DESIGN, DEVELOPMENT AND EVALUATION OF BILAYER TABLET USING NATEGLINIDE FOR THE MANAGEMENT OF DIABETES

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ABSTRACT

The aim of present study is to formulate Nateglinide sustained release (SR) and immediate release (IR) bilayer tablet by different concentration of Hydroxypropyl methylcellulose (HPMC) and HPMC K 100 M to control the release pattern. The sustained release layer of Nateglinide was prepared by using different grades of HPMC like, HPMC K-100, HPMC along with other excipients by direct compression technique. The immediate release layer of Nateglinide was prepared by Cross carmellose sodium and Sodium starch glycolate by direct compression technique. The powders were evaluated for their flow properties and the finished tablets were evaluated for their physical parameters. The both immediate release and sustained release layers of Nateglinide were characterized by FT-IR and in vitro dissolution studies. The drug release study of Nateglinide was evaluated using USP-II paddle type dissolution apparatus. The release rate of Nateglinide in immediate release layer was studied for 1h in 0.1 N HCL media and that of Nateglinide in sustained release layer was studied for 12 h in pH 6.8 phosphate buffer media. From the nine batches S5 batch showed good release behaviour 94.92% of drug is released over 12 hours. Nateglinide is a poorly water soluble (BCS class 2) ant diabetic drug. Due to the poor water solubility of this drug, its bioavailability is dissolution rate-limited. Total four trial batches of each drug have been manufactured to optimize and develop a robust and stable formulation, the stability studies of the products also comply with ICH guideline.

Keywords: Bi-layer tablet, Sustain Release, Immediate Release, Nateglinide.

1. INTRODUCTION

Oral route is the most commonly employed route of drug administration. Although different route of administration are used for the delivery of drugs, oral route remain the preferred mode. The popularity of the oral route is attributed patient acceptance, ease of administration, accurate dosing, cost effective manufacturing method and generally improved shelf-life of the product. Even for sustained release systems the oral route of administration has been investigated the most, because of flexibility in dosage forms design that the oral route offers. With many drugs, the basic goal of therapy is to achieve a steady-state blood level or tissue level that is therapeutically effective and non toxic for an extended period of time.

Bi-layer tablet concept has long been utilized to develop sustained released formulation. Such tablet has a fast releasing layer and may contain one (bi-layer), to sustain the drug release. The pharmacokinetic advantage relies on the criterion that, drug release from the fast releasing layer leads to a sudden rise in the blood concentration. However the blood level is maintained at steady state as the release from sustaining layer.

Nateglinide appears to lower blood glucose acutely by stimulating the release of insulin from the pancreas, an effect dependent upon functioning beta cells in the pancreatic islets. Extra pancreatic effects also may play a part in the mechanism of action of oral sulfonylurea hypoglycaemic drugs. Nateglinide is rapidly and completely absorbed following oral administration in an immediate release dosage form. The absolute bioavailability of Nateglinide was 73% after single oral doses in patients with type 2 diabetes. Nateglinide is eliminated primarily by hepatic biotransformation: less than 10% of a dose is excreted as unchanged drug in urine and feces; approximately 90% of a dose is excreted as biotransformation products in urine (80%) and feces (10%). The mean terminal elimination half-life of glipizide ranged from 1.5 hours after single or multiple doses in patients with type 2 diabetes.

2. EXPERIMENTAL

2.1. Materials and methods

Nateglinide obtained as gift sample from Balaji chemicals, Gujarat and HPMC were obtained as gift sample from Stadmed Pvt.Ltd, Kolkata. Sodium starch glycolate, microcrystalline cellulose, Magnesium stearate and HPMCK100 were obtained as gift sample from Merck specialties Private Limited. PVP K 30 was obtained as gift sample from Lobachem private limited, Mumbai. All other chemicals/reagents used were of analytical grade.

2.1.1. FTIR SPECTROSCOPY:

The drug and optimised formulation were characterized by IR spectroscopy using a FTIR 8400S (Shimadzu, Japan). The spectra were taken by KBr discs method in the range of 4000–500 cm–1.

2.1.2. DIFFERENTIAL SCANNING CALORIMETRY (DSC) STUDY

Differential scanning calorimetry (DSC) study of Bilayer tablets was performed using a

Toledo DSC (Mettler Star SW 9.20) to determine the drug excipients compatibility study.

The analysis was performed at a rate 5 °C min-1 from 50 to 300°C temperature range under nitrogen flow of 25 mL min-1.

2.1.3. FORMULATION AND CHARACTERIZATION OF BILAYER TABLETS:

The bilayer tablets of Nateglinide were prepared by the direct compression method. The drug and polymers for both IR and SR layer were passed through a # 60 sieve before their use in the formulation.

2.1.3.1. PREPARATION OF SOLID DISPERSIONS OF NATEGLINIDE IR LAYER

Various carriers are used to make solid dispersions. In the present study PEG 4000 was used as a hydrophilic carrier in the preparation of solid dispersion. These solid dispersions were prepared by using Solvent Evaporation Method. These were used in different ratios with respect to plain drug. Different drug: polymers ratios were employed as 1:1, 1:2, 1:3,1:4, 1:5 & solid dispersions were prepared by solvent evaporation method Polymers employed was PEG 4000

2.1.3.1.1 Solvent evaporation method

Solid dispersions Nateglinide was prepared using PEG 4000 by solvent evaporation method in various weight ratios.

