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INFLUENCE OF METHOD OF PREPARATION ON PHYSICO-CHEMICAL PROPERTIES AND IN-VITRO DRUG RELEASE PROFILE OF NIMODIPINE-CYCLODEXTRIN INCLUSION COMPLEXES: A COMPARATIVE STUDY.

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ABSTRACT

In the present work, inclusion complex of Nimodipine (ND) and both β-cyclodextrin (β-CD) and hydroxypropyl β-cyclodextrin (HPβ-CD) was obtained to improve the in-vitro bio-availability of the drug. Inclusion complex in solution was studied by phase solubility technique. The apparent stability constants obtained were 293.87 M^{-1} and 1035.78 M^{-1} at 37 ˚C for both β-CD and HP β-CD respectively. Phase solubility study showed an A L-type diagram indicating the formation of an inclusion complex in 1:1 molar ratio. Solid binary mixtures of the ND with β-CD and HP β-CD were prepared by several methods such as physical mixing, kneading and spray drying. Physicochemical characterizations were performed using Differential scanning colorimetry (DSC), Powder X-ray diffraction (XRD), Scanning electron microscopy (SEM), and aqueous solubility and dissolution studies. Preparation method influenced the physicochemical properties of the binary mixtures. An inclusion complex obtained by spray drying method and it showed greater solubility and drug dissolution rate. The physical stability of the complex was studied. After one year storage in glass container at room temperature no significant change were detected in the solubility and dissolution profile of the spray dried product.

Key words: Nimodipine, β-CD, HPβ-CD, Spray drying, Inclusion complex, Dissolution rate.

INTRODUCTION

Nimodipine (ND) is a dihydropyridine calcium channel blocker originally developed for the treatment of high blood pressure. The contractile pressure of smooth muscle cells are dependent upon calcium ions, which enter these cells during depolarization as slow ionic trancemembrence currents. ND inhibits calcium ion transfer into these cells and thus inhibits contractions of vascular smooth muscle. However, ND is practicaly insoluble in water and this poor water solubility seems to be a problem for its bio-availability and gastrointestinal side effects[1,2].

Cyclodextrins (CDs) are crystalline homogeneous non-hygroscopic substances, which have a torus like macro ring shape, built up from glucopyranose units. They are cyclic oligosaccharides, which are produced by enzymatic degradation of starch by a glucoasyltransferase most commonly derived from *Bacillus macerans and* have been recognized as useful pharmaceutical excipients[3]. Complexation with cyclodextrins has been reported to enhance the solubility, dissolution rate and bioavailability of poorly soluble drugs. Especially, HPß-CD is widely used in the pharmaceutical field owing to its high aqueous solubility, ability to stabilize the drug molecule and capable to mask the bad taste and odor.[4, 5, 6] In the present work an attempt was made to compare the influence of method of preparation on physico-chemical characteristics and dissolution rate of drug in its inclusion complex form.

MATERIALS AND METHODS

Nimodipine was received as a gift sample from Sun pharmaceuticals Ltd, (Baroda, India). HPβ-CD and β-CD were generous gift samples received from Gangwal chemical Pvt. Ltd.(Mumbai, India) and Torrent research center (Gandinagar, India) respectively. All Chemicals and solvents used in the study were of analytical reagent grade. Fresh distilled water was used.

Phase solubility study

The stoichiometric ratio and quantitative expression of stability constant were determined by using phase solubility method of Higuchi and Connors[7]. Excess quantity of the drug was transferred to 20 mL of aqueous solution of β-CD and HP-β-CD separately in various molar concentrations between 0 to 14 mM/L in screw capped vials. These solutions were stirred at 100 rpm on electromagnetic stirrer (Remi Ltd.Mumbai, India) at constant temperature of 37°C ± 0.1°C for 72 hrs. After 72 hrs, aliquots were filtered through a 0.45 μm membrane filter. The filtrate was suitably diluted and analyzed spectrophotometrically at 317 nm. The experiments were conducted in triplicate. Data obtained from phase solubility diagram was used to determine stoichiometric ratio by plotting graph of concentration of ND against the concentrations of both cyclodextrins. The stability constant Ks for the complexes were determined from the graph using following equation:

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

Where slope is obtained from the graph and S_0 is the equilibrium solubility of ND in water.

