

Research Article

Enhancement of Dissolution Rate of Poorly Soluble Drug Nateglinide by Complexation Technique

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ABSTRACT

In our present study, the development and evaluation of inclusion complex applying β -cyclodextrin (β -CD), γ -cyclodextrin (γ -CD) and hydroxypropyl- β -cyclodextrin (HP- β -CD) for the improvement of oral bioavailability of Nateglinide was investigated systematically. The inclusion complex of Nateglinide was prepared by physical mixture, solvent evaporation, kneading methods using 1:1, 1:2, 1:3 molar ratios of drug:CD. The three methods (HP- β -CD) 1:2 ratio prepared for nateglinide tablet formulation by direct compression method. powder was evaluated for pre-compression parameters and post-compression parameters. Drug-excipient compatibility studies showed no interaction inclusion complexes were characterized by differential scanning calorimetry (DSC), and evaluated by dissolution studies. This work proved β -cyclodextrins to be effective solubilizing agent in improving the solubility of poorly water soluble drugs.

INTRODUCTION

Any drug from a given dosage form to be absorbed must be present in the form of solution at the site of absorption. Low aqueous solubility is one of the major problems encountered during formulation development of new chemical entities especially in the process of generic product development. More than 40% new chemical entities developed in pharmaceutical industry are practically insoluble in water. Various techniques are used for the enhancement of the solubility of poorly soluble drugs includes physical and chemical modifications of drug like particle size reduction, crystal engineering, salt formation, solid dispersion, use of surfactant, hydrotropy, co-solvency, use of surfactants, complexation etc. Inclusion complexes are formed by the insertion of the nonpolar molecule or the nonpolar region of one molecule into the cavity of another molecule or host molecules. The most commonly use host molecules are cyclodextrins. Cyclodextrins are nonreducing, crystalline, soluble, cyclic oligosaccharides consisting of glucose monomers arrange in a donut shaped ring having hydrophobic cavity and hydrophilic outer surface. Three naturally occurring CDs are α -, β - and γ - cyclodextrins. Cyclodextrins consist

of six, seven and eight D-glucose units, respectively, attached by α -1, 4- linkages. Cyclodextrins consist of (α -1,4)-linked α -Dglucopyranose units and contain a somewhat lipophilic central cavity and a hydrophilic outer surface. Due to the chair conformation of the glucopyranose units, the cyclodextrins are shaped like a truncated cone [2]. In aqueous solutions cyclodextrins are able to form inclusion complexes with many drugs by taking up a drug molecule or more frequently some lipophilic moiety of the molecule, into the central cavity. No covalent bonds are formed or broken during the complex formation and drug molecules in the complex are in rapid equilibrium with free molecules in the solution.

Nateglinide (NTG) is a poorly water-soluble, anti-emetic drug according to the BCS system (Class II), and its dissolution is the rate limit Immediate Release (IR) tablet dosage form. NTG interacts with the ATP-sensitive potassium (K^+ ATP) channel on pancreatic beta-cells. The subsequent depolarization of the beta cell opens the calcium channel, producing calcium influx and insulin secretion. The T_{max} of NTG i.e. time required for maximum plasma concentration is about 1 hour and its BCS system (Class II drug) also indicates the need

for development of solubility and dissolution rate enhanced forms of NTG. It is poorly water soluble drug with mean absolute oral bioavailability of 73% and greater than 98% of absorbed drug being bound to plasma proteins. Increase in solubility and dissolution rate can enhance oral bioavailability and results in quicker onset of action.

Most of the research works were published in the delivery of NTG by particular delivery system like liposome's, microspheres and nano particles etc. Very few research studies published relating to development of solid inclusion complexes dosage forms of NTG based on oral dosage form technology.

Materials and methods

Materials

NTG was a gift sample from Divis Laboratories Ltd (Hyderabad). All cyclodextrin was obtained from Cavasol® w7 HP. HCl, SLS, microcrystalline cellulose, cross providine, magnesium stearate all the chemicals were obtained from Loba Chemi, Mumbai.