Steps in the preparation of solid dispersion by solvent evaporation:

1. Drug & polymer (PEG 4000) mixtures were dissolved in methanol in the ratio 1:1, 1:2, 1:3,1:4, 1:5.
2. The solutions were made homogeneous by continuous stirring and solvent was evaporated by subjecting the solution with constant stirring at 70 to 80^0c on water batch till complete evaporation of solvent.
3. The obtained solid dispersions were air dried & subsequently pulverized by triturating in pestle-mortar & screened through 60 mesh sieve.

Table1. Composition of various solid dispersions by solvent evaporation method

Sr. No.	Drug	Polymer	Ratio	Method of preparation
1.	Nateglinide	PEG 4000	1:1	SE
2.	Nateglinide	PEG 4000	1:2	SE
3.	Nateglinide	PEG 4000	1:3	SE
4.	Nateglinide	PEG 4000	1:4	SE
5.	Nateglinide	PEG 4000	1:5	SE

2.1.3.1.2 Solubility Study

The solid dispersions were subjected for solubility studies to evaluate the effect of different carriers and carrier ratios on the aqueous solubility of Nateglinide. Nateglinide can be practically insoluble in water. An excess amount of the sample solid dispersion was placed in contact with distilled water. The samples were shaken for 48 hours at 37 °C in an orbital shaker. The supernatant was filtered through a whatmann filter paper. The filtrate was suitably diluted to 10 ppm and analyzed spectrophotometrically at 214 nm. All experiments were conducted in triplicate.

2.1.3.1.5 Powder X-Ray Diffraction

PXRD analysis was done by irradiating the samples with monochromatized Cu Kα radiation at a voltage of 40 kV and a current of 50 mA. The samples were scanned in increments of 0.02° from 5° to 60° (diffraction angle 2θ) at a rate of 1s per step using a zero background sample holder, employing a Bruker AXS D8 Advance Diffractometer with Lynx Eye Detector. The diffractogram was produced by using Diffrac plus Software.

2.1.3.1.6 Differential Scanning Calorimetry:

The powdered sample (3 mg) was hermetically sealed in aluminum pans and heated at a constant rate of 10°C/min, over a temperature range of 30–300°C with nitrogen flow rate of 30 ml/min. Thermograms of the samples were obtained using differential scanning Calorimetry (Lab Mettle Star SW 10.00). Thermal analysis data were recorded with Lab Mettler software programs. Indium standard was used to calibrate the DSC temperature and enthalpy scale.

2.1.3.1.7 Selection of most satisfactory formulation

From all the above formulations, Solid dispersion of Nateglinide- PEG 4000 (1:4) showing

Maximum *in-vitro* drug release. Hence this inclusion complex showing maximum dissolution rate was converted to cost effective tablet formulations.

2.1.3.2 Formulation and Preparation of the IR Layer

The IR ingredients (Table 2) were accurately weighed and added into the blender in ascending order. The powder mix was blended for 20 min. to obtain uniform distribution of the drug in formulation and subjected for preformulation studies. . All the formulation components were passed through sieve #60, weighed, mixed, and compressed into tablet using 8mm punch on Rotary tablet minipress-I (Rimek, Karnavati Engineering Ltd., Mehsana, Gujarat).

Table2. Composition of various tablets prepared Nateglinide IR layer

Ingredients	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**
SD (≈60 mg Nateglinide)	148	148	148	148	148	148	148	148	148
SSG	04	04	04	08	08	08	12	12	12
CCS	02	04	06	02	04	06	02	04	06
DCP	41	39	37	37	35	33	33	31	29
Magnesium stearate	02	02	02	02	02	02	02	02	02
Talc	01	01	01	01	01	01	01	01	01
Aerosil	01	01	01	01	01	01	01	01	01
Colour	01	01	01	01	01	01	01	01	01
Total	200	200	200	200	200	200	200	200	200

(All quantities are in mg)

2.1.3.3 Formulation and Preparation of the SR Layer

The SR ingredients were accurately weighed and added into the blender in ascending order. The powder mix was blended for 20 min. to obtain uniform distribution of the drug in formulation and subjected for preformulation studies. Tablets were prepared by direct compression method with 12 mm stainless steel punch using rotary press (Karnavati Minitab, India). Compression force for all the tablets was adjusted to get tablets of hardness 4-6 kg/cm^2. Hardness was measured by Monsanto type hardness tester (Coslab). Weight of were adjusted to 450 mg of all compress tablets.

Table3. Composition of various tablets prepared Nateglinide SR layer

Ingredients (mg)	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**
Nateglinide	333	333	333	333	333	333	333	333	333
HPMC	22.5	45	67	22.5	45	67	22.5	45	67
HPMC K 100 M	10	10	10	20	20	20	30	30	30
PVP K-30	13.5	13.5	13.5	13.5	13.5	13.5	13.5	13.5	13.5
Talc	2	2	2	2	2	2	2	2	2
Magnesium stearate	4	4	4	4	4	4	4	4	4
Microcrystalline Cellulose	65	42.5	20.5	55	32.5	10.5	45	22.5	0.5
Total	**450**	**450**	**450**	**450**	**450**	**450**	**450**	**450**	**450**

(All quantities are in mg)

2.1.3.4 Formulation of Bilayer Tablet

In the present study bilayer tablet was prepared manually using single station punching machine. Accurately weighed amount of SR powder mix was fed manually into die cavity. SR layer was compressed at mild compression force. After that accurately weighed IR powder mix was manually fed into the die on SR layer and compressed using 12mm circular shape concave punch on Rotary tablet minipress-I (Rimek, Karnavati Engineering Ltd., Mehsana, Gujarat). I8 batch from Nateglinide IR layer and S5 batch from Nateglinide SR layer were selected to form Optimized bilayer tablet (I8S5) by direct compression method. Composition of bilayer tablet was shown in table no.4