Preparation of inclusion complexes

Physical mixture (PM)

Pure drug with β-CD and HPβ-CD in the molar ratio of 1:1 was mixed separately in a glass mortar by geometric mixing without applying pressure for about one hour. The mixture was passed through sieve # 100 and stored in the desiccators over fused calcium chloride[8].

Kneading method (KN)

Inclusion complex by KN method was prepared by geometric mixing of ND with β-CD and HPβ-CD separately in 1:1 molar ratio. Then the mixture was kneaded with 1 mL of 0.2:1 mixture of 25% ammonia solution : water to obtain a pasty mass. The pasty mass was then dried in hot air oven at 45 to 50°C for 24 hours. The dried mass was pulverized and passed through sieve # 100 and stored in desiccator over fused calcium chloride.

Spray drying method (SPD)

The inclusion complex of ND-βCD was prepared by spray drying method. A mixture of drug and β-CD was dissolved in 250 ml of water. To the above solution a mixture of water and 25 % ammonia was added drop wise slowly until a clear solution was formed [9,10]. The resultant solution was spray dried using a spray dryer (LU-222, Advanced, Labultima, Mumbai). The spray drying was done at the following sets of conditions; air flow rate at 400 Nl /h, spray nozzle with a diameter 0.7mm under the atomization pressure of 2 kg/cm^2 with a feed rate of 4ml/min. The inlet temperature was kept at 120 ˚C and out let temperature 90 ˚C ± 2 ˚C. The vacuum in the system was 60 mmWc and aspiration rate was 40 m Bar. The same procedure was adopted to prepare inclusion complex of ND-HPβ-CD. The product thus obtained was collected, packed, doubly wrapped in an aluminum foil and stored in a desiccator till further use. The formulation details are given in Table 1.

Table 1: It shows different formulations of Nimodipine with β-CD and HPβ-CD inclusion complexes

Types of CDs	Method of preparation	Product codes
βCD	Spray drying	SPB
	Kneading	KNB
	Physical mixture	PMB
HPβCD	Spray drying	SPH
	Kneading	KNH
	Physical mixture	PMH
Nimodipine	---	ND

Evaluation of inclusion complexes

Carr's index

This property is indirectly related to the relative flow rate, cohesiveness and particle size of the powder and it was calculated using the following equation:

$$Carr's\ index\ (\%) = \frac{Tapped\ density - Bulk\ density}{Tapped\ density} X\ 100$$

Mean particle size

The mean particle size of powdered inclusion complex was determined using fluorescence microscopy (AVC CXRIII 561, Digital Color CCD Camera, Labomed) method. A small amount of sample was placed on a clean glass slide, mounted on the stage of the microscope and particle size was measured in 4X or 20X lens. The experiment was performed for 50 times and mean particle size was calculated.

Percentage Powder yield

The percentage powder yield for the inclusion complex was calculated using the following equation;

$$\%\ Powder\ yield = \frac{Weight\ of\ powder}{Totol\ weight\ of\ solid} X100$$

Drug entrapment efficiency[11]

Inclusion complex equivalent to 20 mg of ND was crushed in a mortar with 2 ml of 0.01M methanolic hydrochloric acid. Then, the solution was adjusted to 100 ml with the same and vigorously shaken for 20 min. The solution was then filtered through 0.45 µm membrane filter, diluted suitably and absorbance was measured spectrophotometrically at 317 nm. The amount of ND present was calculated using the following equation;

$$\%\ Drug\ entrapment\ efficiency = \frac{Actual\ drug\ content}{Theoritical\ drug\ content} X\ 100$$

Aqueous solubility[12]

An excess amount of sample was added to 5 ml of the distilled water in test tubes sealed with stoppers. The test tubes were vortex-mixed for 5 min and then sonicated for 30 min. They were kept in a constant temperature shaking bath maintained at 37 ± 0.5 ˚C until reaching equilibrium (48 h). A portion of solution was withdrawn and then filtered with a nylon disc filter (0.45 µm) and adequately diluted with methanol. The amount of drug solublised was determined at 317 nm by UV-spectrophotometer (UV-Pharmaspec-1700, Shimadzu, Japan).

