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PREPARATION AND CHARACTERISATION OF NATEGLINIDE-CYCLODEXTRIN INCLUSION COMPLEX

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ABSTRACT

The objective of the present work is preparation and physicochemical characterization of nateglinide (NT)-cyclodextrin (CD) inclusion complex both in solution state and solid state and to enhance the dissolution properties of nateglinide via complexation with β-cyclodextrin (βCD). Inclusion complexes are prepared by kneading and micro wave irradiation methods. The prepared inclusion complexes were investigated in solution state by phase solubility studies and solid state by DSC, FTIR, powder XRD and *in vitro* dissolution studies. Phase solubility studies revealed 1:1M stoichiometric inclusion complexes. A true inclusion complex of Nateglinide with β-Cyclodextrin at 1:1 and 1:2M in solid state was confirmed by Differential Scanning Calorimetry, and powder XRD. *In vitro* dissolution data suggests the improvement of dissolution of Nateglinide-β-Cyclodextrin inclusion complexes were superior when compared to pure. Overall microwave irradiation method showed superior dissolution properties when compared to kneaded systems and physical mixtures. Thus, from the research work it was concluded that aqueous solubility and dissolution rate of NAT can be improved by complexation with cyclodextrin.

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INTRODUCTION

An inclusion complex is formed when a macrocyclic compound possessing an intermolecular cavity of molecular dimensions interacts with a small molecule that can enter the cavity[1],[2]. The macrocyclic molecule is called the host and the small included molecule is called the guest. Cyclodextrins are containing a relatively hydrophobic central cavity and hydrophilic outer surface, cyclic (α-1,4)-linked oligosaccharides of α-D-glucopyranose. For complexation, the cavity size of cyclodextrin should be suitable to accommodate a drug molecule of particular size[3]. Cyclodextrins increases the insoluble drugs bioavailability by enhancing the drug solubility, dissolution and absorption/or drug permeability. They increase insoluble hydrophobic drugs permeability by making the drug available at the surface of the biological barrier, from where it partitions into the membrane without disrupting the lipid layers of the barrier[4-7]. Nateglinide is an oral antidiabetic agent used in the management of Type 2 diabetes mellitus also known as non-insulin dependent diabetes mellitus(NIDDM) or adult onset diabetes**.** Nateglinide by blocking ATP-sensitive potassium channels in pancreatic beta cells stimulates insulin secretion. It promotes a more rapid but less sustained secretion of insulin than other available oral antidiabetic agent[8,9]. Nateglinide logP value is 3.824 and practically insoluble in water and aqueous solubility about 0.03mg/ml belongs to class II drugs of BCS (i.e. high permeability and low solubility). The poor wettability and aqueous solubility of nateglinide makes difficulties in formulations and variations in bioavailability. Thus, the therapeutic importance is to increase the aqueous solubility of nateglinide. Cyclodextrins are carriers able to form inclusion complexes with poorly water soluble drugs. These inclusion complexes have been helpful to improve stability, aqueous solubility, dissolution rate and bioavailability. Hydrophilic derivatives such as β-cyclodextrin (βCD) or sulfobutyl ether-β-cyclodextrin, are useful for improving solubility and dissolution rate of poorly soluble drugs[10-13]. The objective of the present study, an attempt has been made to prepare inclusion complex of model drug nateglinide with βCD by conventional methods and characterize the inclusion complexes in solid state and liquid state.

MATERIALS AND METHODS

MATERIALS

The pure Nateglinide (NAT) was procured from Divis laboratories Hyderabad, , β-cyclodextrin(βCD) were obtained from Glenmark Pharma Ltd., Nasik, India and chemicals and solvents were of analytical grade.

METHODS

Physical mixture (PM):

The physical mixture of Nateglinide and βCD in 1:1 and 1:2M obtained by mixing individual components which are previously been sieved (100-120µm) together with a spatula.

Kneading (KNE):

The physical mixture of NT and βCD in 1:1 and 1:2M were triturated in a mortar with a small volume of dried dichloromethane. The thick slurry was kneaded for 60min and dried at 45^{o}C for 48 h. The dried mass was pulverized and sieved through a 100-150µm sieve and stored in dessicator until further evaluation.

