

STUDIES ON THE PREPARATION, CHARACTERIZATION AND SOLUBILITY OF**B-CYCLODEXTRIN-NELFINAVIR INCLUSION COMPLEXES.**HIREMATH S.N.*¹, GODGE G.R.², KHARIA A.A.³, VAIDYA V.R.⁴**ABSTRACT: -**

Nelfinavir is a poor water-soluble antiretroviral drug with relatively low bioavailability. The effect of β -cyclodextrin on the aqueous solubility and dissolution rate of nelfinavir was investigated. Phase solubility studies indicated that the solubility of nelfinavir was significantly enhanced by complexing it with β - cyclodextrin. It was classified as Bs type indicating the 1:2 stoichiometric inclusion complexes. The various solid complexes prepared by physical mixture, Kneading, coprecipitation & common solvent methods

were characterized by DSC, X-RD & FT-IR. Dissolution study showed that the solubility and dissolution rate of nelfinavir was significantly improved by complexation with β -cyclodextrin.

KEYWORDS: - β -cyclodextrin, physical mixture, Kneading Method, coprecipitation & common solvent method.

1. INTRODUCTION: -

Nelfinavir mesylate is an antiretroviral drug which belongs to class of protease inhibitors (PIs), chemically it is [3S-[2(2S*,3S*),3a,4ab,8ab]]-N-(1,1-dimethylethyl)decahydro-2-[2-hydroxy-3-[(3-hydroxy-2methylbenzoyl)amino]-4-(phenylthio)butyl]-3-isoquinoline carboxamide monomethanesulfonate(salt). NFV is white to off-white amorphous powder, practically insoluble in water¹ and freely soluble in methanol, ethanol, isopropyl alcohol and propylene glycol². Because of its poor water solubility, its absorption is dissolution rate limited.^{3,4,5} NFV is one example of drugs that lose their efficacy upon reaching the lower portion of the GI tract. It is soluble in acidic environment. It was reported that portions of the drug which is undissolved cannot be absorbed. Portions of drug that are dissolved but not yet absorbed when they pass from the stomach into small intestine may undergo precipitation and lose their therapeutic efficacy. It was confirmed by the fact that the presence of food in GI tract significantly increases the extent of absorption of oral NFV⁵.

Although scientists had been achieved remarkable success in AIDS therapy, enormous challenges still remains for the researchers to ultimately halt the progression and find a cure for AIDS. Currently available anti-HIV agents have relatively short half-life, low bioavailability, poor CNS penetration, retention and undesirable side effects. By considering above, since HIV is global problem, we have identified NFV-a protease inhibitor and efforts have been directed to develop more suitable formulation with

improved bioavailability so as to reduce dose, side effects and subsequently the cost of HIV/AIDS therapy.

The solubility of a poorly soluble drug can be altered in many ways, such as modification of drug crystal forms, addition of cosolvents, addition of surfactants, complexation with cyclodextrins etc. Out of these methods, the cyclodextrin approach is of particular interest. Hence in this investigation inclusion complexation of nelfinavir was tried with β -cyclodextrin (β -CD) with an aim to improve its pharmaceutical properties (i.e. aqueous solubility and dissolution properties). The complex of NFV with β -CD was prepared by using various methods at 1:2 stoichiometric ratio⁶. The characterization of drug, β -CD & various complexes were done by using powder x-ray diffractometry (PX-RD). FTIR analysis of drug, β -CD & various complexes was carried out to study the interaction between drug & polymer. *In vitro* aqueous solubility and dissolution rate profile of the complexes were studied. Cyclodextrins are cyclic (α -1, 4) - linked oligosaccharides of α -D glucopyranose, containing a relatively hydrophobic central cavity and hydrophilic outer surface. Due to lack of free rotation about the bonds connecting the glucopyranose units, the cyclodextrins are not perfectly cylindrical molecules but are toroidal or cone shaped. Depending upon those models, the primary hydroxyl groups are located on the narrow side of the cone shape, while secondary hydroxyl groups are located on the wider edge. From last two decades, CDs and their derivatives have been of considerable interest in the pharmaceutical field because of their ability to form complexes with a variety of drug molecules⁷. Cyclodextrins are capable to increase the solubility of water insoluble

