World Journal of Pharmaceutical Sciences
ISSN (Print): 2321-3310; ISSN (Online): 2321-3086
Available online at: http://www.wjpsonline.org/
Original Article

Preparation and characterization of nateglinide- β cyclodextrin inclusion complexes

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Received: 21-05-2018 / Revised Accepted: 28-06-2018 / Published: 02-07-2018

ABSTRACT

The objective of the work is physicochemical characterization of nateglinide (NT)- β-cyclodextrin (CD) binary systems both in solution state and solid state and to improve the dissolution properties of nateglinide via complexation with β-cyclodextrin. Binary systems of NT with β-CD, prepared experimentally by different technique (evaporation and kneading),were investigated in solution state by phase solubility studies and solid state by differential scanning calorimetry, Fourier transformation-infrared spectroscopy, and in vitro dissolution studies. Phase solubility studies revealed 1:1M stoichiometric solid binary systems. Stability constants (Ks) were calculated were found to be CD dependent. A true inclusion complex of NT with β-CD at 1:1 and 1:2M in solid state was confirmed by DSC. *In vitro* dissolution data suggests the enhancement of dissolution properties of NT- β-CD binary systems were superior when compared to pure.

Keywords: Nateglinide; Cyclodextrins; FTIR; DSC; Solid binary systems; *in vitro* dissolution;

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How to Cite this Article: Venkatesh, Anand Kumar Y, C. Mallikarjuna Setty. Preparation and characterization of nateglinide- β cyclodextrin inclusion complexes. World J Pharm Sci 2018; 6(7): 51-57.

INTRODUCTION

Nateglinide (NT) N-(Trans-4-Isopropylcyclohexyl-1-Carbonyl)-D-Phenylalanine 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 stimulates insulin secretion by blocking ATP-sensitive potassium channels in pancreatic beta cells. It promotes a more rapid but less sustained secretion of insulin than do other available oral antidiabetic agent. Nateglinide recently has been approved by the United States Food and Drug Administration (FDA) for use in type 2 diabetic mellitus and is most effective if administered 1 to 10 minutes before a meal in 120 mg dose, nateglinide is rapidly absorbed with mean peak plasma drug concentrations (Cmax) generally occurring within 1 hour (Tmax) after dosing[1,2]. Nateglinide is practically insoluble in water and having aqueous solubility about 0.03mg/ml. and its log P value is 3.824 indicates class II drugs of BCS (Biopharmaceutical classification systems i.e. High permeability and low solubility).The poor aqueous solubility and wettability of nateglinide leads to difficulties in formulating oral solutions and variations in bioavailability. Thus, increasing the aqueous solubility of nateglinide is of therapeutic importance. Cyclodextrins (CDs) are cyclic (α-1,4)-linked oligosaccharides of D-glucopyranose containing a relatively hydrophobic central cavity that provides a microenvironment for appropriate sized non polar molecules and hydrophilic outer surface. These carriers have been widely applied as multifunctional pharmaceutical excipients due to their remarkable molecular complexation property with many drugs, modifying their physical, chemical and biological properties[3]. Hydrophilic derivatives such as βCD or sulfobutyl ether-β-cyclodextrin, are useful for improving solubility and dissolution rate of poorly soluble drugs[4].

In the present study an attempt was made to prepare and evaluate nateglinide solid binary systems in order to improve aqueous solubility and dissolution properties. Cyclodextrins are able to form solid binary systems with poorly water soluble drugs. These solid binary systems have been shown to improve stability, solubility, dissolution rate and bioavailability[5,6].

MATERIALS AND METHODS

Materials: The nateglinide pure sample was supplied by Divis laboratories Hyderabad, India, β-cyclodextrin (βCD), was obtained from Glenmark Pharma Ltd., Nasik, India and all other chemicals and solvents were of analytical grade.