Table4. Formulation of optimize of bi-layer tablet of Nateglinide IR Layer (I8) and Nateglinide SR Layer (S5)

Sr. No.	**Ingredients**	**Formulation (I8)**	**Ingredients**	**Formulation (S5)**
	Formulation of Nateglinide IR Layer (I8)		**Formulation Of Nateglinide SR Layer (S5)**	
1	SD (≈60 mg Nateglinide)	148	Drug (Nateglinide)	333
2	Sodium Starch Glycolate	12	HPMC	45
3	Cross Carmellose Sodium	04	HPMC K 100	20
4	Di Calcium Phosphate	31	PVP K 30	13.5
5	Magnesium stearate	02	Magnesium stearate	04
6	Talc	01	Talc	02
7	Aerosil	01	Micro Crystalline Cellulose	32.5
8	colour	01	**Total**	**450**
9	**Total**	**200**		

(All quantities are in mg)

2.1.3.5 Powder characterization

2.1.3.5.1 Angle of repose

Angle of repose was determined by using funnel method. The granules were poured from funnel that can be raised vertically until a maximum cone height ‘h’ was obtained. Then the diameter of the powder cone was measured and the angle of repose was calculated using the following equation. $\theta = \tan^{-1}(h/r)$.

2.1.3.5.2 Bulk density

Bulk Density Apparent bulk density was determined by placing pre-sieved granules into a graduated cylinder and measuring the volume and weight as it is. The bulk density is calculated by using following formula. Bulk density = Weight of powder / volume of packing.

2.1.3.5.3 Tapped density

A quantity of 2 gm of powder from each formula was introduced into a 10 ml measuring cylinder. After a initial volume was observed, the cylinder was allow to fall under its own weight on the hard surface from the height of 2.5 cm at two second intervals. The tapping was continued until no further change in the volume was noted. The tapped density was calculated by using following formula. Tapped density = weight of powder / tapped volume of packing.

2.1.3.5.4 Compressibility index

Compressibility index Compressibility index of granules was determined by Carr's compressibility index. Carr's index: [(TBD – LBD) x 100]/ TBD.

2.1.3.5.5 Hausner ratio of powder blends for Glipizide tablet

Hausner ratio was determined by using the ρ_B is loose bulk density and pt is tapped bulk density. Hausner ratio is greater than 1.25 is considered to be an indication of poor flow ability. Hausner ratio = ρ_T / ρ_B

2.1.3.6 Evaluation of tablets

2.1.3.6.1 Hardness test

Hardness indicates the ability of a tablet to withstand mechanical shocks while handling. The hardness of the tablets was determined using Monsanto hardness tester. It is expressed in kg/cm2. Three tablets were randomly picked and hardness of the tablets was determined values are reported .

2.1.3.6.2 Weight variation

Twenty tablets were randomly selected from each batch and average weight was calculated.

Then individual tablet were weighted and individual weight was compared with an average weight.

2.1.3.6.3 Thickness

Twenty tablets were randomly selected from each batch and their thickness was measured by using vernier calliper. Thickness of three tablets from each batch was measured and mean was calculated.

2.1.3.6.4 Friability

Roche friabilator was used for the purpose. Twenty tablets were weighed and placed in the Roche friabilator, which was then operated for 25 rpm for 4 min. After revolution tablets were deducted and reweighed. Compressed tablets should not lose more than 1% of their weight. Values are reported. The percentage friability was measured using the formula, % F = {1-(Wo/W)} ×100

2.1.3.6.5 Drug Content

20 tablets of each formulation were weighed and powdered. The quantity of powder equivalent to 15mg of Nateglinide was taken and dissolved in 30 ml of methanol with gentle heating on a water bath, cool and add sufficient amount of methanol is added to produce 50 ml. filter and dilute to 5 ml of the filtrate to 50ml with methanol. The absorbance was measured spectrophotometrically at 209 nm.

2.1.3.6.6 In vitro drug release study

In vitro drug release study was performed using dissolution apparatus USP type II paddle method with a stirring speed 50 rpm at 370 C ± 0.5 in 900 ml of 0.1 N HCL for immediate release upto 1 hr and 6.8 pH phosphate buffer up to 12 hr for sustain release. The samples were collected at per selected time intervals with replacement of equal volume of dissolution media. The absorbance of collected samples was measured spectrophotometrically at 209 nm. **2.1.3.7Dissolution profile**

The similarity factor (*f*2) given by SUPAC guidelines for modified release dosage forms was used as a basis for comparing dissolution profiles. Dissolution profiles are considered to be similar when *f*2 is 50 to 100. This similarity factor was calculated by the following formula:

$$f_2 = 50 \times \log\left\{\left[1 + \left(\frac{1}{n}\right)\sum (R_t - T_t)^2\right]^{-0.5} \times 100\right\}$$

Where *Rt and Tt* are the mean percentage of dissolved drug from the reference and test formulations at time t respectively.

2.1.3.8Stability Studies (ICH Geneva 2003): The optimized formulation was subjected for two month stability study according to ICH guidelines. The selected formulations were packed in aluminium foils, which were in

wide mouth bottles closed tightly. They were then stored at room temperature 40ºC / 75% RH for 2 months and evaluated for their permeation study.

2.2 RESULTS AND DISCUSSION:

Bilayer tablet is one of the approaches for biphasic release system. Attempts have been made for preparation of biphasic release with variable concentration of superdisintegrant in IR layer and rate retarding polymer in SR layer for adjusting release pattern according to marketed formulation and USP guidelines of Nateglinide Sustained release tablet. In the bilayer tablet one of the layers was formulated with superdisintegrant CCS and SSG for immediate drug release while another layer was formulated with the hydrophilic polymer HPMC and HPMC K100M for extended drug release.