METHODS

Preparation of Standard Stock solution:

10 mg of NTG was accurately weighed and dissolved in 10 ml volumetric flask containing methanol. The volume was made up to 10 ml with the methanol to get concentration of (1 µg/ml).

Calibration curve in 0.01 N HCl

The standard solution of NTG was subsequently diluted with 0.01 N HCl solution to obtain a series of dilution containing 2, 4, 6, 8, 10 µL of NTG per ml of solution. The absorbance was measured in UV-visible spectrometer at 210 nm using 0.01 N HCl solution as blank. The concentration of NTG and its absorbance values are given in table.

Solubility studies

Solubility profile studies were performed with PH 2 0.01 N HCl, pH 4.6 acetate buffer, pH 5.8 phosphate buffer, pH 6.8 phosphate buffer and 7.4 phosphate buffer in order to determine the aqueous medium that offer good solubility condition for NTG. The solubility of NTG was also determined in 0.1 N HCl PH 2 containing various concentrations of SLS (0.5%) as follows:

Preparation of complexation

Physical mixtures

The physical mixtures of NTG and CDs in 1:1, 1:2 and 1:3 M were obtained by mixing individual components that had previously been sieved (75-150 µm) together with a spatula.

Solvent Evaporation Method

Inclusion complexes of Nateglinide were prepared by dissolving carriers HP β-Cyclodextrin and Nateglinide at their corresponding ratio in common volatile solvent like methanol using a glass mortar. They were mixed by slight pressure for 15 min. Then the solvent was allowed to evaporate in hot air oven at 45 °C for 2h. The dried mass was passed through 100 # mesh and stored in desiccators at room temperature until further use. The complexes were made in different ratios with respect to drug and polymers.

Kneading Method

Nateglinide and carrier HP β-Cyclodextrin were weighed according to their corresponding molar ratio. Nateglinide and carrier were transferred to a mortar pestle. The mixture was reduced the size by continuous stirring with pestle. Water-methanol mixture (1:1 v/v) ratio was added to the above physical mixture and continuously stirred until the slurry mass was formed. Slurry mass was collected and dried in a hot air oven for 2 hrs at 50 °C, dried mass was collected and further dried in desiccators over for 24 hrs. The dried mass was collected and passed through 100 # mesh, and packed it in a closed container. The complexes were made in different ratios with respect to drug and HP β-CDs as shown in Table.

EVALUATION OF FLOW PROPERTIES

Bulk density

It is the ratio of total mass of powder to the bulk volume of powder. It was measured by pouring the weighed powder into a measuring cylinder and the volume was noted. It is expressed in gm/mL and is given by

$$D_b = M/V_0$$

Where, M is the mass of powder
V₀ is the Bulk volume of the powder.

Tapped density

Tapped density was determined by using graduated cylinder. An accurately weighed sample was carefully added to the graduated cylinder with the aid of funnel. The initial volume

was noted and the sample was tapped on a horizontal base. Tapping was continued until no further reduction in sample volume was observed. Volume was noted and tapped density is calculated by using the following formula.

$$D_t = M/V_t$$

Where, M is the mass of powder

V_t is the Bulk volume of the powder.

Compressibility Index(CI) and Hausner's ratio (H)

In recent years the compressibility index and the closely related Hausner's ratio have become the simple, fast, and popular methods of predicting powder flow characteristics. The compressibility index has been proposed as an indirect measure of the bulk density, size, shape, surface area, moisture content and cohesiveness of the materials. Both the compressibility index and the Hausner's ratio were determined by using bulk density and the tapped density of the powder.

$$\text{Carr's index} = \frac{\rho_t - \rho_b}{\rho_t} \times 100 \quad \text{--- (6)}$$

$$\text{Hausner's ratio} = \frac{\rho_t}{\rho_b} \quad \text{--- (7)}$$

Angle of repose

The angle of repose has been used to characterize the flow properties of solids. Angle of repose is a characteristic related to inter

particulate friction or resistance to movement between particles. This is the maximum angle possible between surface of pile of powder or granules and horizontal plane. The angle of repose was determined by funnel method suggested by Newman.