Phase solubility studies: Phase solubility studies were carried out, according to the method described by Higuchi and Connors[7]. Excess amounts of nateglinide (50mg) were added to 25ml of either purified water or β-CD aqueous solutions (ranging in concentration from 0.01 to 0.1M) in a series of 25ml stoppered conical flasks. The mixtures were shaken for 72h at room temperature (28ºC) on a rotary flask shaker. After 72h shaking to achieve equilibrium 2ml aliquots were withdrawn at 12h intervals and filtered immediately using a 0.45µm nylon disc filter. The filtered samples were diluted and assayed for nateglinide by measuring absorbance at 209nm. Shaking was continued until three consecutive estimations were the same. The solubility experiments were conducted in triplicate (coefficient of variation, CV<2%). The blanks were performed in the same concentrations of β-CD in water in order to cancel any absorbance that may be exhibited by the β-CD molecules. The apparent stability constants were calculated from the solubility diagrams, with the assumption of 1:1 stoichiometry, according to the equation:

$$Ks = \frac{\text{slope}}{So(1 - \text{slope})}$$

Where S_o is NT solubility in the absence of β-CD.

Preparation of solid binary systems: The following binary systems of NT and β-CD were prepared at 1:1 and 1:2 molar ratios (1:1 and 1:2M).

Physical mixture (PM): The physical mixture of NT and β-CD in 1:1 and 1:2M were obtained by mixing individual components that had previously been sieved (100-120µm) together with a spatula.

Kneaded system (KNE): The physical mixture of NT and β-CD in 1:1 and 1:2M were triturated in a mortar with a small volume of dried ethanol. The thick slurry was kneaded for 60min and then dried at 45ºC for 48 h. The dried mass was pulverized and sieved and a 100-150µm granulometric sieve.

Coevaporated systems (CE): The aqueous solution β-CD was added to a solution of nateglinide dissolved in dry ethanol. The resulting mixture was stirred for 1hr and evaporated until nearly dryness. The dried mass was further dried at 45ºC for 48 h .The dried mass was pulverized and sieved through a 100-150µm granulometric sieve.

Fourier transmitted infrared spectroscopy: Fourier transform IR spectra were recorded on a Shimadzu FTIR-281-spectrophotometer.The spectra were recorded for nateglinide βCD, physical mixture, and all binary systems. Samples were prepared in KBr disks prepared with a hydrostatic press at a force of 5.2Tcm^{-2} for 3 min.

The scanning range was 450-4000cm^{-1} and the resolution was 1cm^{-1}.

Differential scanning calorimetry: Thermograms of pure materials, their treated samples and all binary systems 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.

Dissolution studies: *In vitro* dissolution studies of pure nateglinide and its solid binary systems (i.e. prepared solid binary systems) were carried out in 900ml of 0.5%w/v sodium lauryl sulphate in 0.01NHCl using a USPXXI type 2-dissolution rate test apparatus by the powder dispersed amount method (powder samples were spread over the dissolution medium).Sample equivalent to 20mg of nateglinide, speed of 50rpm and a temperature of 37^{0}C were used in each test. A 5ml aliquot was withdrawn at different time intervals, filtered using a 0.45µm nylon disc filter and replaced with 5ml of fresh dissolution medium. The filtered samples were suitably diluted, if necessary and assayed for nateglinide by measuring the absorbance at 209nm.The dissolution experiments were conducted in triplicate. The results were computed by using dissolution software PCP DISSO V3.0.

RESULTS AND DISCUSSIONS

Drug content: The coefficient of variation (CV) and standard deviation (SD) in the percent drug content was found to be less than 0.1% in all the batches prepared. The yields of all the preparation were in the range of 95-99% of the initial amounts taken. Small SD and CV values indicate that the method employed gave inclusion complexes with uniform drug content and good yields.

Phase solubility studies: A summary of the findings of the phase solubility diagram was given in Figure 1.The solubility of nateglinide increases linearly with an increase in the concentration of β-CD, giving AL type solubility diagram. The increase in solubility in the systems is due to one or more molecular interaction between NT and β-CD to form complex. The apparent stability constants (K1:1) were calculated for these complexes from phase solubility diagrams. The stability constant value calculated was 6.32M±0.01682 for βCD. The larger constant that was observed with βCD indicates that nateglinide interacts strongly with βCD.