2.2.1FTIR SPECTROSCOPY:

Nateglinide and Optimized formulation same characteristic peaks were observed for

the drug-excipients mixture, indicating that no chemical reaction or interaction between the drug and excipients took place. The FTIR spectra of pure Nateglinide showed characteristics peaks at 1637.26 cm-1 which indicates **C=O** stretching vibration. 2937.20 cm-1 indicates that **C–H** group is present, 3314.59 cm-1 indicates **N-H** bond is present, 1439.12 cm-1 indicates **--CH3** group is present.

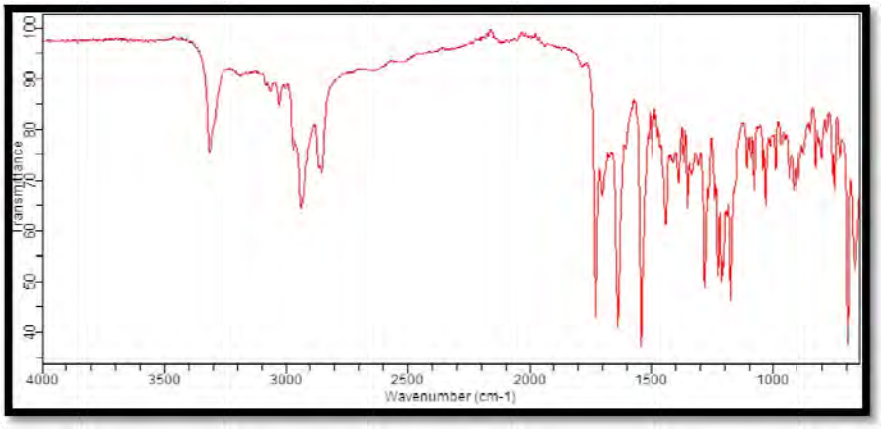

Figure 1: FTIR spectra Nateglinide.

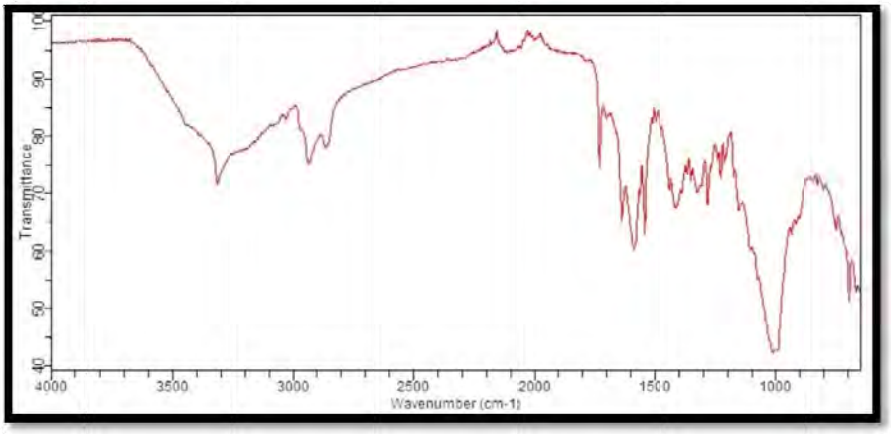

Figure2: FTIR spectra Nateglinide-PEG 4000 solid dispersion (1:4) prepared by SE method

2.2.2 Solubility Study.

The maximum solubility was observed at ratio 1:4.The results are shown in table no: 05.

Table5. Solubility study of various Solid Dispersions

Sr. No.	Drug	Polymer	Ratio	Solubility (mg/ml) ± S.D.
1.	Nate	-----	-----	0.01588±0.012
2.	Nate	PEG 4000	1:1	0.0234±0.001
3.	Nate	PEG 4000	1:2	0.05324±0.008
4.	Nate	PEG 4000	1:3	0.0822±0.004
5.	Nate	PEG 4000	1:4	0.1332±0.01
6.	Nate	PEG 4000	1:5	0.0912±0.008

2.2.4 Powder X-Ray Diffraction:

Optimized solid dispersion was studied for prediction of crystallinity. The PXRD Pattern of Nateglinide is shown in figure 4. Based on the diffractogram it can be suggested that Nateglinide is present in its crystalline form since it exhibits well defined sharp peaks at a diffractogram angle of 2θ. The strong peak of 2θ at 12.64 showing the highly intense peak with 100% intensity indicating presence of crystalline Nateglinide and other peaks were shown at 7.92, 11.67, 20.31, 25.053, 25.721 and 26.036 of 2θ scale.

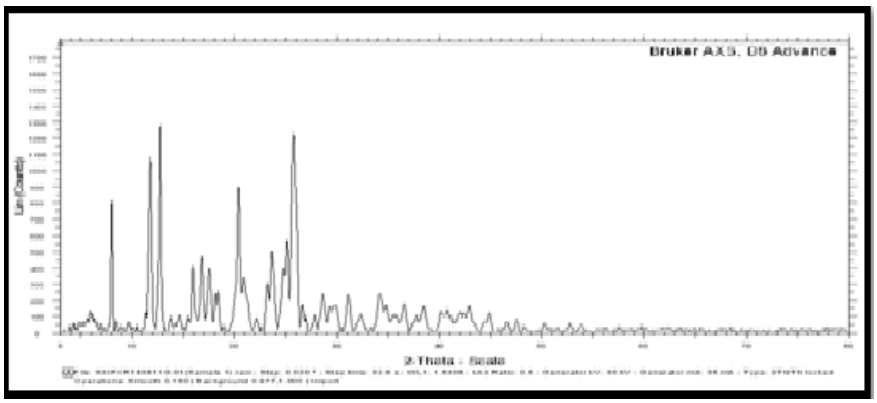

Figure 4: X-ray diffractogram of Nateglinide**.**

PXRD spectra of Nateglinide Solid dispersion (figure 5) the entire characteristic peaks which were shown by the drug were absent in the solid dispersion with PEG 4000. The intense peak at 12.64° indicating crystalline nature of drug. X-ray diffraction pattern SD showed absence of these distinct peaks; it was indicating that crystalline nature of drug was reduced.