Microwave oven irradiation (MC):

The aqueous solution of cyclodextrin was added slowly into a solution of NAT dissolved in dried dichloromethane with constant stirring. These solvents containing glass containers subjected for irradiation in microwave oven for 90sec at 60^{0}C. After reaction completed, adequate amount of dried dichloromethane added to remove the residuals. The resulting mixture stirred for 1h and evaporated under vacuum until dry. The dried mass was pulverized and sieved through a 100-120µm granulometric sieve and stored in dessicator until further evaluation. The different formulae were given in table 1.

Table1: Different formulae of nateglinide physical mixture and inclusion complex.

Batches	API	Cyclodextrin	Ratio	Method
B-1	NAT	βCD	1:1 M	PM
B-2	NAT	βCD	1:2 M	PM
B-3	NAT	βCD	1:1 M	KNE
B-4	NAT	βCD	1:2 M	KNE
B-5	NAT	βCD	1:1 M	MC
B-6	NAT	βCD	1:2 M	MC

EVALUATION

Drug content:

In each case physical mixture and inclusion complex equivalent to 20mg of NT was accurately weighed and transferred into 25ml volumetric flask. Dried ethanol was added and mixed to dissolve the NT. The volume was made up to 25ml with 0.5%w/v sodium lauryl sulphate in 0.01N HCl. From this 1ml is subsequently diluted with 0.5%w/v sodium lauryl sulphate in 0.01NHCl and measure the absorbance at 209nm by using double beam UV spectrophotometer, the NT content was calculated using the calibration curve.

Phase solubility studies:

Phase solubility studies carried out, as per the method described by Higuchi and Connors[14]. Excess amounts of NT (50mg) were added to 25ml of CD aqueous solutions (ranging in the concentration 0.01 to 0.1M) in a series of 25ml stoppered conical flasks. The mixtures were shaken for 72h at room temperature (28^{o}C) on a rotary flask shaker. After 72h shaking to achieve equilibrium.2ml aliquots were withdrawn at 12h intervals and immediately filtered using a 0.45µm nylon disc filter. The filtered samples diluted and assayed for NAT by measuring absorbance at 209nm. Shaking mixtures were continued until three consecutive estimations became the same. The solubility experiments were conducted in triplicate (coefficient of variation, CV<2%). The blanks were performed in the same concentrations of βCD in water in order to cancel any absorbance that may be exhibited by the Cyclodextrin molecules.The apparent stability constants calculated from the solubility diagrams, with the assumption of 1:1 stoichiometry, according to the equation:

$$Ks = \frac{Slope}{So(1-Slope)}$$ Where $S_o$is Nat solubility in the absence of Cyclodextrin.

FTIR:

The FTIR spectra were recorded for NAT, βCD, physical mixture and inclusion complexes on Shimadzu FTIR-281-spectrophotometer. Samples are prepared in KBr disks prepared with a hydrostatic press at force of 5.2Tcm^{-2} for 3 min. The scanning range was 450-4000cm^{-1} and resolution was at 1cm^{-1}.

Differential scanning calorimetry (DSC):

Thermograms of NAT, βCD and inclusion complexes were recorded on a Seiko, DSC 220C model Differential Scanning Calorimeter (Tokyo, Japan).About 10mg of samples were sealed in aluminum pans and heated at a rate of 10^{0}C/min from 30^{0}C-300^{0}C.

Powder X-ray diffractometry (XRD):

The powder X-ray diffraction studies of NAT, βCD, physical mixtureand inclusion complex recorded by using Philips X-ray powder diffractometer (model PW 1710) employing Cu-K_{α}-radiation. The diffractometer were run at 2.4^{0}/min interms of 2θ angle.