In vitro drug release rate studies[13]

Dissolution studies of the selected inclusion complexes were performed to evaluate the in-vitro drug release profile using USP dissolution apparatus type (USPXX III-Electro Lab, TDT 06P) with 500 ml phosphate buffer pH 7.4 as a dissolution medium at 37˚C± 0.5˚C and 50 rpm for 30 min. At different time

intervals, 5 ml aliquots were withdrawn, filtered, suitably diluted, and assayed for ND content by measuring the absorbance at 317 nm using a spectrophotometer (UV- Pharmaspec - 1700, Shimadzu, Japan). Equal volume of fresh medium at the same temperature was replaced into the dissolution medium after each sampling to maintain its constant volume throughout the study. Dissolution studies were performed in triplicate (n=3).The mean values were calculated for cumulative drug release and the same was used while plotting the release curves. The percent drug released at various time intervals was calculated and plotted against time.

Characterization of inclusion complexes

X-ray diffraction study (XRD)

X-ray diffraction study was done to analyze the powder characteristics of ND and its inclusion complexes. X-ray diffractograms were obtained by Philips diffractometer (PW 1140) by measuring 2θ in the range of 4 to 50 with reproducibility of + 0.0001.

Differential scanning calorimetry (DSC)

DSC scans of the selected powdered samples were recorded using DSC-Shimadzu 60 with TDA trend line software. The thermal traces were obtained by heating from 50°C to 250°C at the heating rate of 10°C/min under inert N_2 dynamic atmosphere (100 mL min^{-1}) in open crucibles.

Scanning electron microscopy study (SEM)

The morphology of powdered samples of the selected formulations was studied by SEM using Phillips XL 30 instrument (emission current: 0 to 200 □A, accelerating voltage: 0.2 to 30 kV, resolution with W filament 3.5 nm at 30 kv, specimen movement: X= 50 mm and Y = 50 mm, secondary and gaseous secondary electron detectors).

Statistical analysis

Similarity factor (f_2)

The U.S. FDA's guidance for industry on dissolution testing of immediate release solid oral dose forms (1997), as well as SUPAC-IR (1995), SUPACMR (1997) and bioavailability and bioequivalence study guidance for oral dosage forms, describes the model independent mathematical approach proposed by Moore and Flanner for calculating a dissimilarity factor f_1 and a similarity factor f_2 of dissolution across a suitable time interval. The f_2 is a measure of similarity in the percentage dissolution between two dissolution curves and is defined by following equation[14].

$$f_2 = 50\log\left\{\left[1+\frac{1}{n}\sum_{t=1}^{n} w_t\left(R_t - T_t\right)^2\right]^{-0.5} x100\right\}$$

where n is the number of withdrawal points, R_t is the percentage dissolved of reference at the time point t and T_t is the percentage dissolved of test at the time point t. A value of 100% for the similarity factor (f_2) suggests that the test and reference profiles are identical. Values between 50 and 100 indicate that the dissolution profiles are similar whilst smaller values imply an increase in dissimilarity between release profiles. Similarity factor (f_2) was calculated for all the formulations.

Mean dissolution time (MDT)

MDT reflects the time for the drug to dissolve and is the first statistical moment for the cumulative dissolution process that provides an accurate drug release rate[15]. A higher MDT value indicates greater drug retarding

ability[16]. MDT values for all the formulations were calculated using following equation;

$$MDT_{\text{in vitro}} = \frac{\sum_{i=1}^{n} t_{mid}\,\Delta M}{\sum_{i=1}^{n} \Delta M}$$

Here, i is dissolution sample number, n is number of dissolution times, t_{mid} is time at the midpoint between times t_i and t_{i-1}, and ΔM is the amount of ND dissolved (µg) between times t_i and t_{i-1}.

$DP_{30\ min}$

It shows percent of drug dissolved within 30 minutes. $DP_{30\ min}$ was calculated from dissolution curves of prepared inclusion complexes.

