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Development of Nebivolol-β-Cyclodextrin-HPMC Ternary inclusion complex for enhancement of Solubility and Dissolution rate

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Abstract

The present study was aimed to improve the solubility and dissolution rate of poorly soluble drug nebivolol through ternary inclusion complex using β-cyclodextrin and HPMC.

Physical mixture and Binary inclusion complexes were prepared with varying drug to β-Cycodextrin molar ration (1:1, 1:2, 1:3 and 1:4) by kneading method. Binary inclusion complexes system showed multifold increase in solubility of drugs compared to its native form. Binary complexes at molar ratio of 1:2 was further utilised for the formation of ternary inclusion complex using HPMC. Different concentration of HPMC (2.5%, 5%, 10% and 20% w/w) were added for complex formation in order to study their effect on solubility of drug. Inclusion complex was evaluated for solubility study, drug content, Fourier-transform infrared (FTIR), differential scanning calorimeter (DSC), powder X-ray diffractometry (PXRD), scanning electron microscopy (SEM) and dissolution studies.

The phase solubility study exhibited A_L type solubility curve, which suggested the formation of soluble complexes. Solubility study suggested that use of HPMC markedly increase the drug solubility with increased in concentration. The FTIR and DSC show compatibility between drug and polymer. Broadening of DSC peak suggested the reduction in intensity and amorphous conversion of drug. XRD study also confirmed the crystallinity reduction in complex. The SEM of ternary complex showed homogeneous surface compare to its rod shape in native form.

All binary and ternary inclusion complex prepared with β-cycodexrtin and HPMC showed increase in drug solubility and dissolution rate as compare to pure drug. Ternary complex with 10% of HPMC showed fastest drug release of 99.15±2.10 in 60 min. Finally, it was concluded that nebivolol- β-cycodexrtin-HPMC at a weight ratio of 1:2:10% w/w was the best combination for improving solubility and dissolution rate of Nebivolol.

Key words : Nebivolol, β-cycodexrtin, HPMC, DSC, XRD and SEM.

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BCS class II (low solubility and high permeability) drugs possesses poor water solubility and dissolution is key challenges in the formulation development of safe and effective dosage form. More and more hydrophobic drug molecules were coming in recent time in the treatment of different diseases. Poor solubility of such drugs leads of incomplete or lower absorption and biological activity. To overcome the limitations of BCS Class II drugs, earlier several methods have been reported, like solid dispersion, particle size reduction salt formation, lipid-based formulations, complexation with cyclodextrins etc.[7,11,14,] Beta-cyclodextrin (β-cyclodextrin) is a cyclic oligosaccharide composed of seven glucose units linked by α-1,4 glycosidic bonds. It has a hydrophobic inner cavity and a hydrophilic outer surface, allowing it to form inclusion complexes with poorly soluble compounds, improving their solubility and stability. β-cyclodextrin is widely used in pharmaceuticals, cosmetics, and food industries to enhance the delivery, taste, and bioavailability of active ingredients.[5] Beta-cyclodextrin (β-cyclodextrin) plays a crucial role in enhancing the solubility and bioavailability of poorly water-soluble drugs. Its hydrophobic inner cavity can encapsulate lipophilic drug molecules, forming inclusion complexes that protect the drug from degradation and improve its dissolution in aqueous environments. This property allows for better drug absorption, stability, and controlled release. β-cyclodextrin is widely used in formulations to reduce drug doses, minimize side effects, and improve therapeutic outcomes.[9] Binary inclusion complexes involve the encapsulation of a drug molecule within the cavity of a single cyclodextrin, such as β-cyclodextrin. This interaction improves the solubility, stability, and dissolution rate of poorly water-soluble drugs by protecting them from environmental degradation and facilitating better absorption.[3] Ternary inclusion complexes go a step further by incorporating a third component, like a hydrophilic polymer (e.g., PVP, PEG, HPMC, Pectin) alongside the drug and cyclodextrin. These additional hydrophilic agents further enhance the solubility and dissolution rate by stabilizing the complex, preventing drug recrystallization, and maintaining a more favorable pH or ionic environment. Ternary systems are especially beneficial when binary complexes alone do not achieve the desired solubility or bioavailability, providing a synergistic effect in drug delivery.[12] Nebivolol is a cardioselective beta-1 adrenergic receptor blocker primarily used to treat hypertension and heart failure. Nebvolol was found to be insoluble in water leads to it limited and incomplete absorption. The present work designed to study the effectiveness of novel inclusion complexes of the nebivolol with β-CD in absence and presence HPMC to improve the solubility and dissolution characteristic of the drug.