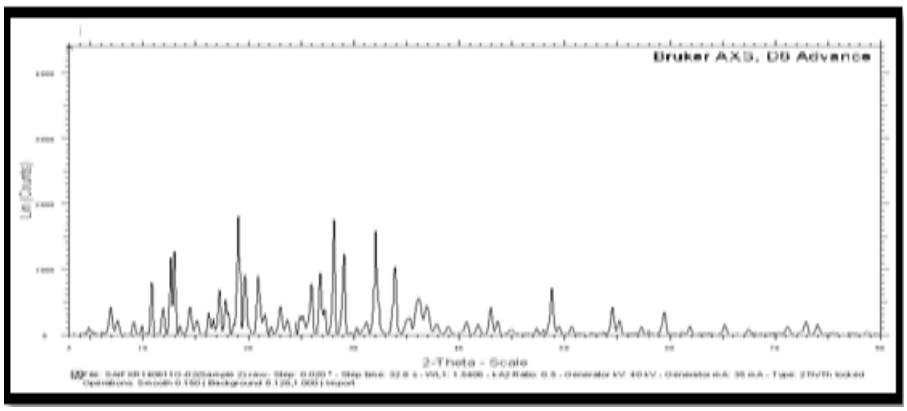

Figure 5. X-ray diffractogram of physical mixture (Nateglinide-PEG 4000).

2.2.5 Differential Scanning Calorimetry:-

The DSC thermogram of pure drug indicated a sharp endothermic peak at139.93°C corresponding to melting of pure Nateglinide. It can be concluded that the excipients and drug do not interact with each other. Also the drug did not form a complex with the excipients as the endothermic peaks remained unchanged in position. An endothermic peak corresponding to the melting point of pure drug was prominent in the physical mixture of optimized formulation with respect to PEG 4000 peaks, which clearly suggests that the drug was present in an unchanged form.

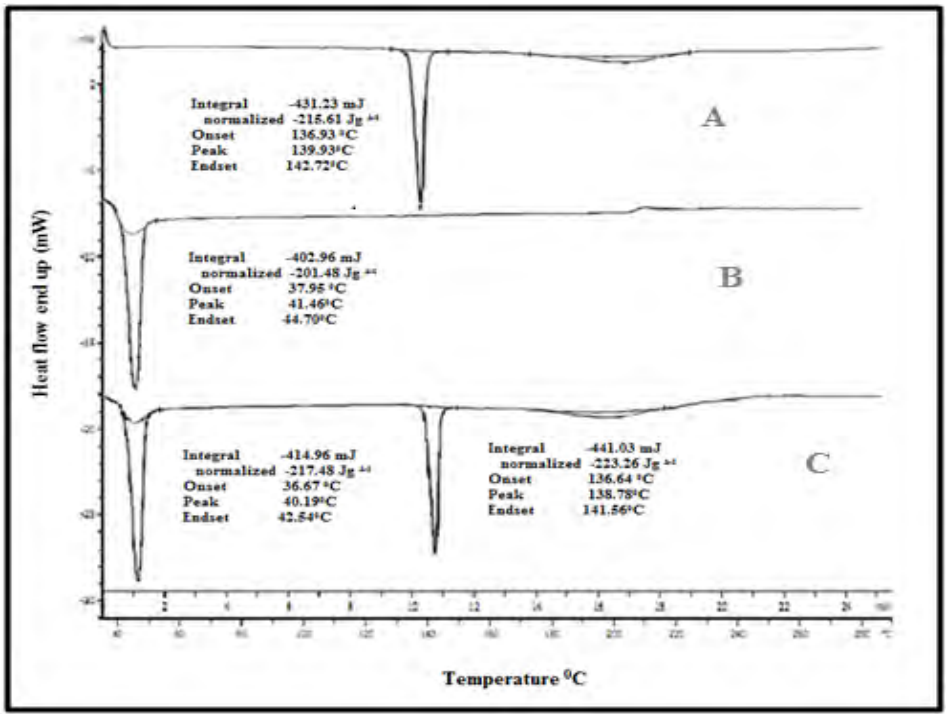

Figure 6. DSC thermograms of A-Nateglinide, B- PEG 4000 and physical mixture

2.2.6 Evaluation parameters of powder blend for IR & SR layers:

The powder blends of both IR and CR layers of different formulations of bilayer tablets were

Evaluated for various physical properties (Table 7&8). The bulk densities for the powder blend of IR and SR layer of various formulations values indicated satisfactory flow behaviour.