drugs, by means of inclusion complex formation^{8,9,10,11}. The hydrophobic cavity of cyclodextrin is capable of trapping a variety of molecule within to produce inclusion complexes. Many advantage of drugs complexed with cyclodextrins have been reported in scientific literature which includes-improvement of solubility, enhancement of dissolution rate and bioavailability, active stabilization, odour or taste masking, reduced volatility, transformation of liquid or gas into solid form, compatibility improvement, irritation reduction and reduced side effects^{12,13}. They have found wide application in the food and flavor industry¹⁴, cosmetic industry¹⁵, packing industry¹⁶, textile¹⁷, fermentation¹⁸, and catalysis fields¹⁹.

2. MATERIALS AND METHODS: -

2.1 Materials: - Nelfinavir mesylate was obtained as a gift sample from Emcure pharmaceuticals Ltd., Pune, India and β -CD was from SA pharmachem Pvt. Ltd. Mumbai. All other reagents and solvents were of analytical grade.

2.2 Phase Solubility Studies: -

The phase solubility technique illustrates the evaluation of the affinity between β -CD and nelfinavir in water. The solubility measurements of nelfinavir mesylate with β -CD were performed according to Higuchi and Connors²¹. An excess amount of drug (50mg) were added to 25 ml portions of distilled water, each containing variable amount of β -CD such as 2,4,6,8,10 and 12×10^{-3} moles/liter. All the above solutions were shaken for 72hrs (100rpm) at $25 \pm 0.5^\circ\text{C}$. After shaking, solutions were filtered and their absorbance was noted at 254 nm using UV spectrophotometer (UV-1700, Shimadzu, Japan). For each concentration

two sets are prepared and average of two readings was considered. The solubility of the NFV in every β -CD solution was calculated and phase solubility diagram was drawn between solubility of NFV and different concentrations of β -CD. The apparent stability constant (K_c) according to the hypothesis of 1:1 stoichiometric ratio of NFV: β CD complex was calculated from the phase solubility diagrams using following equation²⁰.

$$K_{(1:1)} = \frac{\text{Slope}}{S_0 (1 - \text{slope})}$$

The slope is obtained from the initial straight-line portion of the plot of NFV concentrations against β -CD concentration, and S_0 is the equilibrium solubility of nelfinavir in water

2.3 Preparation of Solid inclusion Complexes:-

Solid inclusion complexes of nelfinavir with β -CD were prepared at 1:2 molar ratios.

2.3.1 Physical Mixture: -

NFV with β -CD in 1:2M ratio was mixed in a mortar for about one hour with constant trituration, passed through sieve no.100 and stored in desiccators over fused calcium chloride.

2.3.2 Kneading Method: -

NFV with β -CD in molar ratio of 1:2M was taken. First cyclodextrin is added to the mortar, small quantity of 50% ethanol (water: ethanol; 1:1v/v) is added while triturating to get slurry like consistency. Then slowly drug is incorporated into the slurry and trituration is further continued for one hour. Slurry is then air dried at 25°C for 24 hrs, pulverized and passed through sieve no.100 and stored in desiccators over fused calcium chloride.

2.3.3 Co-precipitation Method: -

NFV was dissolved in ethanol at room temperature and β -CD was dissolved in distilled water. Different molar ratios (i.e.1: 2M) of NFV and β -CD was taken. The mixture was stirred at room temperature for one hour and then slowly evaporated on a boiling water bath. The inclusion complex precipitated as a crystalline powder was pulverized and passed through sieve no.100 and stored in desiccators till free from any traces of the organic solvent.