Fourier transmitted infrared spectroscopy: More evidence of complex formation was obtained from FTIR study, which investigated the functional groups of NT involved in the complexation. Examples of FTIR spectra are presented in figure 2. In the FTIR spectra of NT shows the principle peaks at the wave numbers of 1244-1382cm^{-1} justifying the presence of carboxyl, carboxylate groups and carbonyl stretching at 1650cm^{-1}, C-H stretching between 2857-3034 cm^{-1}, C=O vibration at 1723cm^{-1} and NH stretching appeared at 3369 cm^{-1}.The FTIR spectra of CDs showed intense band at 3600-3200cm^{-1}(3322cm-^{1} for βCD), corresponding to absorption by hydrogen bonded OH groups and at 3000-2800cm^{-1}(2922cm-^{1} for βCD) were assigned to stretching vibrations of the bonds in –CH and –CH_2 groups.

However, the spectra of solid binary system prepared by kneading and coevaporation methods showed rightward shifts of the band corresponding to hydrogen bonded group(3415 to 3381cm^{-1}).This result suggest that some of the existing bonds formed between the OH groups on the narrow side of cyclodextrin molecules might be distributed after the formation of solid binary systems. In the nateglinide FTIR spectra, a strong absorption peak was observed at 1660cm^{-1}, which was assigned to drug carbonyl stretching. In case of physical mixture the FTIR spectra was superimposed to pure nateglinide spectra where as in solid binary systems prepared with βCD by coevaporation method the characteristic aromatic carbonyl stretching band of drug appeared shifted to lower wave number at 1635-1649cm-^{1} for NAT-βCD. In kneading method the characteristic aromatic carbonyl stretching band of drug appeared shifted to lower wave number at1649-1653cm-^{1} suggesting the formation of hydrogen bonds between the carbonyl groups of nateglinide and the hydroxyl groups of the host cavities, during solid binary systems[8,9] cyclodextrins. These findings are in full agreement with other authors[10] 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.

Differential scanning calorimetry: The DSC curves of the NT, β CD compared with its solid binary systems prepared by coevaporation and kneading method are presented in Figure 3. The method confirms not only an interaction between the drug and β-CD, but also a real inclusion. The DSC thermogram of NT exhibited an endothermic peak at 132.18^{o}C corresponding to its melting point and DSC thermograms of βCD showed broad endothermic peaks at 96.99 ^{o}C. Lowering of the endothermic peaks in β-CD are mainly due to their dehydration process during analysis and a small peak observed at 218.96 and 220.91^{o}C for βCD corresponds to its melting point indicating, major portion of βCD undergoes dehydration during analysis. The DSC curves of NT were compared

with the NAT- β-CD binary systems prepared by coevaporation and kneading method. DSC curves of NAT-βCD 1:1 and 1:2M ratios indicate progressive reduction in NT endothermic peak intensity and shifted to a lower temperatures passing from physical mixture to kneaded and coevaporated systems. This marked reduction in intensity and/or broadening and shift to a lower temperature of the NT endotherm in kneaded and coevaporated systems indicates a partial inclusion of NT in the β-CD cavities. On the contrary, in the binary systems prepared by the kneading and coevaporation methods showed the complete disappearance of the NT endothermic peak indicating the formation of an amorphous solid dispersion, the molecular encapsulation of the drug inside the β-CD cavity, i.e. a true solid binary systems

Dissolution studies: When an assumed drug- β-CD inclusion complex is dispersed in a dissolution medium, a very rapid dissolution is often observed. Dissolution rate tests are based on this observation in order to characterize the solid binary systems between drug and cyclodextrin. The most often used dissolution rate tests are the rotating disk method and dispersed amount method. In the present investigation, dispersed amount method is used to investigate the various dissolution parameters of nateglinide and its solid binary systems. The usual method of evaluation of in vitro dissolution testing is the 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 for comparison such as percent released in 30 minutes i.e.DP_{30} and also relative dissolution rate at 30minutes i.e. RDR_{30} are calculated to assess improvement in extent of dissolution rate enhancement. Another parameter suitable for the evaluation of *invitro* dissolution has been suggested by Khan[11] who introduced the idea of 'Dissolution Efficiency' (D.E.). 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.