Table7. Results of precompression evaluation of Nateglinide IR layer

Batch No.	Bulk density (gm/cm^3) ±SD	Tapped density (gm/cm^3) ±SD	Compressibility index (%)±SD	Hausner's ratio± SD	Angle of repose(ɵ) ±SD
I1	0.5283±0.007	0.6053±0.0127	12.681±1.126	1.1456±0.015	22±0.040
I2	0.5559±0.007	0.6710±0.022	17.059±2.063	1.2071±0.029	21.02±0.523
I3	0.5475±0.004	0.6148±0.005	10.933±1.199	1.1231±0.001	26.05±0.888
I4	0.5683±0.007	0.6413±0.009	11.367±0.149	1.1283±0.001	24.00±0.714
I5	0.5464±0.006	0.6204±0.013	12.259±2.79	1.1419±0.035	23.08±1.069
I6	0.5570±0.071	0.6522±0.0056	14.787±1.305	1.1740±0.018	22.05±0.373
I7	0.5475±0.004	0.6638±0.0005	17.521±0.128	1.2124±0.001	24.08±0.690
I8	0.5361±0.010	0.6099±0.0085	12.094±1.745	1.1384±0.022	24.06±1.390
I9	0.5860±0.004	0.6638±0.0059	11.701±1.279	1.1330±0.016	22.01±1.172

Table8. Results of post compression evaluation of Nateglinide IR layer

Batch No.	Thickness (mm) ±SD	Hardness (kg/cm^2) ±SD	Friability (%)	Weight variation (%)±SD	Disintegration Time (Min)	Drug content (%)
I1	4.09±0.024	3.89±0.067	0.9908	0.22±0.008	6.98	90.56
I2	4.06±0.019	3.96±0.063	0.8323	0.25±0.006	6.04	90.00
I3	4.06±0.018	3.93±0.061	0.7927	0.24±0.004	7.23	101.45
I4	4.09±0.026	4.01±0.067	0.9116	0.21±0.002	5.87	91.83
I5	4.01±0.027	3.98±0.069	0.8719	0.25±0.002	7.17	93.93
I6	4.05±0.078	3.95±0.065	0.7530	0.25±0.001	7.27	95.93
I7	4.02±0.079	3.92±0.053	1.0305	0.20±0.001	6.59	97.80
I8	4.04±0.080	4.06±0.033	0.9105	0.25±0.004	6.55	94.10
I9	4.03±0.104	3.99±0.045	0.7892	0.25±0.006	5.44	90.73

Table9. In-Vitro drug release of Nateglinide IR tablet

Time (min)	Formulation Batches (% CDR ±SD)								
	I1	**I2**	**I3**	**I4**	**I5**	***I6***	**I7**	**I8**	**I9**
5	16.24±0.001	26.45±0.0001	28.19±0.001	31.46±0.002	30.65±0.015	35.88±0.001	36.51±0.001	37.56±0.001	33.65±0.001
10	21.86±0.002	33.20±0.002	35.50±0.01	39.11±0.001	36.85±0.016	43.12±0.013	44.78±0.01	46.05±0.001	44.23±0.001
20	30.17±0.016	40.53±0.014	41.77±0.012	45.54±0.016	43.47±0.019	50.68±0.0001	52.62±0.026	58.08±0.002	59.94±0.026
30	39.51±0.001	48.29±0.014	50.63±0.001	53.84±0.021	51.14±0.016	59.22±0.001	61.27±0.016	67.96±0.014	68.82±0.001
40	47.83±0.024	54.88±0.001	59.59±0.001	61.78±0.0001	59.44±0.029	67.15±0.034	73.61±0.001	75.13±0.001	77.13±0.017
50	53.67±0.001	61.30±0.0002	64.22±0.002	69.80±0.002	66.18±0.015	73.58±0.031	81.61±0.001	88.09±0.001	80.19±0.001
60	60.78±0.002	69.56±0.001	70.45±0.016	77.42±0.015	73.57±0.024	81.42±0.039	90.43±0.001	94.68±0.0001	86.46±0.002

Table10. Results of precompression evaluation of Nateglinide SR layer

Batch No.	Bulk density (gm/cm^3) ±SD	Tapped density (gm/cm^3) ±SD	Compressibility index (%)±SD	Hausner's ratio± SD	Angle of repose(ɵ) ±SD
S1	0.4283±0.007	0.7053±0.0127	12.681±1.126	1.1456±0.015	21±0.040
S2	0.3559±0.007	0.6710±0.022	17.059±2.063	1.2071±0.029	22.02±0.523
S3	0.4475±0.004	0.7148±0.005	11.933±1.199	1.1231±0.001	24.05±0.888
S4	0.4683±0.007	0.7413±0.009	11.367±0.149	1.1283±0.001	24.00±0.714
S5	0.5464±0.006	0.5204±0.013	12.259±2.79	1.1419±0.035	26.08±1.069
S6	0.3570±0.071	0.6522±0.0056	14.787±1.305	1.1740±0.018	22.05±0.373
S7	0.4475±0.004	0.6638±0.0005	15.521±0.128	1.2124±0.001	22.08±0.690
S8	0.4361±0.010	0.7099±0.0085	12.094±1.745	1.1384±0.022	24.06±1.390
S9	0.4860±0.004	0.7638±0.0059	11.701±1.279	1.1330±0.016	22.01±1.172

Table11. Results of Post compression evaluation of Nateglinide SR layer

Batch No.	Thickness (mm) ±SD	Hardness (kg/cm^2) ±SD	Friability (%)	Weight variation (%)±SD	Drug content (%)
S1	5.059±0.024	4.89±0.067	0.9908	0.252±0.008	90.56±0.001
S2	5.076±0.019	4.96±0.063	0.8323	0.250±0.006	90.00±0.002
S3	5.068±0.018	4.93±0.061	0.7927	0.249±0.004	101.45±0.0001
S4	5.039±0.026	5.01±0.067	0.9116	0.251±0.002	91.83±0.0001
S5	5.031±0.027	4.98±0.069	0.8719	0.254±0.002	93.93±0.001
S6	5.052±0.078	4.95±0.065	0.7530	0.253±0.001	95.93±0.0002
S7	5.028±0.079	5.22±0.053	1.0305	0.250±0.001	97.80±0.002
S8	4.045±0.080	4.06±0.033	0.9105	0.252±0.004	94.10±0.001
S9	4.033±0.104	3.99±0.045	0.7892	0.253±0.006	90.73±0.0002