RESULTS AND DISCUSSION

The phase-solubility of drug with β-CD and HPβ-CD (Fig.1) indicated linear increase of solubility with an increased concentration of CDs. The phase solubility diagrams were of A_L type which indicated that the formation of 1:1M complex in solution. The apparent solubility constant (K_s) obtained from the slope of the linear phase solubility diagrams was found to be 293.87M^{-1} and 1035.74M^{-1} for ND:β-CD and ND:HPβ-CD complexes. This value of stability constant (Ks) indicated that, the complexes formed is quite stable. It is report by Higuchi and Connors that the ks valves in the range of 200 to 5000M^{-1} shows improved dissolution properties and hence better bioavailability.

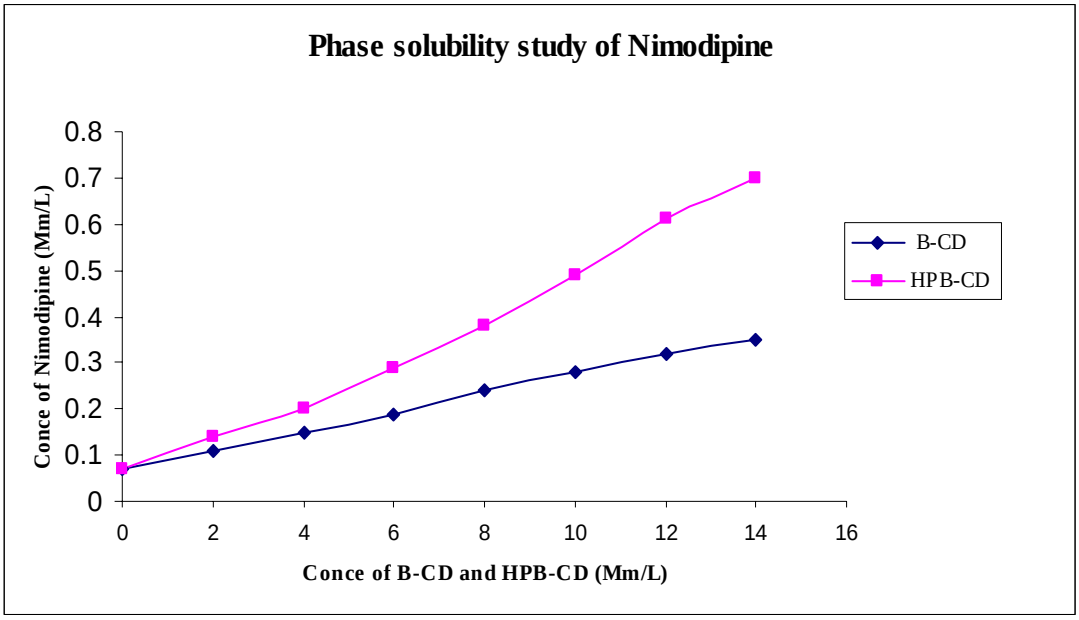


Fig 1. It shows phase solubility study of ND with β-CD and HPβ-CD

The aqueous solubility values of the pure drug and inclusion complexes prepared by said methods (table 2) were indicated that there is an enhancement of aqueous solubility which was high in case of spray dried complexes followed by kneading, physical mixture and pure drug. A 5 and 7 fold enhancement in solubility was observed with βCD and HPβCD respectively. The reasons for this enhancement may be due to uniform particle size obtained in the spray dried complex formulations.

Table 2: It shows aqueous solubility of ND, β-CD and HPβ-CD inclusion complexes

Product codes	ND	SPB	KNB	PMB	SPH	KNH	PMH
Aqoeous solubility (mcg/ml)	136	556	467	402	724	534	412