Dissolution studies:

In vitro dissolution studies of pure NAT, physical mixture and inclusion complex were carried out in 900ml of 0.5%w/v sodium lauryl sulphate in 0.01N HCl using a USP type 2 dissolution test apparatus using powder dispersed amount method (powder samples were spread over the dissolution medium). In each case sample equivalent to 50mg of NAT were used at optimum conditions viz., 900ml dissolution medium, speed of 50rpm and a temperature of 37^{0}C. A 5ml aliquot was withdrawn at predetermined intervals of time, filtered using a 0.45µm nylon disc filter and then replaced with 5ml of fresh dissolution medium. The filtered samples suitably diluted, assayed for NAT by measuring the absorbance at 209nm using double beam UV spectrophotometer. The dissolution experiments conducted in triplicate and the results were computed by using dissolution software PCP DISSO V3.0.

RESULTS AND DISCUSSION

Drug content:

The drug content was found to be in the range of 99.12% to 99.85% with low coefficient of variation and standard deviation i.e., less than 0.15% in all the batches prepared. Small SD and CV values shows the method employed gave inclusion complex with uniform drug content.

Phase solubility studies:

The solubility of NAT increases linearly with increase in the concentration of βCD, giving A_L type solubility diagram, the increase in solubility in the systems is due to one or more molecular interaction between NT and βCD to form complex. The βCD seems to optimal for entrapment of NT molecules and consequently provides the greatest solubilization effect. Solubility of NAT without βCD was 0.678±0.0012M×10^{-3} and the apparent stability constant obtained was 6.32M±0.01682 for βCD. The larger constant value indicates that NAT interacts strongly with βCD produces ($K_{1:1}$) stoichiometric constant. Phase solubility diagram was given in figure 1.

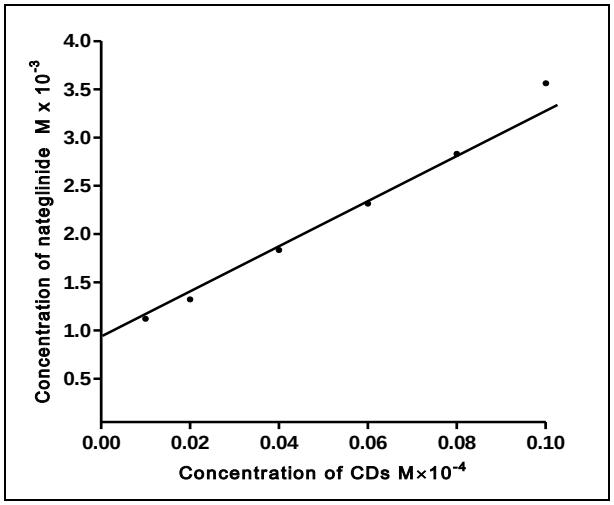

Figure 1: Phase solubility of NAT in 0.5%w/v sodium lauryl sulphate in 0.01N HCl.

FTIR study:

More evidence of complex formation obtained from FTIR study, investigated the functional groups of NAT involved in the complexation. The FTIR spectra of NAT shows the characteristic bands at 1244-1382cm^{-1} for carboxyl, carboxylate groups; 1650cm^{-1} for carbonyl stretching; 2857-3034 cm^{-1} for C-H stretching; 1723cm^{-1} for C=O vibration; 3369 cm^{-1} for NH stretching. The FTIR spectra of βCD showed intense band at 3600-3200cm^{-1} corresponding to absorption by hydrogen bonded OH groups and at 3000-2800 cm^{-1} stretching vibrations of -CH and -CH_2 groups. However, the spectra of inclusion complex showed rightward shifts of the band corresponding to hydrogen bonded group suggest existing bonds formed between the OH groups on the narrow side of cyclodextrin molecules which might be distributed after the formation of inclusion complex. In case of physical mixture the FTIR spectra was superimposed to pure NAT spectra where as in inclusion complex the aromatic carbonyl stretching band of drug appeared shifted to lower wave number 1635-1649cm-^{1} and 1649-1653cm-^{1} respectively for kneading and microwave irradiation inclusion complex. The FTIR results suggest the formation of hydrogen bonds between the carbonyl groups of NAT and the hydroxyl groups of the host cavities, during inclusion complexation[15,16] with cyclodextrin. These findings are in agreement with other authors[17] who previously reported that the carbonyl group is joined to a hydroxylic compound by hydrogen bonds, the stretching band is displaced to lower frequency due to a weakening of the carbonyl radical double bond.Comparative FTIR spectra were presented in figure 2.