Table11. Results of Post compression evaluation Nateglinide SR layer

Time (min)	Formulation Batches (% CDR ±SD)								
	S1	S2	S3	S4	S5	*S6*	S7	S8	S9
1	6.43±0.0001	9.04±0.003	6.92±0.0002	9.75±0.0001	9.75±0.0031	9.63±0.0002	9.08±0.0016	9.66±0.0021	9.43±0.001
2	11.77±0.002	12.51±0.001	10.58±0.003	13.89±0.002	15.38±0.0001	15.86±0.003	15.70±0.002	15.54±0.0016	15.37±0.0025
3	20.67±0.001	19.33±0.003	16.27±0.0002	20.68±0.0016	21.35±0.002	22.06±0.0001	21.07±0.003	21.35±0.0024	23.13±0.0002
4	26.92±0.002	24.78±0.001	20.72±0.001	26.93±0.0002	27.06±0.0061	28.01±0.002	29.01±0.0001	28.00±0.0001	29.76±0.005
5	32.17±0.0001	30.56±0.015	26.07±0.004	32.59±0.002	35.41±0.0002	35.63±0.002	36.28±0.002	34.68±0.0026	36.78±0.001
6	38.25±0.001	38.23±0.001	30.17±0.001	41.00±0.0012	42.71±0.0002	42.11±0.001	43.83±0.001	41.46±0.0023	40.82±0.002
7	42.73±0.002	45.96±0.003	35.93±0.015	48.32±0.0026	51.28±0.001	50.13±0.0021	51.85±0.002	49.80±0.0019	47.82±0.002
8	47.69±0.0021	52.64±0.0001	40.42±0.024	54.50±0.003	60.70±0.0001	58.87±0.0003	59.47±0.001	56.21±0.0031	54.59±0.001
9	52.94±0.001	60.01±0.003	45.72±0.0012	61.57±0.0012	69.51±0.0002	64.01±0.002	65.65±0.0012	63.29±0.001	59.23±0.003
10	57.84±0.001	65.90±0.001	53.17±0.0013	69.25±0.004	76.97±0.003	69.28±0.001	71.94±0.0021	71.60±0.0031	65.77±0.004
11	65.83±0.0002	70.99±0.013	59.75±0.002	76.33±0.001	85.48±0.0001	74.11±0.002	80.04±0.0020	79.95±0.0001	72.10±0.002
12	72.97±0.002	76.44±0.002	67.54±0.001	84.46±0.001	93.29±0.002	80.54±0.001	89.55±0.001	87.22±0.002	85.80±0.0002

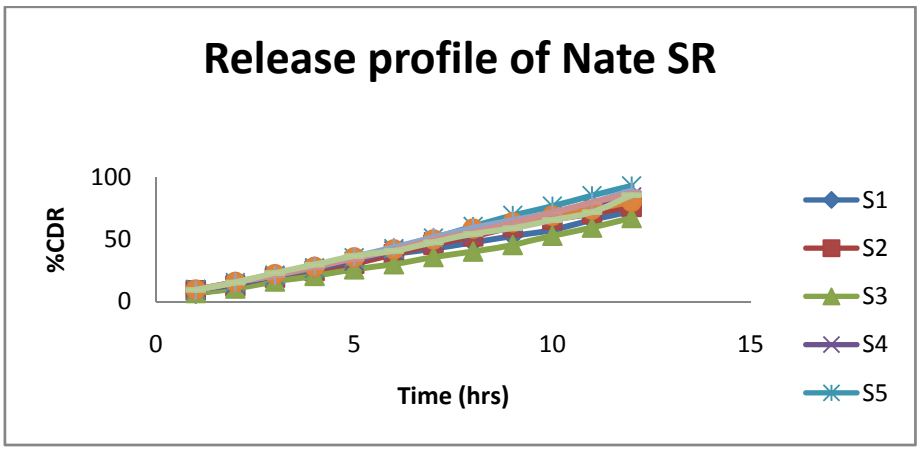

Figure8. Dissolution profiles of Nateglinide SR layer

2.2.7 Evaluation of optimized bilayer tablet of Nateglinide IR layer and Nateglinide SR Layer:
Optimized bilayer tablet was prepared from optimized Formulation of Nate IR Layer (I8) and Nate SR Layer (S5). This tablet was subjected only to in vitro drug release study to check the drug release was as per specifications given in official compendia or not.

2.2.7.1 Evaluation of precompression and post compression parameters of Bilayer Tablet

All the Prepared tablet formulations were subjected for precompression and post compression evaluation such as bulk density, tapped density, Hausner's ratio and Carr's index. Results of precompression evaluations of formulation mixtures are shown in table no.13.From the results of Compressibility (Carr's) index and Hausner's ratio it can be clearly concluded that the Nateglinide tablet blend were having excellent flow properties, fair to good compressibility All the prepared bilayer tablets were subjected to compendial test for post compression evaluation such as friability, hardness, thickness, uniformity of weight, disintegration time & content uniformity results. Evaluation optimized batch is given in table no.13.