Material :

Nebivolol was obtained as a gift sample from Cipla Ltd., Mumbai, India. β-CD was gifted by Signet Ltd Mumbai. All other chemicals and solvents used were of pharmaceutical and analytical grade.

Saturation solubility study :

Saturation solubility study was tested in different solvent like and distilled water, 0.1 N Hcl and Phosphate buffer pH 6.8 as per

method reported by huguchi and Connors. Extra amount of drug was added in to the 10 ml of glass vial, which was shaken mechanically on shaker for 72 hrs. Filter sample was then analysed spectrophotometrically at 282 nm after suitable dilution.[8]

Phase solubility study :

Higuchi and Connors method was employed for Phase solubility studies.[6] An excess amount of nebivolol was added to 10 ml screw-capped vials containing different molar ratio (5 to 20 mM/L) of β-CD with or without hydrophilic polymer at different concentration (0, 2.5, 5, 10 and 20%w/w) in water. The suspensions were vigorously shaken in a shaking water bath at 37±0.5°C for 72 hrs to reach equilibrium. Filtration of the suspension was carried out using 50 mm millipore filters. An aliquot portion of the filtrate was diluted suitably and analysed by measuring its absorbance spectrophotometrically at 282 nm against a blank solution containing the same concentrations of the carrier. Each experiment was conducted in triplicate.[2]

Preparation of Binary inclusion complexes:

Binary inclusion complex drug was made with βCD at 1:1, 1:2, 1:43 and 1:4 weight (w/w) ratios by physical mixing and kneading method. Physical mixture was performed by properly mixing the weighed amounts of nebivolol and βCD in a mortar and pestle for up to one hour to ensure a uniform mixture. In the kneading (KN) method, the drug and βCD were physically kneaded for 1 h in a mortar with ethanol–water (1:1 v/v) to obtain a paste. It was placed at 45 °C in an oven to dry. The resultant mass was crushed and sieved to get uniform size powder.

Preparation of Ternary complexes by Kneading method :

Binary inclusion complexes ratio that showed highest solubility was further utilised for the preparation of ternary inclusion complexes by adding the hydrophilic polymer (HPMC) at different concentrations (2.5%, 5%, 10% and 20%) of dry weight (w/w) of binary inclusion complexes. Hydrophilic polymers (HPMC) were added to the prepared binary inclusion complex and similar procedure as mention above were repeated for the preparation of ternary complex by kneaded method.[10]

Characterization of the Ternary inclusion Complex :
Solubility study :

Shake flask method was used to carry out the solubility study. The excess amount of prepared binary and ternary inclusion complexes was added into 10 ml distilled water in in conical flask and shaken for 24 h at room temperature on rotary flask shaker. After shaking resultant samples containing undissolved solid suspended in the test medium were centrifuged at 10,000 rpm for 5 min, the clear supernatants obtained were filtered through whatman filter paper. Filtered sample ware analyzed by spectrophotometer at 282 nm after dilution.[15]

Fourier Transform Infrared Spectroscopy (FTIR) :

The complex formation was assessed by evaluating the change in peak shape, position, and intensity using a spectrophotometer

(FTIR Shimadzu 8400S, Lab Wrench). The spectra of FBX, SBE7-βCD, selected hydrophilic polymer, FBX-ternary inclusion complex, and physical mixture (PM) of raw materials were compared to interpret the spectra. The analysis was performed between 4000 and 400 cm^{-1} and the conformational changes were observed.

Differential Scanning Calorimetry (DSC):

The thermal behavior of pure nebivolol and nebivolol-ternary inclusion complex were examined using a differential scanning calorimeter (Metler) including with aluminum-sealed pan cell. Samples were placed in hermetically sealed aluminium pans and heated in a temperature range of 30 to 300 °C with 10 °C/ min increment rate in a nitrogen atmosphere.[4]

Powder X-Ray Diffraction :

X-ray powder diffraction patterns of drug, carrier and solid dispersion was recorded on an X-ray powder diffraction system (Rigaku, Mini Flex 600). The scanning was done over range of 5° to 60°. The position and intensities of diffraction peaks were considered for the comparison of crystallinity[8].