Fixed funnel method was adopted for measuring the angle of repose. In this method, powder was passed through a funnel (8 cm diameter at top and 1.7cm diameter at efflux tube) that is fixed at predetermined height (2cm) and allowed to pass with or without shaking to get precise vibration. Following equation was used for the calculation of angle of repose value

$$\text{Angle of repose} = \tan^{-1} \left(\frac{h}{r} \right)$$

Where 'r' is the radius of pile and 'h' is height of pile measured.

Table-20 lists flow characterization of powders based on values of CI, HR and Angle of repose.

FTIR Interference Studies

The FTIR spectrum of pure Nateglinide showed an absorption band at 2924 cm⁻¹ (aliphatic C-H stretching; asymmetric), 2859 cm⁻¹ (aliphatic CH stretching; symmetric), 1713 cm⁻¹ (C = O stretching for Ketone), 3064 cm⁻¹ (aromatic C-H Stretching), 3086 cm⁻¹ (aromatic C=H Stretching). The FTIR spectrum of physical mixture and pure Nateglinide show all the peaks for drug and other excipients, hence no interaction was observed between them. The results were shown in Fig

Table: Reference ranges to assess flow properties of powders

S. No.	Flow character	Compressibility index	Hausner's ratio	Angle of repose
1	Excellent	≤10	1.00-1.11	25-30
2	Good	11-15	1.12-1.18	31-35
3	Fair	16-20	1.19-1.25	36-40
4	Passable	21-25	1.26-1.34	41-45
5	Poor	26-31	1.35-1.45	46-55
6	Very poor	32-37	1.46-1.59	56-65
7	Very very poor	>38	>1.6	>66

IN-VITRO EVALUATION TESTS

Estimation of drug content:

An accurately weighed quantity of Solid Dispersions equivalent to 10mg of NTG, was taken into a 10 mL volumetric flask and dissolved in methanol and filtered through a Whatman No.1 filter paper (0.45μ). The filtrates were diluted suitably 0.01 N HCl with 0.5% w/v SLS solution. The content of NTG was determined spectrophotometrically at 210 nm against suitable blank using UV-visible Spectrophotometer (UV-3000, LABINDIA).

Weight variation test

10 individual tablets of each formulation were weighed and their weights were recorded. Percent deviation from average weight was calculated for each formulation. The limits of deviation allowed as per IP were listed in table.

Specifications for uniformity of weight of capsules

Average weight	Percent deviation allowed
Less than 130mg	10
More than 130mg but less than 324mg	7.5
324mg or more	5

Hardness

The Hardness of the tablets was measured with a Monsanto hardness tester (M/s Campbell Electronics, model EIC-66, India). The results reported were average value with standard deviation of 10 tablets for each formulation.

Friability

For each formulation 10 tabs were weighed, placed in Friabilator (M/S Cambell Electronics, India) and were subjected to 100 rotations in 4 min. The tablets were reweighed and Friability was calculated along with mean and the standard deviation. The results are given in

$$\text{Friability} = \frac{W_1 - W_2}{W_1} \times 100$$

Where " W_1 " is the initial weight and " W_2 " is the final weight of the tablets.

In-vitro disintegration test

One tablet in to each tube was introduced and disc was added. The assembly was suspended in a beaker containing 1000mL of water and the

apparatus was operated for 30 minutes. The time taken for complete disintegration of each tablet was noted. The tablets pass the test if all of them have disintegrated within the time (30 min).

In-vitro dissolution test

The following conditions, as suggested by FDA, were used for dissolution testing of NTG epitant formulations.