Table13. Precompression & Postcompression parameter of bilayer tablet

Sr. No.	Precompression parameters	Observation	Precompression parameters	Observation
1	Bulk density (gm/ml)	0.669	% Weight variation (mg)	Complies
2	Tapped density (gm/ml)	0.8126	Thickness (mm)	6.20±0.04
3	Compressibility index %	17.636	Hardness (kg/cm2)	5-6
4	Hausner's ratio	0.823	Friability (%)	0.0799
5	Angle of repose	19.91	Drug content (%) of Nateglinide IR Tablet	98.83
6	-		Drug content (%) of Nateglinide SR Tablet	95.86
7	-		Disintegration Time of Nateglinide IR Tablet (Min)	07.48

2.2.7.2 Comparison of drug release of marketed formulation and bilayer tablet formulation

Marketed formulation dissolution profile was compared with the reproducible batch (I8S5) to study similarity factor. Comparison of dissolution profile of Nate IR layer and Nate SR layer from bilayer tablet with that of Marketed bilayer tablet formulation of same drugs is shown in figure no.9 & table no.14

Table14. Dissolution profile of Marketed formulation & Bilayer tablet batch I8-S5 (Nateglinide)

Time (hrs)	I8-S5 (Bilayer tablet)	Marketed Formulation
1	16.89±0.001	12.92±0.001
2	18.64±0.001	17.24±0.0001
3	28.18±0.0002	27.94±0.001
4	35.31±0.015	36.37±0.002
5	40.24±0.002	45.19±0.0013
6	47.16±0.002	52.16±0.0015
7	55.14±0.011	61.33±0.0031
8	62.43±0.003	67.28±0.002
9	69.73±0.0001	76.78±0.002
10	78.56±0.002	80.64±0.003
11	86.25±0.002	87.41±0.002
12	94.28±0.002	96.35±0.001

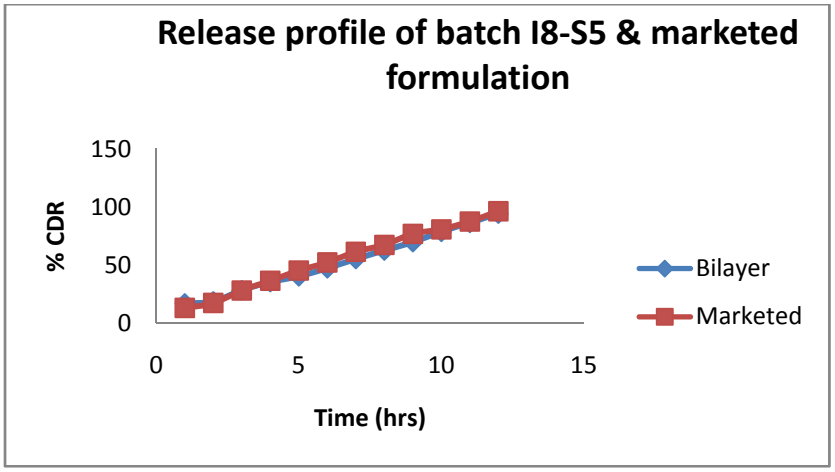

Figure9. Comparative dissolution of Nateglinide IR & SR from bilayer tablet (I8-S5) with that of Marketed formulation

2.2.8 Similarity factor (f_2) study

FDA and the European Agency for the Evaluation of Medicinal Product, suggest that two dissolution profiles are declared similar if f_2 is between 50 and 100.

$f_2$values for reproducible bilayer tablet formulation I8-S5 was determined using excel and it was shown in table no.15.

Table15. f_2 value for reproducible bilayer tablet (H2M12-B) formulation

Sr.No	Formulation	f_2 value
1.	H2M12-B(Metoprolol Succinate)	78

As per FDA guideline H2M12-Bformulations can be considered as showing similar dissolution profile as that of reference products. Based on above observations it can be concluded that formulations H2M12-Bis the most suitable formulations among the all the formulations as it show similar dissolution profile as that of marketed formulations. Hence, it was further considered for stability study.

2.2.9 Stability study

Optimized formulations of bilayer tablet were subjected to stability studies as per ICH guidelines. Various parameters such as Physical appearance, drug content, disintegration time and in vitro dissolution profile release were measured before and after 30, 60 and 90 days of stability. Results of stability studies are shown in table no.16.Physical appearances of all formulations were unaffected or did not show any significant changes.

Table 16. Stability study of optimized formulation

Stability parameter at 40±2 ^{0}C/ 75±5% RH	Time (Days)			
	0	30	60	90
Nateglinide Bilayer tablet				
1) Disintegration of Nate IR layer (Min.)	10.48	10.33	10.25	10.11
2) Drug content %	95.86	95.79	95.66	95.09
3) In vitro dissolution	94.28	94.01	93.88	92.67

Results of stability studies showed that there is no significant change in above mentioned parameters after elevated temperature and humidity conditions during stability studies. Thus it can be proved from the stability studies that the prepared formulation is stable and not much affected by elevated humidity and temperature conditions.

2.3 CONCLUSION AND FUTURE SCOPE

Based on the above study, it can be concluded that Nateglinide, a conventional drug for type 2 diabetes can be successfully formulated in the form of bilayer tablet by optimizing drug polymer ratio using different grades of common polymers like HPMC, HPMC K 100 M etc. This is basically done to improve bioavailability of the drug and better therapeutic compliance. The sustained layer of the drug showed steady state release behaviour

over a prolonged duration of time which may reduce dose related side effects. In future, natural biodegradable polymers can be used to improve therapeutic efficacy of the drug and further minimizing side effects.

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