Surface morphology study :

The surface morphology of the pure drug nebivolol and optimized ternary inclusion complex was investigated using a scanning electron microscope. The study was performed using an electron microscope (JOEL, Tokyo, Japan). The samples were coated with gold and detected under the microscope at high resolution to reveal the change in morphology.

In vitro dissolution study :

Dissolution study was conducted on pure nebivolol and prepared secondary and ternary complexes. USP dissolution test apparatus (Electrolab, India) type II was used for the study. The study was performed at 37 °C ±0.5 °C temperature with a paddle rotation speed of 50 rpm using 0.1 N HCl (900 ml) as dissolution medium. Aliquots of 5 ml was withdrawing from the dissolution sample at fixed time interval of 10, 20,30,40,50 and 60 min. The equivalent amount of fresh dissolution medium was added in order to maintain sink conditions. These aliquots were diluted suitably and analysed by UV–Visible spectroscopy at 282 nm using blank. All these experiments were carried out in triplicate.[1,13]

Saturation solubility of the nebovolol was tested in different solvent like distilled water, 0.1 N Hcl and phosphate buffer solution pH 6.8. Nebivolol showed very poor solubility (0.0632 ± 2.06 mg/ml) in water because of its basic hydrophobic nature. Solubility of nebivolol in 0.1 N Hcl and in phosphate buffer solution pH 6.8 was found as 0.429 ± 3.16 and 0.202 ± 2.92 mg/ml respectively (fig. 1). Study demonstrat ed pH dependent solubility of nebivolol.

Phase solubility study of Drug Nebivolol :

Phase solubility study of drugs was performed by adding excess amount of drug in 10 ml vial that was holding varied concentration of β Cyclodextrin in water. Study revealed that the solubility of nebivilol increased linearly along with the concentration of β-CD. This might be due to the solubilizing efficiency and cavity forming ability of β-CD. The phase-

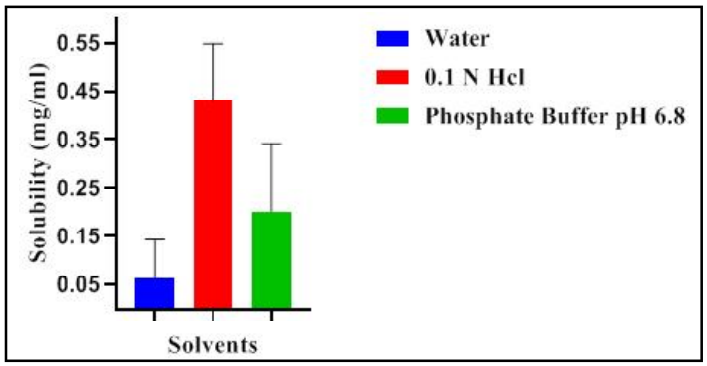

Figure 1. Solubility Profile of Nebivolol in different Solvents

solubility curve determined for β-CD in distilled water was linear, giving A_L type solubility curve, which suggested the formation of soluble complexes. Increased in molar concentration of drug was might be because of complexing impact of β-CD which further suggesting strong affinity between drug and β-CD. By utilizing phase solubility curve, the value of apparent stability constant (Kc) was determined. The values of stability constant (Kc) of Nebivolol with β-CD was found to be 1058 M^{-1}. Higher value of stability constant indicate stronger association between the drug and β-CD along with higher drug entrapment.

Further to analysed a favourable or non-favourable effect of β-CD on the drug solubility was determined from Gibbs-Free energy calculation. Gibbs free energy of drug was calculated from phase solubility study data. Study revealed that the $\Delta G°$tr of drug at all concentration of β-CD were negative (Table-1). Negative value of Gibbs-free energy indicates better dissolution of drug. Increased in negative $\Delta G°$tr value with increasing proportion of β-CD suggests that interaction between drug and polymer was favourable and spontaneous that gives improved solubility of drug, indicating formation of more stable inclusion complexes. The phase solubility study of Nebivolol with β-CD in water was given in table 1 and phase solubility curve of Nebivolol is shown in figure 2.