2.4 Physicochemical characterization of Nelfinavir-CD solid binary systems

2.4.1 Powder X-ray Diffractometry: -

The X-RD patterns of drug, β -CD & complexes were recorded by using an automated Philips PW 1729 X ray generator with filter Ni, Cu radiation over intervals 10-80⁰/2 θ . The operation data were follows: -filter- Ni, x ray target-Cu, wavelength-1.542A and scanning speed 1⁰/min.

2.4.3 Fourier Transform Infrared Spectroscopy: -

Infrared spectroscopy is one of the most powerful analytical techniques that offer the possibility of chemical identification. The IR spectra of NFV and their complexes were obtained by KBr pellet method by JASCO-FTIR-5300 spectrophotometer. The various IR spectra of inclusion complexes prepared by Physical mixture, Kneading method, Common solvent method and coprecipitation method were obtained and studied for interaction patterns.

2.5 In-vitro Dissolution Studies: -

The In-vitro dissolution of the complex was compared with those of pure drug and PM. The dissolution rate studies were carried out in 900ml of (I) distilled water and (II) phosphate buffer (pH=4) using USPXXIII dissolution apparatus (Electrolab) with a pedal stirrer, according to United States pharmacopoeia (USP). The complex equivalent to 250mg of NFV was used in each test. The dissolution medium was 900 ml of distilled water & phosphate buffer (pH=4). The stirrer was adjusted at 50 rpm, and the temperature was maintained at $37^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ throughout the experiment. The samples (5ml) were withdrawn at various time intervals by means of a syringe fitted with pre-filter at known intervals of time and analyzed for drug release by measuring the absorbance at 254nm after suitable dissolution with distilled water or phosphate buffer.

3. RESULTS AND DISCUSSION: -

3.1 Phase Solubility Study: -

The phase solubility diagram for NFV: β -CD system in water can be characterized as Bs type phase solubility curve, which suggests that the molar ratio of the complexes is 1:2M. Phase solubility diagram of NFV: β -CD (Bs type), illustrates the solubility enhancing capability of CDs. It shows a typical curve whose initial rising portion is followed by a plateau region. Further increase in β -CD concentration resulted in decrease in solubility but again increase in β -CD concentration gives an increase in the solubility of nelfinavir. The apparent stability constant, Kc, obtained from the slope CDs. Linear increase of drug solubility as function of CD concentration suggest A_L type of the linear phase solubility diagram was found to be 149.45M^{-1} for β -CD.

3.2 Preparation of Nelfinavir-CD solid binary systems: -

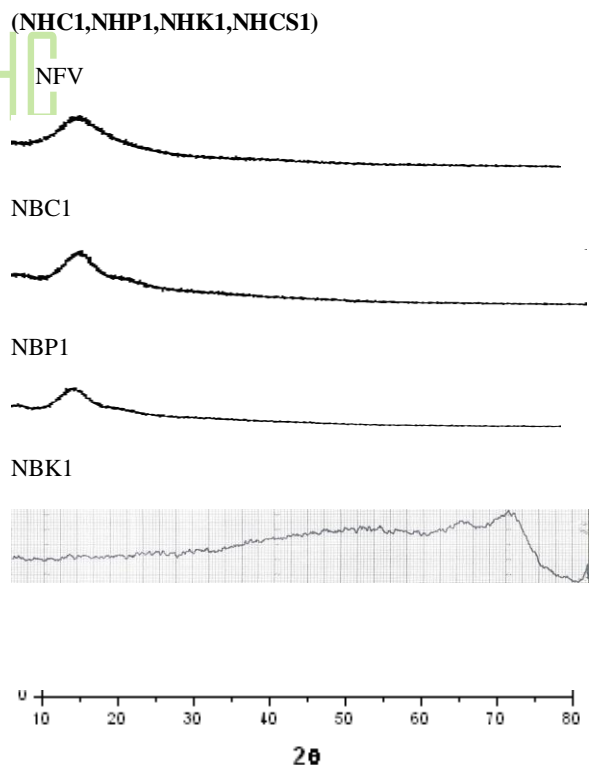
Solid binary systems of nelfinavir with β -CD were prepared using physical mixture, kneading, coprecipitation. Based on the results obtained through the phase solubility

studies, which proved the possibility of formation of higher order complexes between NFV and different cyclodextrins, 1:2 molar ratio was chosen for the preparation of solid binary systems. Physical mixtures were also investigated in the same molar ratios for comparison.