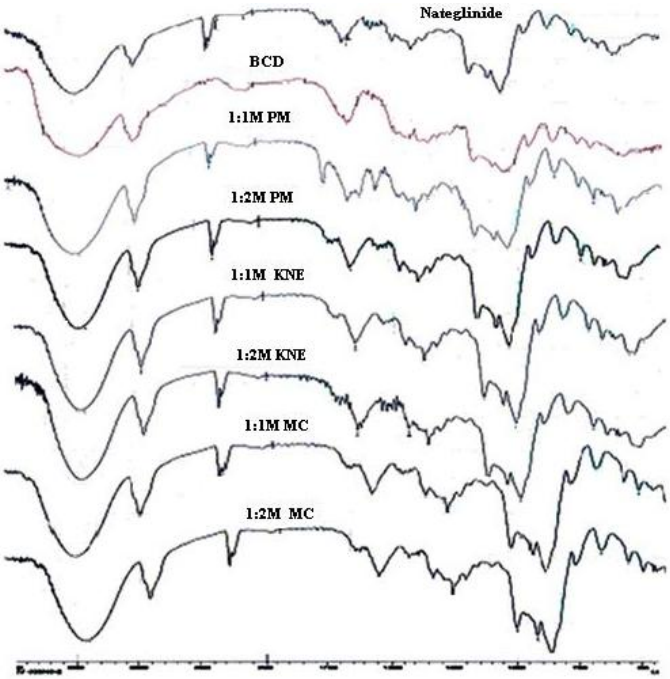

Figure 2: ComparativeFTIR spectra of NAT, physical mixture and its inclusion complex.

DSC study:

The DSC curves of the NAT, βCD and its inclusion complex and are given in figure 3. The method confirms not only an interaction between the NAT and βCD, but also a real inclusion. The DSC thermogram of NAT exhibited an endothermic peak at 132.18^{o}C corresponding to its melting point and DSC thermogram βCD showed broad endothermic peaks at 68.58^{o}C. Lowering of the endothermic peaks in βCD is mainly due to their dehydration process during analysis. The DSC curves of NAT-βCD 1:1 and 1:2M ratios shows progressive reduction in NAT endothermic peak intensity and shifted to lower temperatures, indicating the formation of an amorphous solid dispersion i.e., molecular encapsulation of NAT inside the βCD cavity suggest a true inclusion complexation.