Table-1. Phase Solubility Study and Gibbs Free Energy of Nebivolol with β- Cyclodextrin Complex

Sr. no	β-CD Concentration (mM/L)	Concentration of Nebivolol (mg/ml)	Gibbs Free Energy ΔG^0 (kcal mol^{-1})
1	0	0.0632±1.16	-
2	5	0.356±0.78	-0.750
3	10	0.668±1.22	-1.024
4	15	0.976±0.68	-1.188
5	20	1.320±1.04	-1.319
Stability Constant (Kc)		1058	

Values are mean ± SD (n = 3)

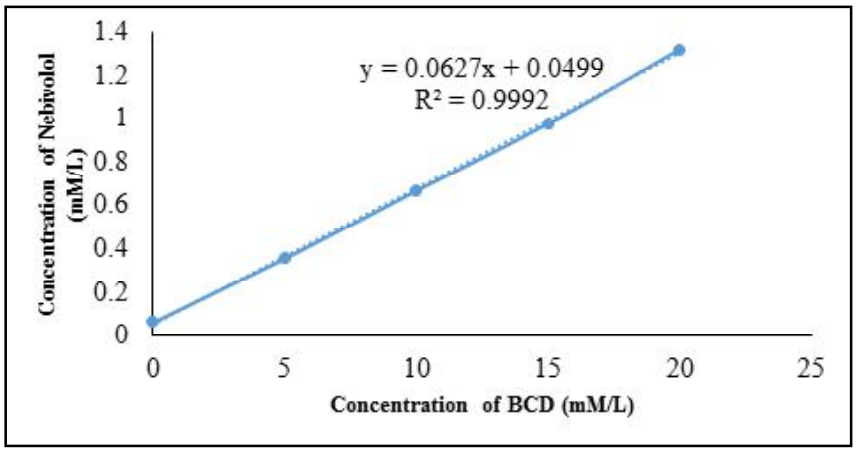

Figure 2. Phase Solubility Curve of Nebivolol with β-CD

To study the effect of incorporation of hydrophilic polymers over the solubility of drug, showed positive effects on binary complexes. It was observed that as the concentration of hydrophilic polymer HPMC increases from 2.5% to 20%, the solubility of drug also increased. This could be because of hydrophilic nature of polymer. The stability constant values of complexes were also demonstrated the higher value with higher concentration of HPMC. The stability constant value ranged from 2159 to 6894, indicating higher affinity of HPMC for the drug. The higher values of stability constant were also confirmed the existence of strong interaction between nebivolol and inclusion derivatives, which suggested the formation of ternary complexes. (fig. 3).

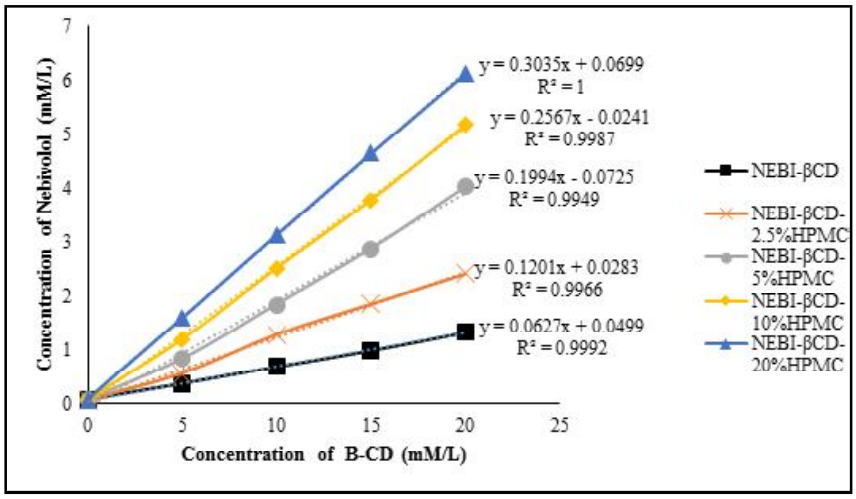

Figure 3. Phase Solubility Curve of Nebivolol in aqueous solution of β-CD with HPMC

Complexation efficiency is also an another crucial parameter in evaluating the solubility of inclusion complexes. The complexation efficiency of the ternary systems was found higher upon the incorporation of HPMC, than the binary system. The values of CE of complex increased with the addition of HPMC to the complexation media, indicating greater effectiveness of ternary systems over the binary one. The results are shown in table-2.