3.3 Powder X-Ray Diffraction: -

The X-RD patterns of NFV: β -CD systems are represented in **fig. (1)**. The diffractograms of NFV and β -CD exhibited a series of intense peaks which is an indicative of their crystalline nature. X-RD pattern of PM (NBP_1) is simply the superimposition of each component indicating no formation of new structure. Complex prepared by coprecipitation (NBC_1) methods showed a diffraction pattern quite similar to that of PM, while those obtained from kneading method (NBK_1) shows less peaks with low intensity. This indicates that the inclusion complex prepared by kneading method is less crystalline than the complexes prepared by physical mixture, common solvent and coprecipitation method.

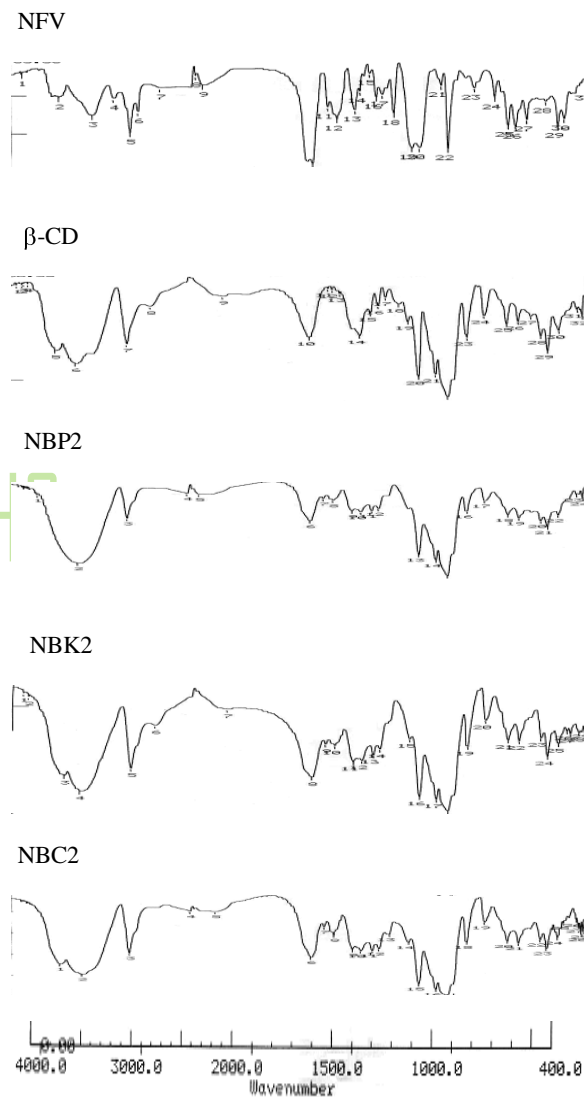
Fig.1: -X-RD Spectrum of pure drug, polymer & formulations



Time(min)	Pure Drug	NBP1	NBP2	NBK1	NBK2	NBC2
0	0	0	0	0	0	0
5	4.8	38.64	42	42.96	40.32	43.44
10	6.8	56.64	54.96	67.43	58.08	62.39
15	8.3	71.27	71.52	78.23	74.15	76.31
20	8.35	91.43	86.15	85.91	88.55	84.13
30	10.60	96.23	95.51	93.83	96.95	93.59
45	11.54	96.95	99.59	98.39	98.63	96.23
60	11.59	105.35	101.03	99.59	101.51	99.35
90	11.90					
120	12.46					

drug in 120 min. in distilled water & phosphate buffer respectively. (As shown in Table no.1 & 2).