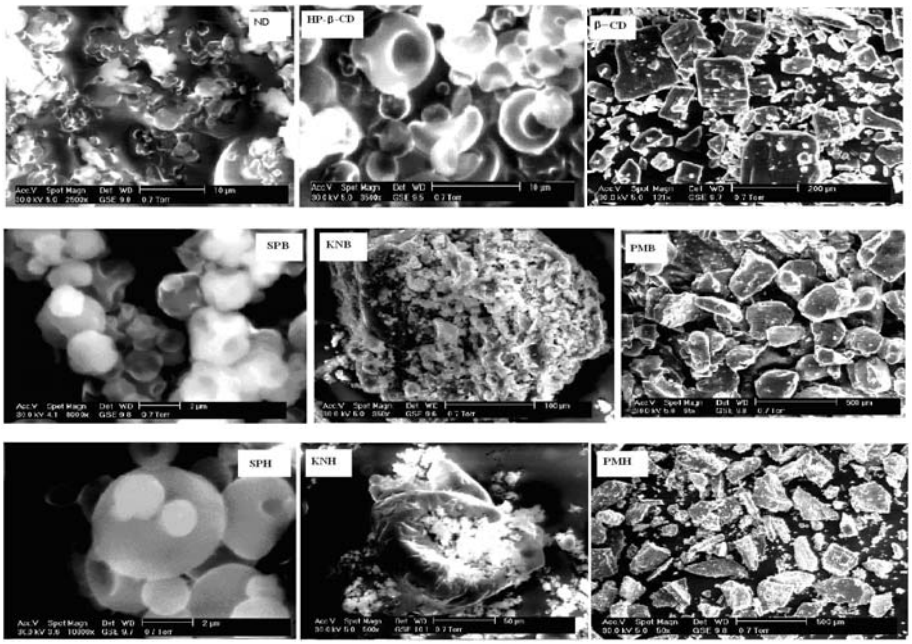
The Values of Carr's index (table 3) for inclusion complexes prepared by PM method for both CDs were the least values when compared to other formulation, which indicating good flow properties. The mean particle size for spray dried inclusion complexes for both CDs showed the least mean particle size in μm. So, solubility of these complexes was thought to be high compared to other formulations. The percentage yield for PM inclusion complexes were higher followed by kneading method and spray drying method. The percentage of Drug entrapment efficiency for all formulations prepared by spray drying is higher followed by kneading and physical mixture method.

Table 3: It shows comparison of some physical properties and % Drug entrapment efficiency

Types of CDs	Product codes	Carr's index (%)	Mean particle size (μ)	Powder yield (%)	Drug entrapment efficiency (%)
βCD	SPB	16.67	10.83	60.86	92.85
	KNB	16.13	19.42	86.75	84.56
	PMB	14.8	23.35	96.52	74.67
HPβCD	SPH	17.02	12.91	61.21	96.15
	KNH	16.56	22.46	87.40	88.74
	PMH	14.45	25.17	95.80	79.41

SEM photographs (Fig 2) clearly indicated that the crystellinity of the drug was reduced highly in spray dried and kneading complexes than the physical mixtures and particles obtained by spray dried method were spherical in shape.

Fig. 2: It shows SEM Photographs of ND, HPβ-CD, β-CD, SPB, KNB, PMB, SPH, KNH & PMH



The DSC graphs (Fig. 3 & 4) showed a melting point of the drug at 205.4°C and 90 °C to 120°C for both CDs due to loss of water from their molecules. The less intensity of peaks for spray dried formulations indicating that they had superior inclusion complexation property than all other formulations. This may be due to complete entrapping of drug in the CDs cavity. This confirms that the spray drying method was the best method for the preparation of inclusion complexes.