Table-2. Stability Constant and Complexation Efficiency of Inclusion Complexes

Inclusion Complex	Stability Constant (M^{-1})	Complexation Efficiency
NEBI:βCD	1058	0.066
NEBI:βCD:HPMC (2.5%)	2159	0.136
NEBI:βCD:HPMC (5%)	3940	0.249
NEBI:βCD:HPMC (10%)	5464	0.345
NEBI:βCD:HPMC (20%)	6894	0.435

Solubility study :

The solubility of pure drug nebivolol, physical mixure (PM), binary and ternary inclusion complexes ware determined using the shake flask technique. Nebivolol showed very less amount of water solubility (0.0632 ±1.94 mg/ml) when computed in solubility study. Little aqueous solubility of nebivolol confirmed its crystalline nature. Compared to pure nebivolol, the physical mixture drugs with β-Cycodextrin showed improved drug solubility, indicating a positive interaction among the drug and β-Cycodextrin. It was observed that as the concentration of β-Cycodextrin increases the solubility nebivolol also increases. Solubility of physical mixture (PM) prepared with different ratio of drug: β-CD (1:1, 1:2, 1:3 and 1:4) was found to be 2.11 ±1.47, 5.46 ±0.81, 6.58 ±2.21, 7.16 ±1.24 respectively.

Binary inclusion complexes drug with β-Cycodextrin in molar ration (1:1, 1:2, 1:3 and 1:4) showed multifold increase in solubility of drugs compared to its native form. Binary complexes at molar ratio of 1:1, 1:2, 1:3 and 1:4, showed drug solubility of 7.12 ±1.69, 12.41 ±0.66, 13.14 ±1.63 and 13.87 ±0.84 mg/ml respectively. From the study it was noticed that at molar ratio of 1:2 showed highest solubility of drug. Beyond the molar ratio of 1:2, the nebivolol solubility was not increase significantly with further increase in β-CD concentration. All binary complexes formulations made by kneading method showed higher solubility of drug. This could be because of formation of stable complex between drug and β-CD. Based on the solubility data, the molar ratio of nebivolol: β cycodextrin (1:2) were optimized and used for the preparation of ternary complexation with HPMC.

Ternary complexes exhibited superior results than binary complexes, because of incorporation of hydrophilic polymers. HPMC were added as hydrophilic polymer in to different concentration (2.5%, 5%, 10% and 20% w/w) in order to study their effect on solubility of drug. From the solubility study data, it was come to know that, HPMC markedly increase the drug solubility with increased in concentration. At concentration 10% w/w, the

solubility of drug was found to be 24.21 ±1.15 mg/ml, above that concentration solubility was not increased significantly. Increased in solubility of nebivolol in to ternary complexes might be due to conversion of crystalline drug in to amorphous form. HPMC enhanced the solubility of nebivolol from 16.52 ±1.36 mg/ml to 25.43 ±2.18 mg/ml. Hence, it can be concluded that HPMC is a polymer of choice in enhancing the solubility of Nebivolol. Solubility study data pure nebivolol, physical mixture, binary complexes and ternary complexes was shown in figure 4.

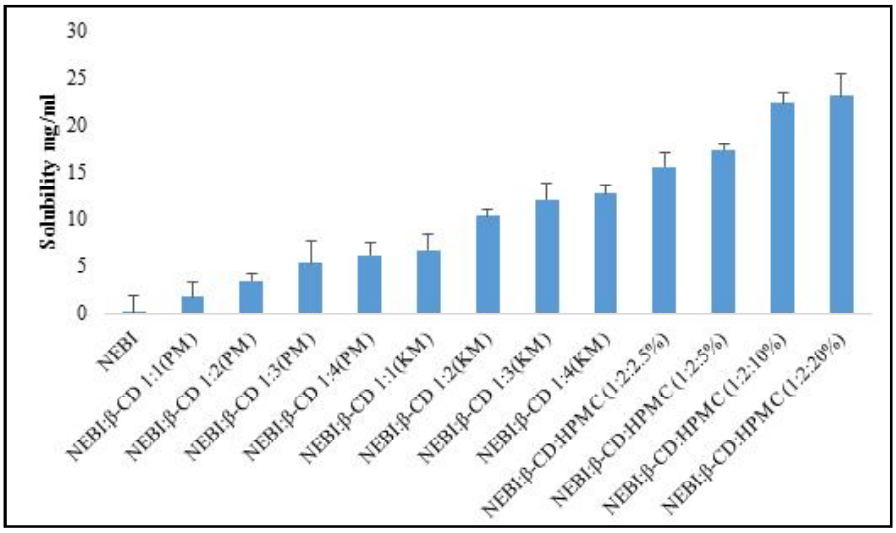

Figure 4. Solubility profile of Nebivolol, Binary and Ternary Inclusion Complexes in Distilled Water