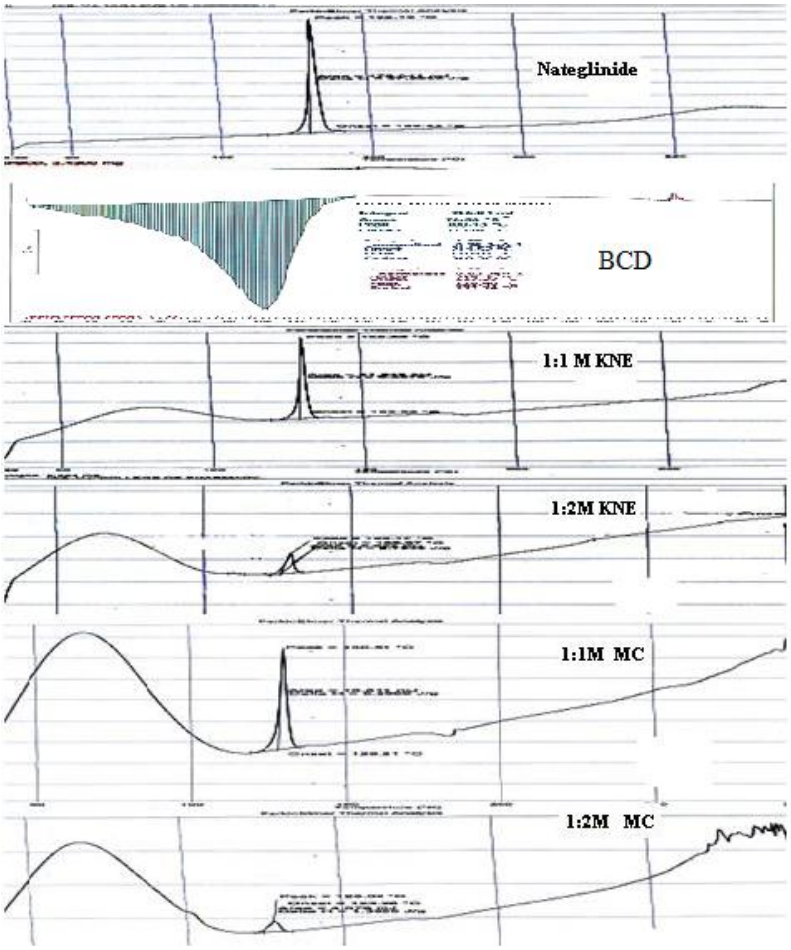

Figure 3: Comparative DSC spectra of NAT and its inclusion complex.

X-ray diffractometry studies:

Powder X-ray diffractometry is a useful tool for the detection of cyclodextrin complexation in powder or microcrystalline states. The diffraction pattern of the complex should be distinct from the superimposition of each of the components if a true inclusion complex were to form.X-ray diffraction pattern of NAT and its inclusion complexes prepared by all methods are presented in figure 4. By comparing some representative peak heights in the diffraction patterns of the inclusion complexes with those of a reference crystallinity was determined. The relationship used for the calculation of crystallinity was relative degree of crystallinity (RDC) $=I_{sam}/I_{ref}$, where I_{sam} is the peak height of the sample under investigation and I_{ref} is the peak height at the same angle for the reference with the highest intensity. NAT peak at 25.31^{0} (2θ) values was used for calculating RDC of kneaded and microwave irradiation inclusion complex. The RDC values were found to be 0.555; 0.407; 0.450; 0.297 for kneading and microwave irradiation method inclusion complex at 1:1 and 1:2M ratios. From the RDC values it is seen that when NAT was considered as reference sample, in case of NAT-βCD inclusion complexes lot of decrement in the crystalline structure of NAT in 1:1 and 1:2M NAT-βCD prepared by kneading and microwave irradiation method. In these systems all the characteristic peaks were suppressed and a nearly smooth peaks were observed indicate the formation of amorphous state of the complex. These results suggest that NAT undergoes a strong interaction with βCD at both the molar rations prepared by kneading and microwave irradiation method resulting formation of true inclusion complexes. Further these results were justified by DSC studies.