Fig. 3: It shows DSC spectra of ND, HP-β-CD, SPH, KNH, and PMH

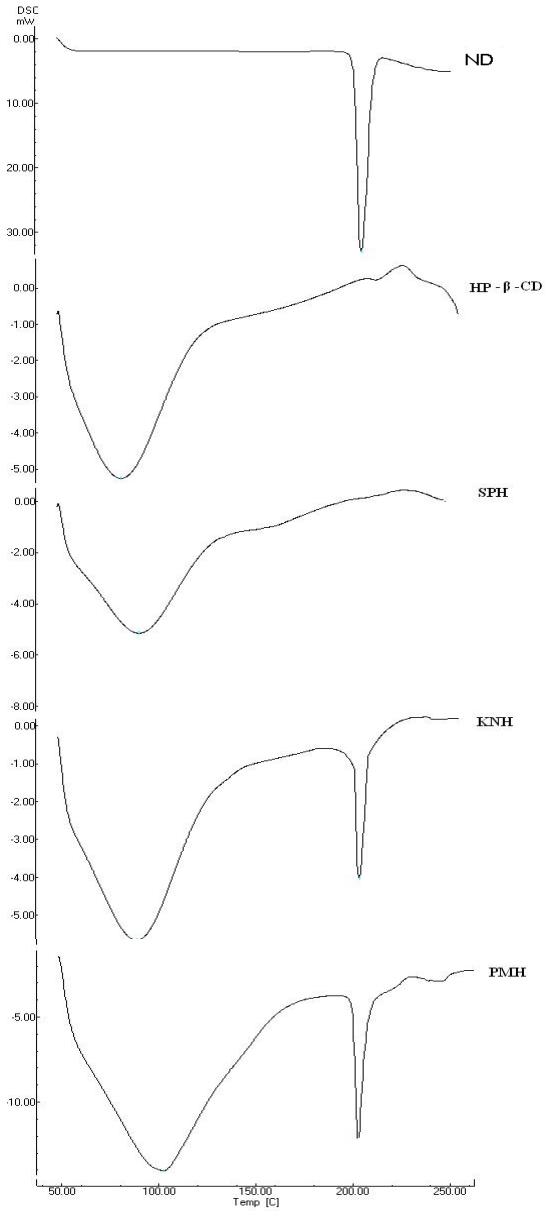