Fig.2: -FTIR Spectrum of pure drug, polymer & various formulations



3.4 Fourier Transform Infrared Spectroscopy: -

Table.No.1 Dissolution Studies of Nelfinavir Complexes

The FT-IR spectrum Fig 2 of NBP₂ and NBK₂ have showed characteristic peaks at 3732.60 cm⁻¹, 3383.80cm⁻¹, 1647.36cm⁻¹ and 3528.12cm⁻¹, 3385.38cm⁻¹, 1647.36cm⁻¹ for -OH, -NH, -C=O stretching respectively. Shift of peaks in the FT-IR spectrum of these formulations indicates the interaction between drug and polymer.

3.6 In-vitro Dissolution Studies: -

The release rate profiles were drawn as the percent drug released (vs) time. The inclusion complexes of NfV: β-CD released upto 93 to 96% & 75 to 96% of drug in 30 minutes in distilled water and phosphate buffer respectively. Whereas pure drug could release only 12.46% & 34.54% of

Table No.2 -Dissolution Studies of Nelfinavir Complexes

Time(min)	Pure Drug	NBP1	NBP2	NBK1	NBK2	NBC2
0	0	0	0	0	0	0
5	10.0	44.64	45.84	49.44	42.96	34.32
10	12.48	46.15	71.51	63.11	63.11	47.52
15	14.02	75.83	75.83	78.95	74.87	54.96
20	20.40	86.39	88.55	88.55	81.83	65.99
30	21.33	96.23	91.67	93.35	90.95	75.83
45	32.40	98.87	94.31	101.51	93.35	84.71
60	34.08	104.63	99.83			90.95
90	34.08					
120	34.54					

LIST OF ABBREVIATIONS USED

CD	Cyclodextrin
βCD	Beta-cyclodextrin
NFV	Nelfinavir mesylate
PM	Physical mixture
KM	Kneading method
CM	Co-precipitation method
HIV	Human Immune deficiency syndrome virus
NRTI	Nucleoside reverse transcriptase inhibitor
NNRTI	Non-nucleoside reverse transcriptase inhibitor
PIs	Protease inhibitor

X-RD of complexes of NFV: β-CD the PM (NBP₁) is simply the superimposition of each component indicating no formation of new structure. Complex prepared by coprecipitation (NBC₁) method showed a diffraction pattern quite similar to that of PM, while those obtained from KM (NBK₁) showed less peaks with low intensity. This indicates that the inclusion complex prepared by KM is less crystalline than the complex prepared by physical mixture

& coprecipitation method. When compared with the characteristic peak values of pure drug. This shift in the values of peaks indicates the interaction between drug & polymer. The enhancement in the dissolution rate has been found due to the formation of an inclusion complex in the solid state and reduction in the crystallinity of the product as confirmed by X-RD studies. β-CD can be used to prepare NFV inclusion complexes. Solubility of NFV in water & phosphate buffer was improved greatly as a result of complex formation with β-CD in comparison to pure NFV. A marked increase in the dissolution of NFV with β-CD at 1:2M ratios prepared by kneading and coprecipitation method was obtained. NFV-CD complexation results in an increase of solubility & dissolution rate for the drug suggesting a possible enhancement of its oral bioavailability.

