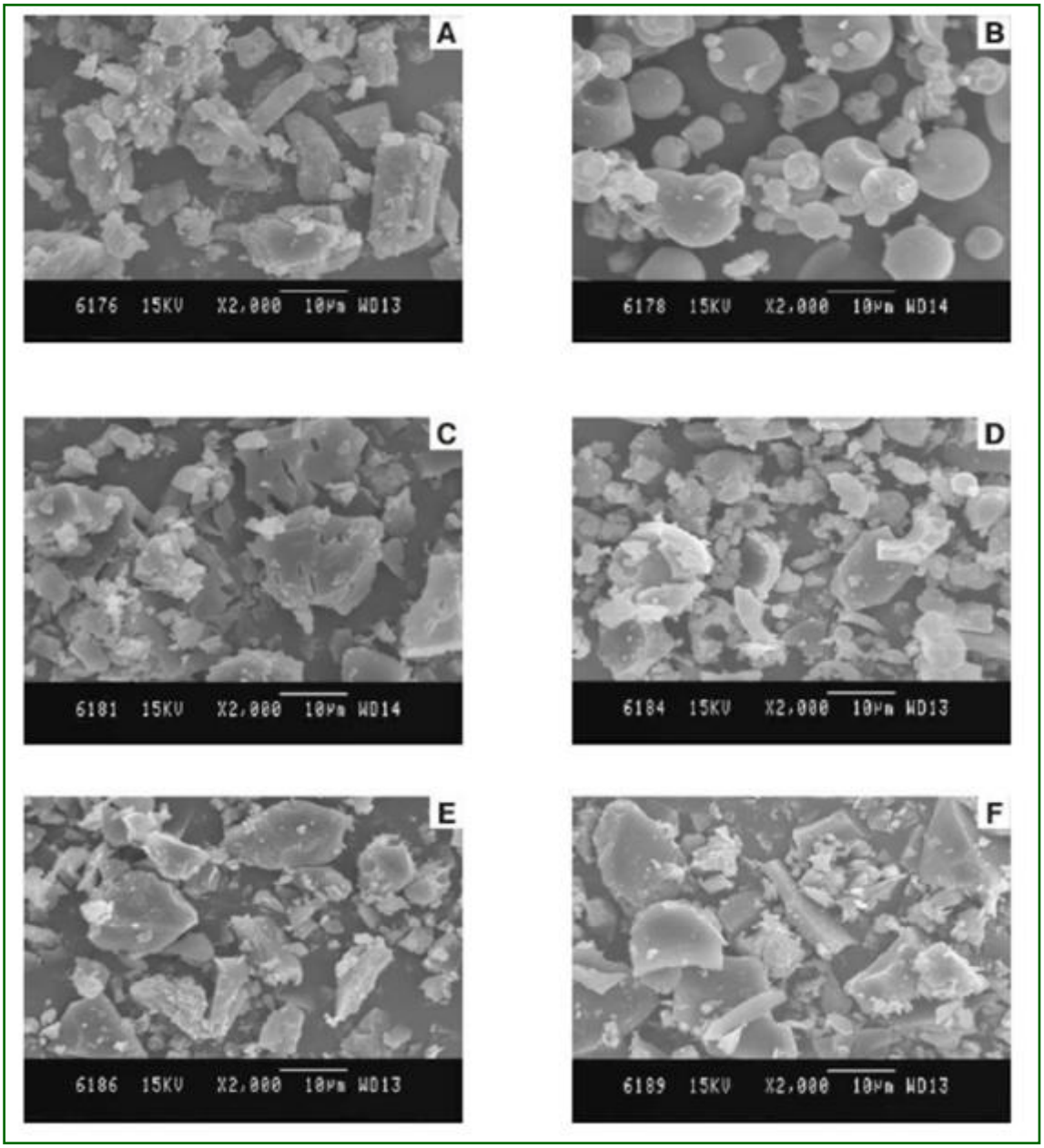

Fig 9: Scanning electron microscopy of rifampicin, M- β-CD and their inclusion complexes