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

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_{10}, DE30 and DE_{60} values were calculated from the dissolution data of each product and used for comparison.

The dissolution data of nateglinide and its solid binary systems were studied by using dissolution software **PCP DISS0 V.3.0** and data are computed in **Table 2** and the dissolution profiles are shown in **figure 4.** The dissolution data obtained were subjected to model fitting and the model which fits the observed dissolution data was evaluated by correlation coefficient (r) between the variables involved. The calculated `r' values in various models for all complexes are summarized in **Tables.** The dissolution of standard nateglinide and from various solid binary systems 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 were calculated from the dissolution software and are given in **Tables.**

The results of the dissolution rate studies indicated higher dissolution rate of nateglinide from solid binary systems when compared to nateglinide itself and the corresponding physical mixtures. One-way ANOVA was 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 solid binary systems prepared by coevaporation method when compared to standard nateglinide and other solid binary systems. The slight increase in dissolution rate and efficiency values recorded for the physical mixture may be explained on the basis of the solubility of the drug in aqueous β-CD solutions. Since the β-CD dissolve more rapidly in the dissolution medium than the drug alone, it can be assumed that, in early stages of the dissolution process, the β-CD molecule will operate locally on the hydrodynamic layer surrounding the particles of the drug[12,13,14,15]. This action results in an insitu inclusion process, which produces a rapid increase of the amount of the dissolved drug. Overall the rank order of improvement in dissolution properties of nateglinide with β-CD is, concentration 1:2>1:1M and with methods CE>KNE>PM.

Conclusions: Physicochemical characterization of NT- β-CD binary systems in solution state by phase solubility revealed 1:1M complexation of nateglinide with the β-CD. A true inclusion of NT with BCD at 1:1 and 1:2 M kneaded and coevaporated binary system in solid state was confirmed by DSC studies. Dissolution properties of NT- β-CD binary systems were superior when compared to pure NT. Overall coevaporated systems showed superior dissolution properties when compared to kneaded systems and physical mixtures. Thus, the pharmaceutical like aqueous solubility and dissolution rate of NT can be improved by complexation with cyclodextrins.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the staff of V.L. College of Pharmacy, Raichur and Principal and staff of Sarada Vilas College of Pharmacy, Mysuru for their constant support and cooperation during research work.

Table 1: Phase solubility of nateglinide in 0.5%w/v SLS in 0.01NHCl.

Concentration of CDs M in 0.5%w/v SLS in 0.01NHCl	**Concentration of nateglinide in βCD solution M×10^{-3}**	
	Mean	**SD**
0.001	0.926	0.0012
0.002	1.190	0.0012
0.003	1.275	0.0116
0.004	1.440	0.0012
0.005	1.634	0.0017
0.010	1.923	0.0015
Solubility of nateglinide without CDs: 0.678± 0.0010 M×10^{-3}		

Table 2: Dissolution parameters for pure drug, physical mixtures and NAT-βCD Solid binary systems

Dissolution Parameter	**NAT –βCD Solid binary systems**						
	Pure drug	**Physical Mixture**		**Kneading**		**Coevaporated**	
	---	**1:1M**	**1:2M**	**1:1M**	**1:2M**	**1:1M**	**1:2M**
T_{50}(min)	>120	113.6	107.4	34.9	31.7	29.7	31.7
D.E_{10} (%)	0.68	5.61	6.53	11.46	12.38	17.31	12.38
D.E_{30} (%)	6.39	16.53	17.66	28.93	31.08	39.27	31.08
D.E_{60} (%)	11.41	24.91	26.08	44.58	47.04	54.36	47.04
RDR_{30}	1	2.05	2.14	3.46	3.72	4.42	3.72
First-order-model`r'	0.8991	0.8169	0.8991	0.9956	0.9966	0.9742	0.9966
Hixson-Crowell's cube root model`r'	0.8915	0.7871	0.8915	0.9700	0.9696	0.9102	0.9696