Fourier Transform Infrared Spectroscopy (FTIR) :

The FTIR analysis has been carried out to study the interaction between nebivolol with β-CD and HPMC polymers. The pure nebivolol showed characteristic at 3905 – 3432 cm^{-1} (O-H Stretching for alcohol and Phenol), 2920 – 2850 cm^{-1} (C-H Stretching Vibration for aromatic or unsaturated alkenes compounds), 1777 cm^{-1} (C=O stretching), 1214 cm^{-1} (aromatic C=C stretching or N–H bending), 1214 cm^{-1} (C–O stretching), 1075 cm^{-1} (C–O stretching) and 871, 817, 778 cm^{-1} suggested presence of aromatic ring. (Fig. 5) Typical principle peaks was detectable and not showed any shift in IR spectra in binary or ternary solid complex, confirmed no interaction between drug, β-CD and hydrophilic polymer HPMC.

Differential Scanning Calorimetry (DSC):

The DSC thermogram of pure nebivolol showed a sharp single endothermic peak at 228.80°C, (fig. 6) which corresponds to the melting temperature of nebivolol, the sharpness of the peak indicating and confirmed the crystalline nature of the nebivolol. Optimized ternary inclusion complexes formulation (NTC 3) drug: βCD: HPMC (1:2:10%w/w), (fig. 7)

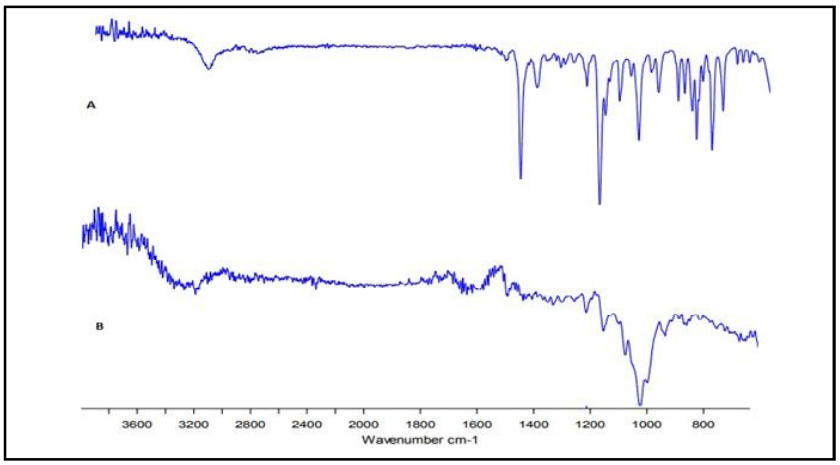

Figure 5. FTIR Spectra of pure nebivolol (A) and Ternary inclusion complex with βCD and HPMC (NTC 3) (B)

showed broadening of endothermic peak of drug nebivolol also sharpness of peak gets reduced, indicating that the crystallinity of the drug was reduced considerably and drug was converted to amorphous form.

Powder X-Ray Diffraction :

XRD spectra of pure nebivolol (figure 8 A) showed prominent high-intensity diffraction peaks at 2θ values of 25.29, 25.30, 25.32, 25.33, 25.35, 25.36, 25.38 and 25.39 showing the highly crystalline nature of nebivolol. XRD diffractgrams of optimized ternary inclusion complex (NTC 3) (figure 8 B) showed overall decrease in peak intensity and crystallinity of drug, indicating amorphous conversion of drug in ternary inclusion complex in presence of β-CD and HPMC.