Scanning electron microscopy of rifampicin (A) pure rifampicin; (B) M β-CD; (C) M β- CD-rifampicin physical mixture; (D) M β-CD-rifampicin prepared by kneading and (E) Mβ-CD-rifampicin prepared by common solvent

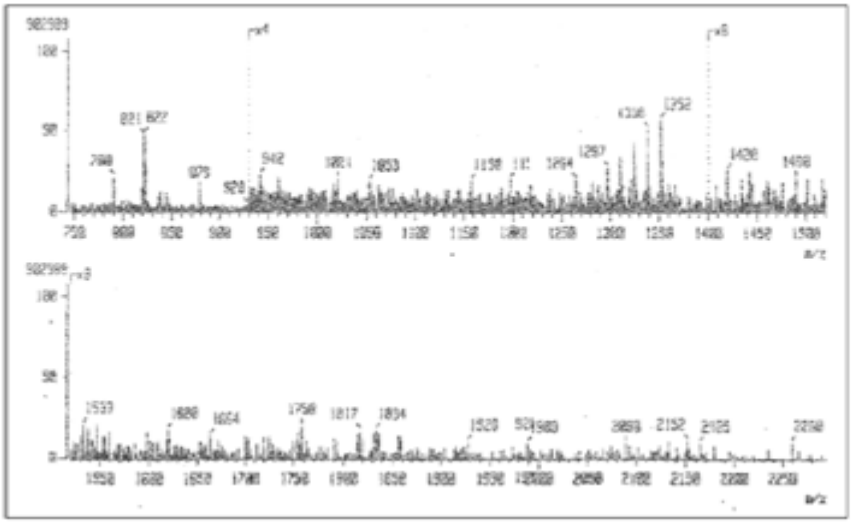

Fig. 10: Mass spectrum of rifampicin-M- β-CD at 10 kV, m-nitrobenzyl was used as matrix

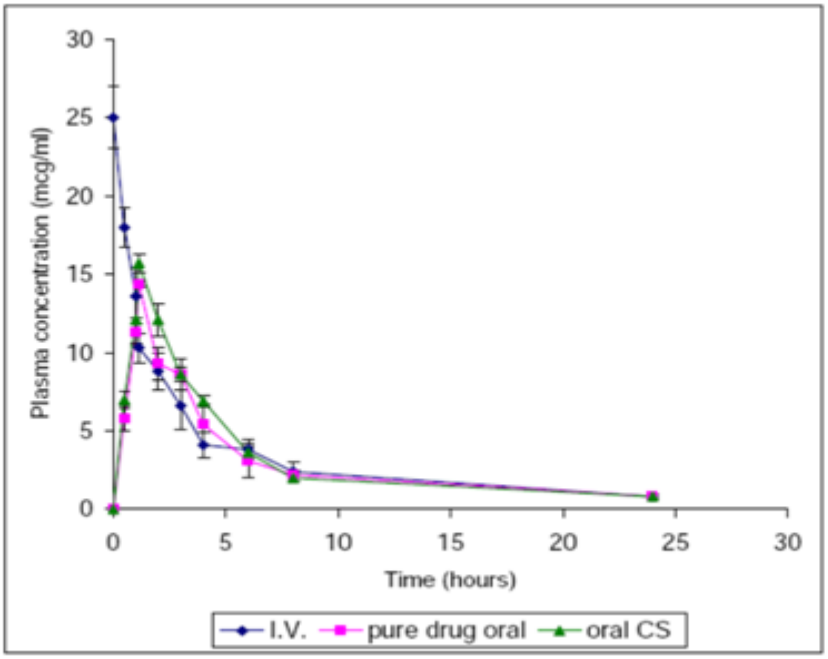

Fig. 11 : Bioavailbility of rifampicin IV, its oral and CS of M - β-CD I.V. - Plasma profiles Intravenous Injection Pure drug oral- Plasma profiles of rifampicin Oral CS- Plasma profiles of CS complex of M β-CD