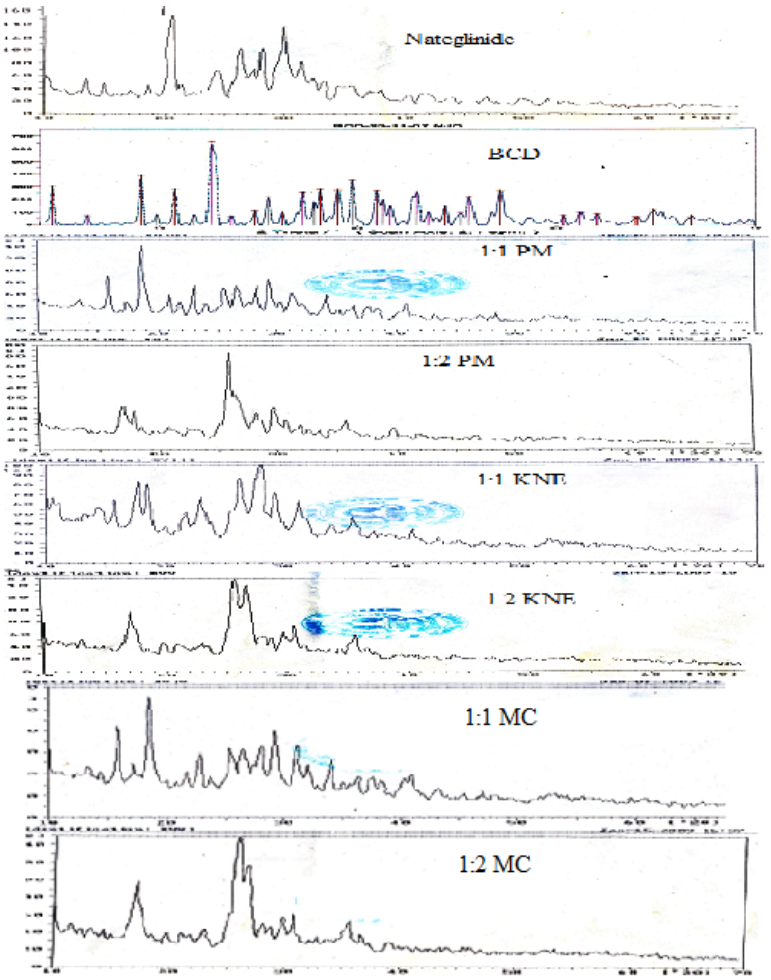

Figure 4: Comparative XRD spectra of NAT, physical mixture and its inclusion complexes.

Dissolution studies:

When an assumed drug-βCD inclusion complex dispersed in a dissolution medium, a very rapid dissolution was observed. Dissolution rate tests are based on this observation in order to characterize the inclusion complexation between drug and cyclodextrin. In the present investigation, dispersed amount method was used to investigate the various dissolution parameters of NAT and its inclusion complexes. The usual method of evaluation of *in vitro* dissolution testing is comparison of time taken for given proportions of active drug to be released into solution and parameters such as t_{50} values are often used. Alternatively, the fraction of drug in solution after given time is used to compare percent released in 30 minutes i.e. DP_{30} and also relative dissolution rate at 30min i.e. RDR_{30} are calculated to assess increase in extent of dissolution rate enhancement. Another parameter suitable for the evaluation of *invitro* dissolution has been suggested by Khan[18,19] who introduced the idea of 'Dissolution Efficiency' (DE). Dissolution efficiency is defined as the area under the dissolution curve upto a certain time 't', expressed as a percentage of the area of the rectangle described by 100% dissolution in the same time. The dissolution efficiency can have a range of values depending on the time interval chosen. In any case, constant time intervals should be chosen for comparison. In the present investigation DE_{30} and DE_{60} values were calculated from the dissolution data of each product and used for comparison.

$$\textbf{Dissolution efficiency (DE)} = \left(\frac{\int_0^t y\,dt}{y100^t}\right) 100$$

The dissolution data of NAT and its inclusion complexes were studied by using dissolution software PCP DISS0 V.3.0, the dissolution profiles are shown in figure 5. The dissolution data obtained, subjected to model fitting and the model which fits the observed dissolution data, evaluated by correlation coefficient (r) between the variables involved. The dissolution of standard NAT and from various inclusion complexes obeyed both Hixson-Crowell's cube root dissolution rate law and first-order dissolution models. $t_{50,}$ RDR_{30}, $DE_{10,}$ DE_{30} and DE_{60} values are calculated from the dissolution software and are given in table 2. The results of the dissolution rate studies indicated higher dissolution rate of Nat from inclusion complexes when compared to nateglinide itself and the corresponding physical mixtures. One-way ANOVA, used to test the statistical significance of difference between pure and treated samples. Significant differences in the means were tested at 95% confidence. The DE_{30} and DE_{60} values were significantly higher ($P<0.05$) in inclusion complexes prepared by microwave irradiation method when compared to standard NAT and other inclusion complexes.

The slight increase in dissolution rate and efficiency values were recorded for the physical mixture may be explained on the basis of the drug solubility in aqueous βCD solutions. Since the βCD dissolve more rapidly in the dissolution medium than the drug alone, can be assume that, in early stages of the dissolution process, the βCD molecule will operate locally on the hydrodynamic layer surrounding the particles of the drug[20, 21, 22]. This action results in an *insitu* inclusion process, which produces a rapid enhancement of the amount of the dissolved drug. Overall the rank order of increase in dissolution properties of nateglinide with βCDconcentration 1:2>1:1M and with methods MC>KNE>PM.