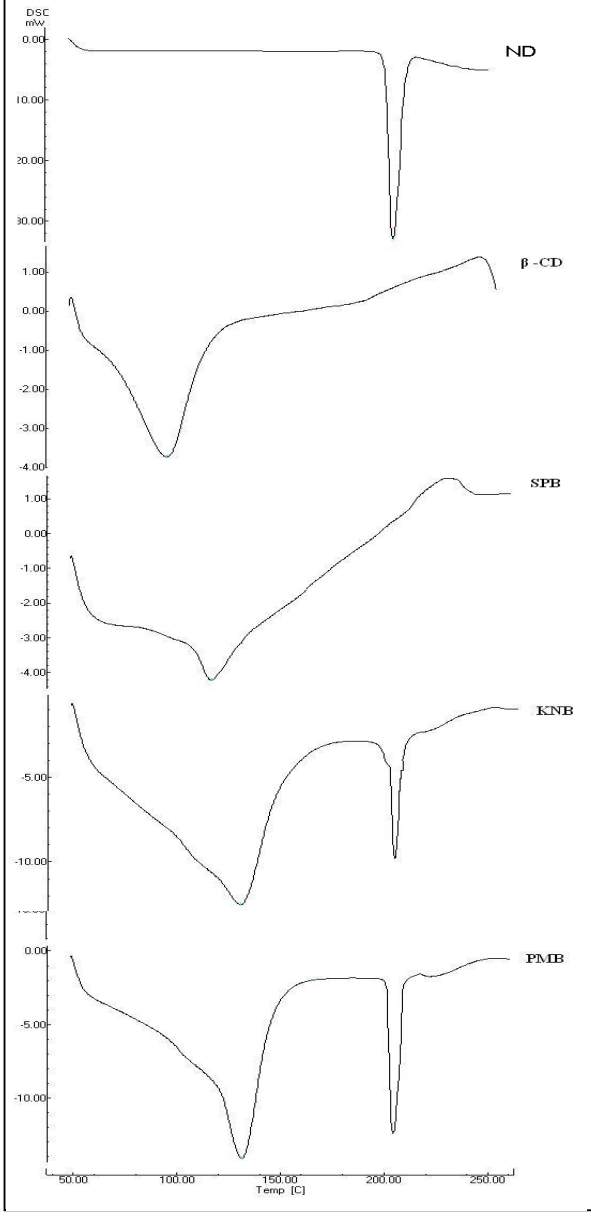
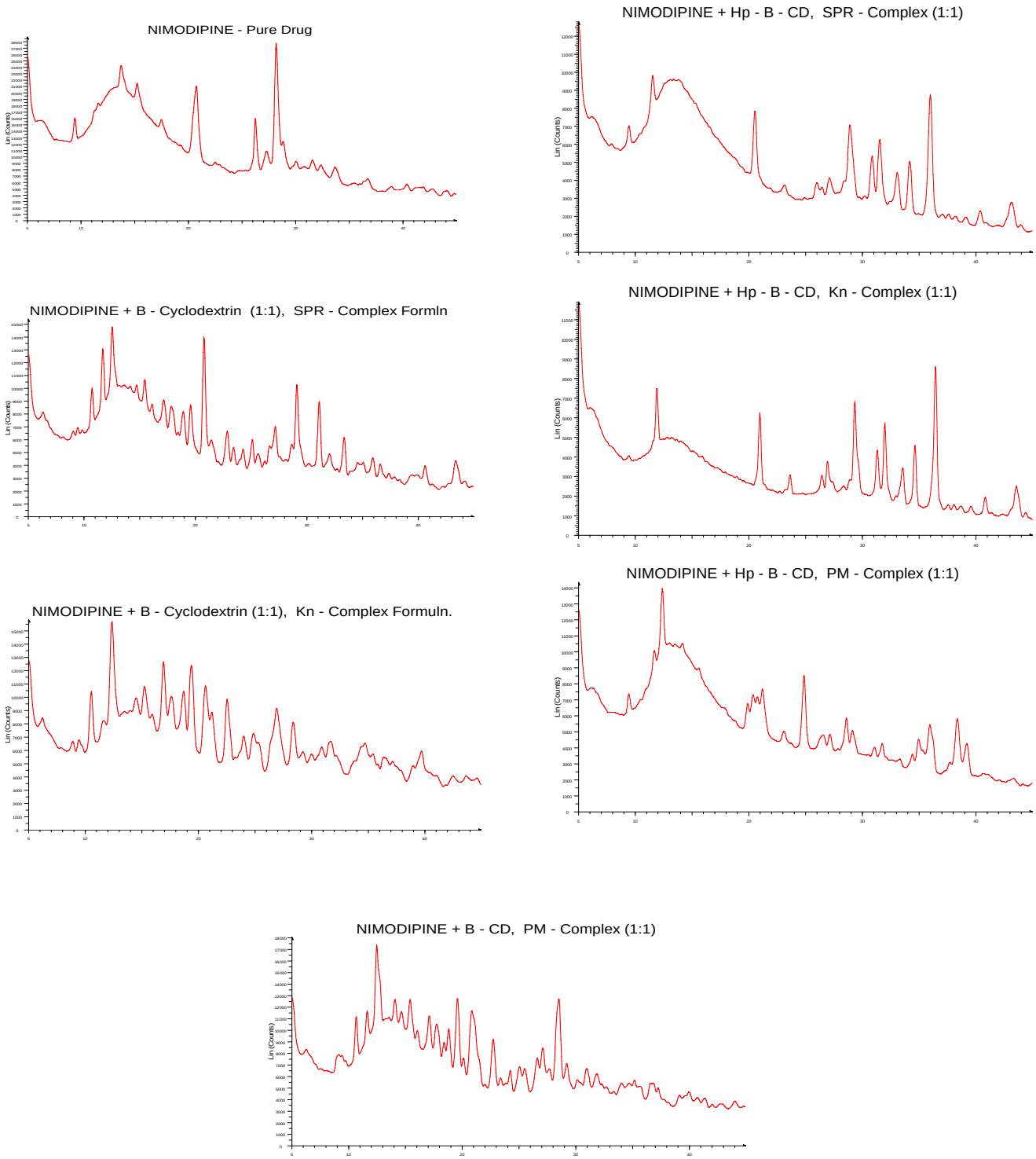