Dosage form - tablet
 Apparatus - USP Type-2 (paddle)
 Mediu - 0.5% SLS in 0.01N HCl
 RPM - 50
 Temperature - $37 \pm 0.5^\circ\text{C}$
 Volume - 900 mL
 Sampling 5,10, , 20, 30 45 and 60 min.

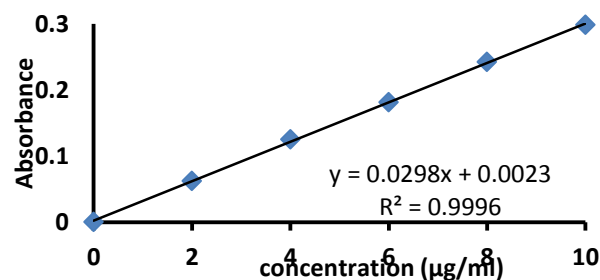
Aliquots of 5 ml were withdrawn at predetermined time interval and equivalent amount of fresh medium was replaced to maintain sink condition after each sampling and analyzed Spectrophotometrically at 210 nm against 0.5% SLS in 0.01N HCl as blank using UV-visible Spectrophotometer (UV-1800, Shimadzu). The inclusion complexes and liquid-solid formulations were taken for comparative study with marketed NTG tablets to observethe dissolution characteristics.

RESULTS AND DISCUSSION

RESULTS

Calibration curve data for the estimation of nateglinide in 0.01 n hydrochloric acid (pH-2)

S. No.	Concentration(μg/ml)	Absorbance
1	0	0.000
2	2	0.062
3	4	0.125
4	6	0.181
5	8	0.242
6	10	0.298



Calibration curve of Nateglinide solubility studies of NTG

AQUEOUS FLUIDS	Amount of NTG solubilized(mg/ml)
PH 2 0.01N HCl	0.0123
PH 4.6 acetate buffer	0.0064
PH 5.8 phosphate buffer	0.0015
PH 7.4 phosphate buffer	0.0100

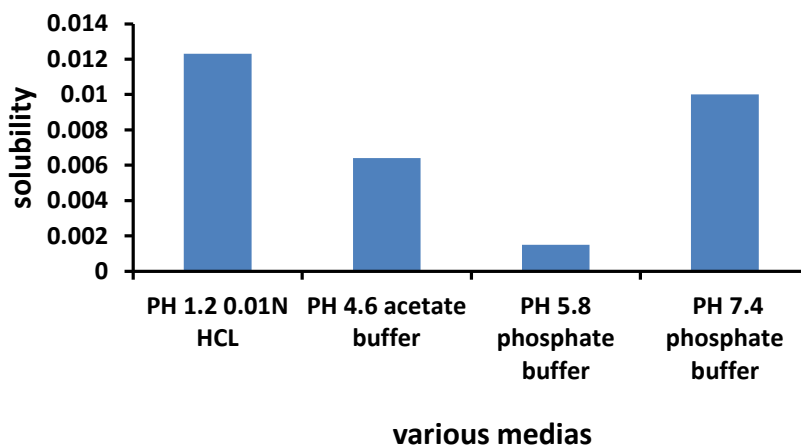


Figure: Solubility studies of NTG

Preparation of NTG tablets employing its complexes

Table: Formulae of NTG tablets prepared employing its complexes

S. No.	Ingredients (mg/tablet)	Formulation			
		F1	F2	F3	F4
1	Nateglinide	60	-	-	-
2	NTG: HP CD (1:2) KM	-	580	-	-
3	NTG: HP CD (1:2) SE	-	-	580	-
4	NTG: HP CD (1:2) PM	-	-	-	580
5	Lactose	40	20	20	20
6	SSG	40	40	40	40
7	Talc	5	5	5	5
8	Magnesium stearate	5	5	5	5
	Total (mg)	350	650	650	650