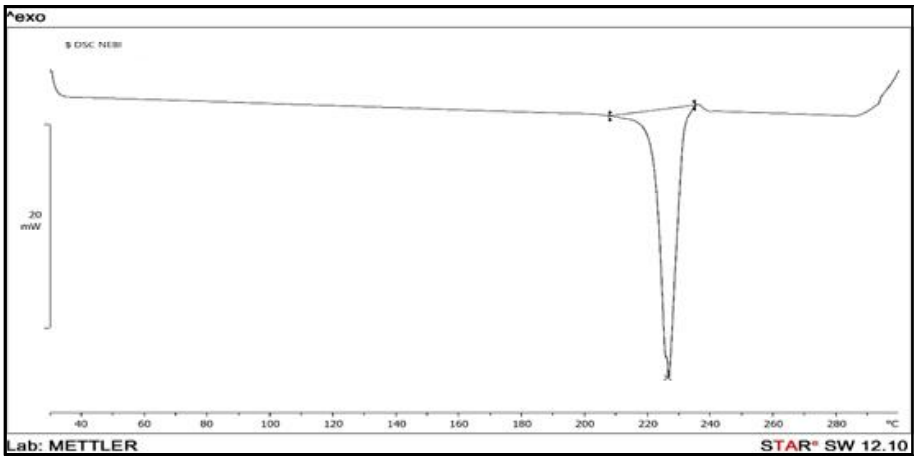

Figure 6. DCS thermogram of pure Nebivolol

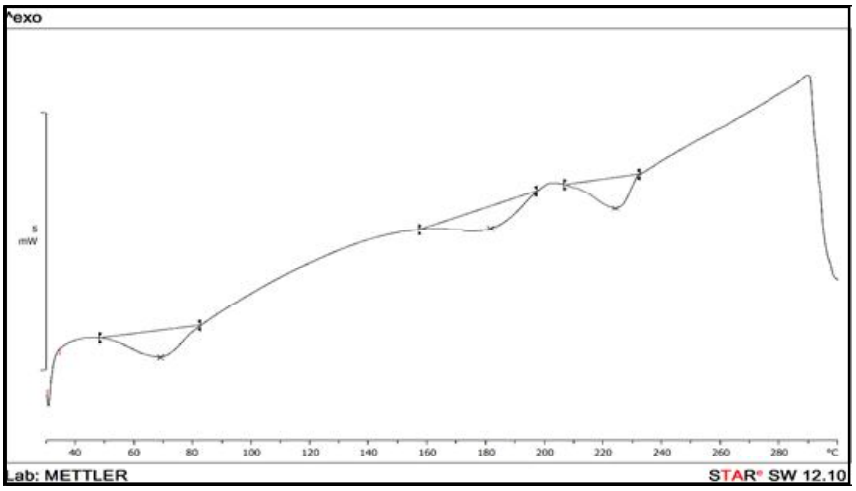

Figure 7. DCS thermogram of optimized Ternary Inclusion Complexes (Nebi:βCD:HPMC 1:2:10%w/w) (NTC 3)

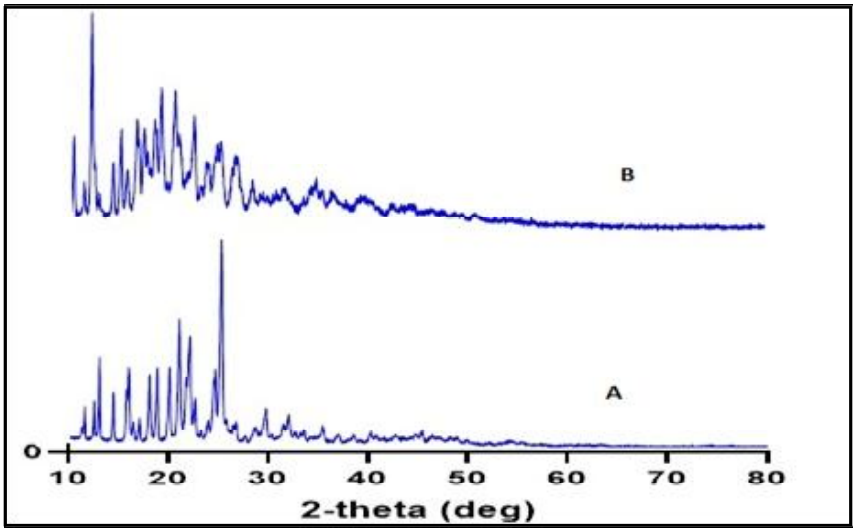

Figure 8. XRD spectra of Pure Nebivolol (A) and Optimized Ternary Inclusion Complexes (B) (Nebi: βCD: HPMC 1:2:10%w/w) (NTC 3)

Surface morphology study (SEM) :

The surface morphology study SEM was done to evaluate surface morphology of the pure drug nebivolol and optimized ternary inclusion complex. SEM image of pure drug nebivolol appeared as rectangular needle-shaped indicating its crystalline nature (fig. 9A). SEM micrograph of the optimized ternary inclusion complex (NTC3) appeared as smooth surface showed amorphous surface, indicated that drug crystallinity was reduced to much higher extend. (fig. 9B) This suggested the formation of stable inclusion complex.