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LIST OF ABBREVIATIONS USED

FTIR	Fourier transform infrared spectroscopy
PXRD	Powder x-ray diffractometry
DE	Dissolution deficiency
NBP₁	Inclusion complexation of Nelfinavir & beta cyclodextrin using physical mixture in 1:1 ratio
NBP₂	Inclusion complexation of Nelfinavir & beta cyclodextrin using physical mixture in 1:2 ratio
NBK₁	Inclusion complexation of Nelfinavir & beta cyclodextrin using kneading method in 1:1 ratio
NBK₂	Inclusion complexation of Nelfinavir & beta cyclodextrin using kneading method in 1:2 ratio
NBC₂	Inclusion complexation of Nelfinavir & beta cyclodextrin using co-precipitation in 1:2 ratio

REFERENCES: -

1. The Merck Index, 13th Edn., 2001; 6471.
2. K. Vanitha P., Rao JV and N. Appalaraju, RP-HPLC method for the estimation of nelfinavir mesylate in tablet dosage form, E.Journal of Chemistry., 4(3),2007 July,302-306.

3. Physicians Desk Reference, 54th edn., Published by Medicinal economics co., White house Station, NJ, (2000)
4. Szente L., Szetli J. and Kis G.L., Spontaneous opalescence of aqueous cyclodextrin solutions: complex formation or self-aggregation, *J.Pharm.Sci*, 87, 1998, 778-781.
5. Gonzalez-Gaitono G., Rodriguez P., Lsasi J.R. et al, The aggregation of cyclodextrins as studies by studies by photon correlation spectroscopy, *J. Inclusion.Phenom.Macrochem*, 44, 2002, 101-105.
6. Uekama K. and Hirayama F, Methods of investigating and preparing inclusion compounds. In: Duchene D, editor. *Cyclodextrins and their industrial Uses*. Paris: Editions de Sante, 1987, 131-172.
7. Szejtli J., *Cyclodextrin Technology*. Dordrecht, The Netherlands: Kluwer Academic Publishers, 1988, 81-83.
8. Loftson, T. and Brewster M.E. Pharmaceutical application of cyclodextrins. 1. Drug solubilization and stabilization, *J.Pharm.Sci*, 85, 1996, 1017-1025.
9. Connors K.A., The Stability of cyclodextrin complexes in solution. *Chem.Rev*, 97, 1997, 1325-1357.
10. Uekama K., Hirayama F. and Irie T., Cyclodextrin drug carrier systems, *Chem.Rev*, 98, 1998, 2045-2076.
11. Stella V.J. and Rajiwski R.A., Cyclodextrins: Their future in drug formulation and delivery. *Pharm. Res*, 14, 1997, 556-567.
12. Uekama, K. and Hirayama, F., Methods of investigating and preparing inclusion Compounds. In Duchene D, editor. *Cyclodextrins and their industrial uses*. Paris, Editions de Sante, 1987, 131-172.
13. Erden, N. and Celebi, N., A study of the inclusion complexes of naproxen with β -cyclodextrin. *Int.J.Pharm*, 48, 1988, 83-89.
14. Mabuchi, N. and Noga, M., Controlled release powdered flavor preparation and confectioneries containing preparation, Japanese Patent JP, 2001, 128:638.
15. Buschmann, H.J. and Schollmeyer, E.J., Application of cyclodextrins in cosmetic products, *A Rev. Cosmet.Sci*, 53, 2002, 185-191.
16. Hirose T. and Yamamoto Y., Hinokitol containing cyclo-olefin polymer Compositions and their mouldings with excellent antimicrobial and gas barrier properties, 2001, Japanese Patent JP 55480.
17. Hedges, A.R., Industrial applications of cyclodextrins, *Chem.Rev*, 98, 1998, 2035-2044.
18. Kumar, R., Dahiya, J.S., Singh, D. and Nigam, P., Biotransformation of cholesterol using lactobacillus bulgaris in glucose controlled bioreactor, *Bioresour. Technol*, 28, 2001, 209-211.
19. Bar R., A new cyclodextrin-agar medium for surface cultivation of microbes on lipophilic substrates, *Appl. Microbiol Biotechnol*, 32 (4), 1990, 470-472.
20. Higuchi T. and Connors K.A., Phase Solubility Diagram. *Adv.Anal.Chem.Instrum*, 4, 1965, 117-21

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