Table: Flow properties of complexation

Formulations	Bulk density	Tapped density	Carr's index	Hausner's ratio	Angle of repose	Flow comment
F1	0.5066	0.6909	26.67	1.36	46.04	Poor
F2	0.5000	0.6666	25.00	1.33	42.61	Passable
F3	0.5263	0.5882	10.52	1.11	32.61	Good
F4	0.5308	0.7962	33.33	1.50	46.16	Poor

Table: Drug content of formulations

F1	97.98 ± 1.09
F2	98.05 ± 2.15
F3	98.51 ± 1.66
F4	98.05 ± 2.23

Table: Post-compression evaluation

Formulations	Weight variation (mg)	Hardness (Kg/cm ²)	Friability (% wt. loss)	DT (see)
F1	349.67 ± 0.38	5.5 ± 0.95	0.82 ± 0.04	381±0.05
F2	648.15 ± 1.03	5.6 ± 0.63	0.66 ± 0.02	186±0.02
F3	646.37 ± 0.89	5.4 ± 0.51	0.74 ± 0.01	256±0.04
F4	650.02 ± 0.38	5.5 ± 0.45	0.59 ± 0.03	381±0.05

FORMULAE NATEGLINIDE TABLET DOSEGE FORMS (1:2)

Time(min)	% NTG dissolved		
	PM 1:2	SE1:2	KM1:2
0	0	0	0
5	44.35±0.79	28.06±0.74	45.61±1.06
10	52.33±0.63	41.23±1.19	60.90±1.78
20	59.98±0.59	65.60±1.21	73.55±0.66
30	71.19±0.74	71.26±1.07	80.30±1.01
45	81.37±0.80	83.31±2.33	87.30±1.09
60	83.60±0.93	87.7±1.18	98.44±0.19

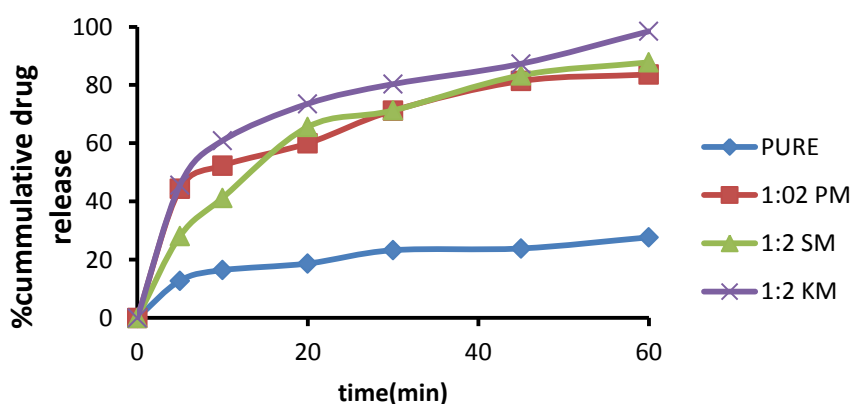


Figure: dissolution profiles of NTG complexes kneading method (1:1),(1:2)and (1:3) comprising NTG pure drug ratios

DISCUSSION

Calibration curve

The present analytical method obeyed beer's law in the concentration range of 2-10 µg/mL and it is suitable for the estimation of the NTG solution. The value of R^2 (regression coefficient) for the linear regression equation were found to be range of 0.993-0.999. results showed in table 6.1 and fig 6.1.1
 $y=0.029x-0.0002$

pH-Solubility studies

From the pH-solubility profile studies, it is understood the increase in the pH decreasing the solubility of NTG as it is weakly basic in nature. The solubility of NTG is high in pH-1.2 & increase in pH from 4.6-7.4 decreased the solubility. The solubility of NTG in pH 4.6-7.4 fluids is almost same and the solubility did not change with increase in pH. Results reported in table 6.2 and pH solubility profile is shown in fig 6.1.2.