Fig. 4: It shows DSC spectra of ND, β-CD, SPB, KNB, and PMB



The XRD Spectra (Fig.5) of formulations prepared by spray drying and kneading method showed that the number of peaks and their intensity are less indicates amorphous form of the drug compared to other formulations.

Fig. 5: It shows XRD Spectra of ND, β-CD, SPB, KNB, PMB, SPH, KNH, and PMH

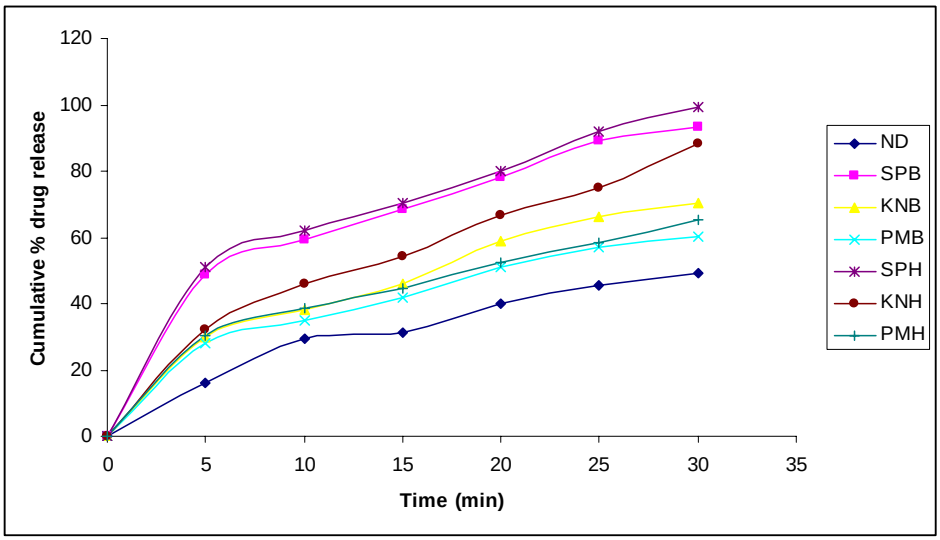


The in-vitro release profile (table 4) of pure ND in phosphate buffer solution pH 7.4 was found to be 49.21% after 30 min. which is very low, where as the spray dried formulations containing HPβ-CD and β-CD showed higher drug release profile of 99.26 and 93.16% respectively followed by kneading complexes and physical mixtures. The possible reasons for improved dissolution profiles include reduction of crystal size, a solublization effect of carrier and amorphous state of drug in inclusion complexes.

Table 4: It shows in- vitro drug release profile of ND and inclusion complexes in 7.4 pH buffer.

Time in min	Cumulative percent drug release in 30 min.						
	ND	SPB	KNB	PMB	SPH	KNH	PMH
5	16.01	48.57	30.00	28.12	50.90	32.40	30.45
10	29.50	59.12	38.25	35.15	62.22	46.05	38.69
15	31.31	68.36	46.12	42.03	70.45	54.33	44.42
20	39.80	78.13	58.90	51.18	80.12	66.56	52.36
25	45.31	89.30	66.14	57.10	92.16	75.15	58.52
30	49.21	93.16	70.21	60.15	99.26	88.12	65.26

Fig. 6: It shows in -vitro dissolution profile of ND and inclusion complexes in 7.4 pH Buffer



Statistical analysis

Calculated f_2 values for ND and all formulations are shown in table 6. All the formulations showed different drug release profile from pure drug. The f_2 values of spray dried formulations containing HPβ-CD and β-CD were 13.68 and 15.38 respectively. This indicates that the drug release profile of these formulations is highly different from pure drug. The MDT value of pure drug is 45.96 min and these values decreased to greater in the inclusion complexes (table 5). The MDT values of spray dried complexes containing HPβ-CD and β-CD showed least values of 19.57 and 20.35 min. respectively.

$DP_{30\ min}$, values for pure drug and all inclusion complexes are reported in the table 5. From this data, it is evident that onset of dissolution of pure drug is very low in dissolution medium i.e., 7.2 % within 30 min. where as for spray dried formulations showed highest drug release of 71.12 % and 60.2 % and least drug release of 34.67 % and 30.95 % for HPβ-CD and β-CD respectively.

Table 5: It shows percent of drug dissolved $DP_{30\ min}$ and MDT values from ND and Inclusion complexes

Product codes	$DP_{30\ min}$ (%)	MDT (min)
ND	7.2	45.96
SPB	60.2	20.35
KNB	45.5	27.62
PMB	30.95	26.85
SPH	71.12	19.57
KNH	50.55	24.97
PMH	34.67	26.50

Table 6: It shows data of similarity factor (f_2) for release profile of ND and inclusion complexes.

Sl. No	Product code	Similarity factor (f_2)
01	ND	66.76
02	SPB	15.38
03	KNB	21.81
04	PMB	32.17
05	SPH	13.68
06	KNH	19.78
07	PMH	29.23

CONCLUSION

Hence, it was concluded from the above study that the inclusion complexes of nimodipine- HPβ-CD and β-CD was successfully developed in order to improve the solubility and fast drug release rate by using spray drying technique as an efficient tool. The spray drying technique was concluded to have profound influence on the physico-chemical properties and in-vitro release profile of Nimodipine-cyclodextrin inclusion complexes.

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