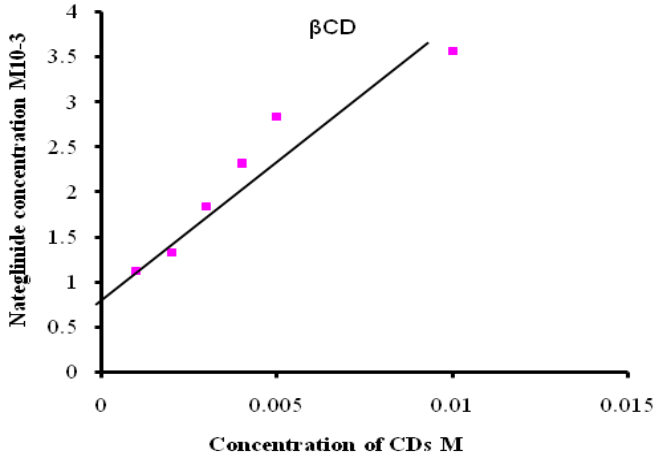

Figure 1: Phase solubility of nateglinide in 0.5%w/v sodium lauryl sulphate in 0.01N HCl

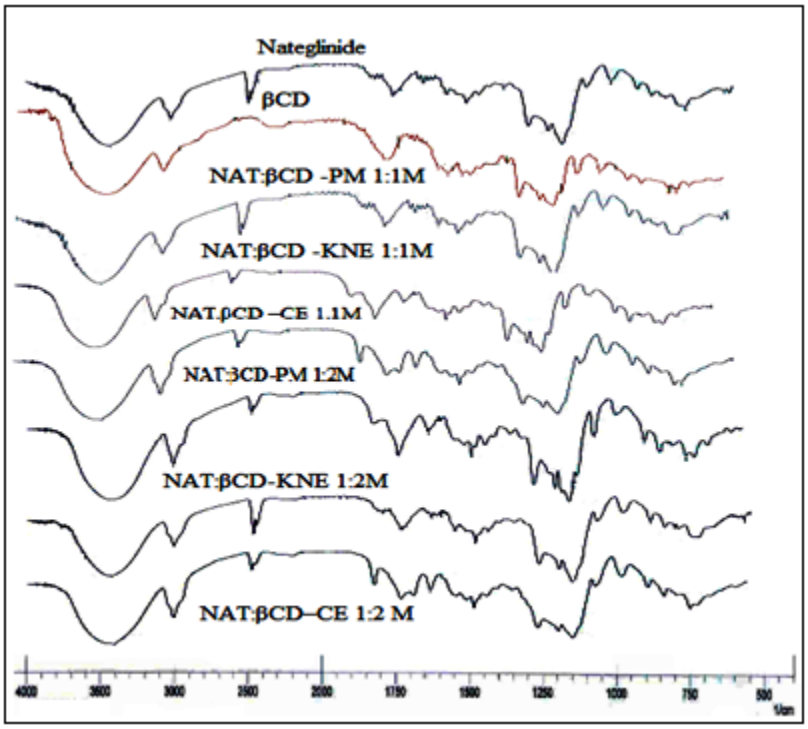

Figure 2: Comparison between FTIR spectras of nateglinide, βCD, NAT: βCD physical mixture and its solid binary systems. A)NAT B)βCD C) NAT:βCD -PM 1:1M D) NAT:βCD -KNE 1:1M E) NAT:βCD –CE 1:1M F) NAT:βCD-PM 1:2M G) NAT:βCD-KNE 1:2M H) NAT:βCD–CE 1:2 M.