Hence 0.01N HCL of pH-1.2 is selected for further development of dissolution medium as it afforded good solubility condition when compared to the other fluids

Evaluation of flow properties of complexation

Flow properties of complexation like bulk density, tapped density, Hausner's ratio, Carr's index and Angle of repose were evaluated and were given in table 6.4

Among the formulation prepared with drug and HPβ-CDs, F1 formulation containing showed poor flow characteristics while improved flowability was observed with increase in ratio of polymer.

Among the complexation prepared F2 and F3 formulations containing NTG and HPβ-CDs(1:2) ratio showed passable and good flow characteristics, whereas, no much improvement in flowability was observed with increase in the ratio of polymer. F4 containing lowest amount of polymer showed only passable flow properties which indicate the need for further increment in ratio of polymer

Preparation of Tablets by Direct Compression Method

All the materials required as per the formulae were blended in a closed polyethylene bag. The blends were directly compressed into tablets on a 16- station tablet punching machine (M/s Cadmach machineries Pvt. Ltd., Ahmedabad) to a hardness of 5- 6 kg/cm² using 9 mm flat

punches. In each case 10 tablets were compressed formulae given in table 6.3

Drug content

Drug content in each formulation was evaluated in order to assess uniformity of drug distribution throughout the polymer. The percentage drug content for all the prepared dispersions were found to be in the range of 97.98 ± 1.09 to 98.05 ± 2.23 indicating uniform drug distribution given in table 6.5

Post-compression parameter

Weight variation (mg) Hardness Friability, Test was carried out with 10 tablets of each formulation. Percent deviation from average weight was calculated for each formulation. All the formulations were found to be within limits of deviation allowed as per IP given in table 6.9

In vitro dissolution studies

Dissolution studies were carried out using 900 mL of 0.01N HCl with 0.5%w/v SLS as dissolution medium. USP type 2 apparatus, temperature of 37°C and 50 rpm test conditions were used in each study. NTG tablets were prepared as per formulae given in table. The results were given in table and shown in fig.

The cumulative percent release for F1, F2, F3 and F4 was found to be 27.82%, 98.4%, 87.79% and 83.60% respectively at the end of 60 min. The comparative dissolution profiles were shown table.

Increase in dissolution rates for formulations prepared from binary mixtures (F2, F3 and F4) compared to pure drug (F1) can be attributed to improved solubility of drug in dissolution medium resulting in improved dissolution rates. Since the CDs dissolve more rapidly in the dissolution medium than the pure drug, it can be assumed that in early stage of the dissolution process, the CDs molecule will operate locally on the hydrodynamic layer surrounding the particles of the drug. The action results in situ in the closed process, which produced a rapid increase of the amount of dissolved drug.

The superior dissolution properties observed with formulation prepared from kneading method (F2) over formulations from SE (F3) and PM (F4) may be due to the better interaction of NTG-HP β CDs during the kneading method binary system.

Overall, superior dissolution rates were observed with tablets prepared from binary mixtures compared to pure drug. Among the

formulations prepared from binary mixtures, kneading method gave superior dissolution rates compared to PM and SE. Tablet formulations showed increased dissolution rates compared to powered forms.

CONCLUSIONS

Enhanced physical stability of the prepared complexation formulations is attributed to drug-HP β -CDS interactions. complexation formulations are less susceptible to recrystallization, perhaps due to the solubilising effect. 1:2 kneading method good interaction. Physical mixture, solvent evaporation method and kneading method is investigated in present study improved the solubility and dissolution for NTG by conversion of crystalline nature to high energy amorphous NTG during complexation process. Physical mixture, solvent evaporation method and kneading method 1:2 ratios showed wetting agent properties.

The dissolution rates of physical mixture, SE and KM were higher than that of pure drug. The *in-vitro* drug release from the physical mixture, when compared to that of the SE and KM, was improved to a lesser degree. The mechanisms responsible for this improvement could be a solubilization effect of the carrier, miscibility of the drug in melted carrier and conversion of the drug from crystalline to amorphous form.

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