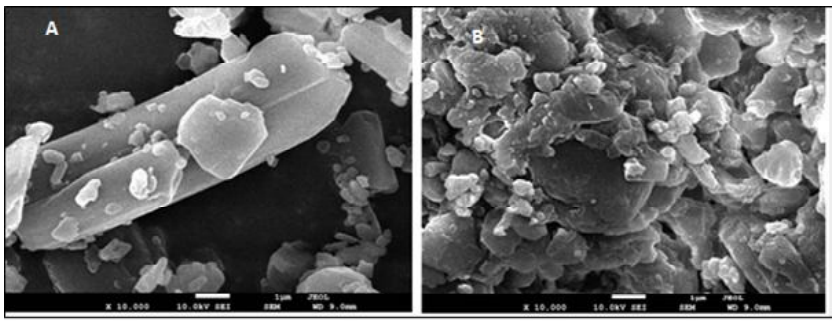

Figure 9. SEM image of Pure Nebivolol and Optimized Ternary Inclusion Complexe (NTC 3)

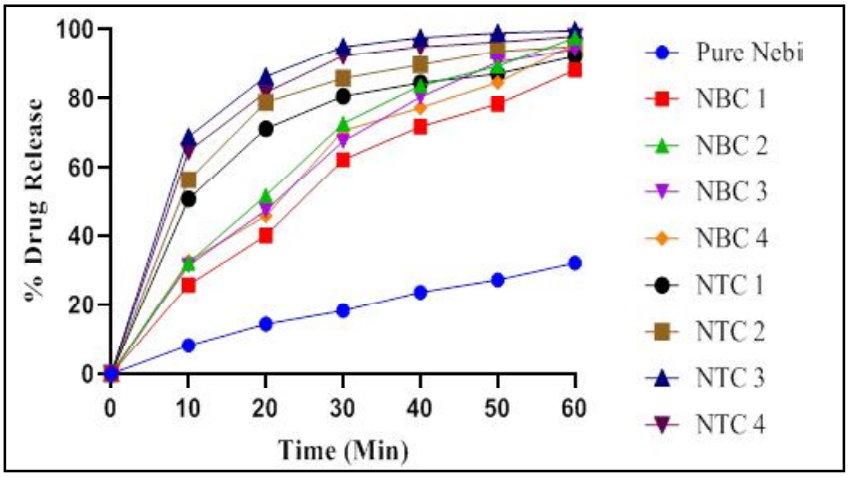

Figure 10. Dissolution profile of pure, Secondary Inclusion Complex and Ternary Inclusion Complexes of Nebivolol with β-cyclodextrin and HPMC

In vitro Dissolution study :

Pure Nebivolol showed slowest drug release profile. By 60 minutes, the cumulative release reached 32.24% ± 0.76. The release increases gradually over time, showing a linear pattern with no sudden spikes. The slower release in Pure Nebi might be due to poor solubility and higher crystallinity. Binary inclusion complex of drug with different concentration of β-CD give faster release than pure Nebi, with significant increases between 10 to 40 minutes. NBC 2 shows the highest release in the NBC series, reaching 97.58% ± 1.54 by 60 minutes. NBC 1 starts slower 40.14% ± 1.45 at 20 minutes, but catches up later, reaching 88.12% ± 1.87 by 60 minutes. All NBC samples reach around 70–97% dissolution by the end, indicating effective release under this condition. Ternary inclusion complex of the drug prepared using different concentration of HPMC exhibits the fastest and highest release profiles among all groups. All ternary complex formulation (NTC)

formulations exhibit the fastest dissolution, with most samples exceeding 90% release within 30–40 minutes. Formulation NTC 3 and NTC 4 perform exceptionally well, reaching 99% release by 60 minutes, indicating an optimized formulation for rapid drug release. The burst release seen with NTC samples suggests the possible solubilizing effect of hydrophilic polymeric agents like HPMC.

The present study demonstrates the successful formulation of ternary inclusion complex of drug with β-CD and hydrophilic polymer HPMC (Nebi: βCD: HPMC 1:2:10%w/w). Solubility and dissolution study indicates formation of more stable and amorphus complex with of drug with βCD and HPMC that enhanced the multifold solubility and drug release compare with pure nebivolol. Hence ternary inclusion complexes prepared by kneading method found to be effective technique of enhancement of drug solubility and dissoluration rate.

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