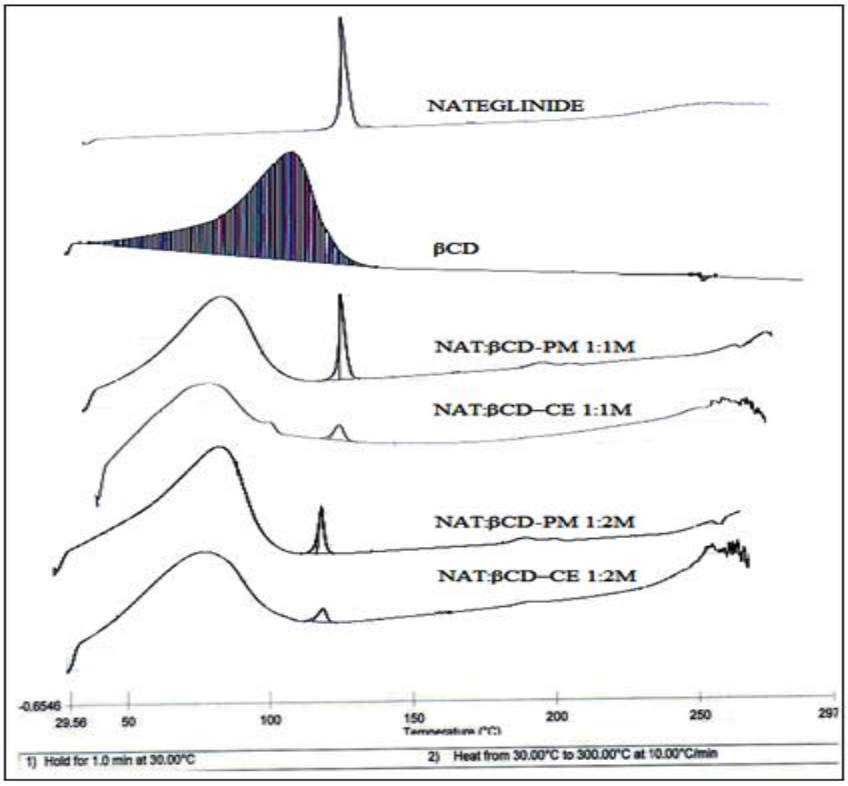

Figure 3: Comparison between DSC spectras of nateglinide, βCD NAT:βCD physical mixture and its solid binary systems. A)NAT B) βCD C) NAT:βCD-PM 1:1M D) NAT:βCD–CE 1:1M E) NAT:βCD-PM 1:2M F) NAT:βCD–CE 1:2M.

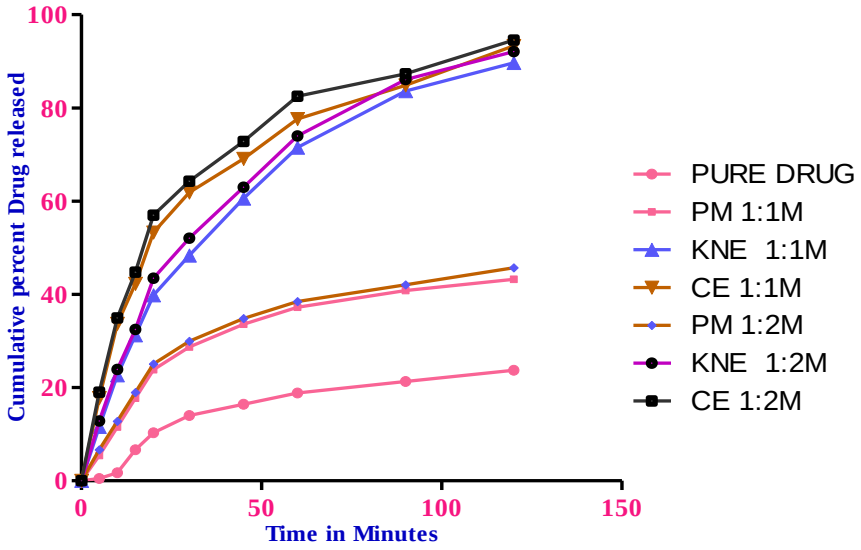

Figure 4: Dissolution profiles of NAT-βCD 1:1M and 1:2M solid binary systems.

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