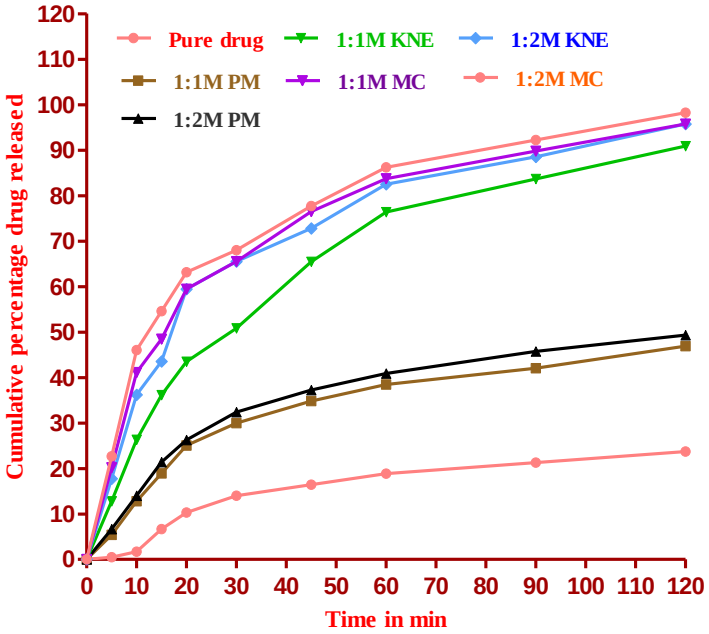

Figure 5: Comparative dissolution profiles of pure NAT, physical mixture and inclusion complex.

Table 2: Dissolution parameters data for pure NAT, physical mixtures and NAT-βCD inclusion complex.

Dissolution parameter	NAT – βCD Inclusion complex						
	Pure drug	Physical Mixture		Kneading		Microwave	
		1:1M	1:2M	1:1M	1:2M	1:1M	1:2M
t_{50}(min)	>120	113.6	107.4	34.9	31.7	42.9	39.7
DE_{30} (%)	6.39	16.53	17.66	24.21	26.26	28.93	31.08
DE_{60} (%)	11.41	25.98	27.97	48.21	57.73	59.83	62.54
RDR_{30}	1	2.05	2.14	3.10	3.28	3.46	3.72
First order`r'	0.8991	0.8169	0.8170	0.9956	0.9966	0.9855	0.9862
Hixson Crowell's cube root `r'	0.8915	0.7871	0.7829	0.9700	0.9696	0.9637	0.9614

CONCLUSIONS

Physicochemical characterization of NAT-βCD inclusion complexin solution state by phase solubility revealed 1:1M complexation of NAT with βCD. A true inclusion of NAT with βCD at 1:1 and 1:2 M kneaded and microwaves irradiated inclusion complexes and in solid state were confirmed by FTIR, DSC, powder XRD studies. Dissolution properties of NAT-βCD inclusion complexes were superior when compared to pure NAT. Overall microwave irradiation method showed superior dissolution properties when compared to kneaded systems and physical mixtures. Thus, from the research work it was concluded that aqueous solubility and dissolution rate of NAT can be improved by complexation with cyclodextrin.

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Conflict of interest

Nil

Abbrevations

ATP	Adenosine Tri Phosphate
BCS	Biopharmaceutical Classification System
CD	Cyclodextrin
DE	Dissolution Efficiency
DE_{10}	Dissolution Efficiency at 10 min
DE_{30}	Dissolution Efficiency at 30 min
DE_{60}	Dissolution Efficiency at 60 min
DP_{30}	Percent of Drug Dissolved at 30 min
DSC	Differential Scanning Calorimetry
FTIR	Fourier transform IR spectra
HCl	Hydrochloric acid
KBr	Potassium Bromide
KNE	Kneading (KNE):
Ks	Apparent stability constants
MC	Microwave oven irradiation (MC):
NIDDM	non-insulin dependent diabetes mellitus
NT	nateglinide
PM	Physical mixture
RDC	relative degree of crystallinity
RDR_{30}	Relative Dissolution Rate
SD	Standerd Deviation
XRD	X-Diffraction Chromatography
βCD	β-cyclodextrin

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