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Formulation and Evaluation of Biphasic Gastro Floating Tablets of Nateglinide and Atenolol

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Abstract: The aim was to design, formulate, and evaluate bilayer gastro floating tablets of an antidiabetic agent, nateglinide (immediate-release layer), and antihypertensive agent, atenolol (sustained-release layer). The solubility of model drug nateglinide was enhanced by using cremophor RH 40 and characterized by FTIR, DSC, XRD, SEM, and *in vitro* dissolution. It was found that selected ingredients were compatible, and crystalline nateglinide transits to an amorphous state. The gastro-bilayer tablets were directly compressed using the optimized nateglinide (solid dispersion equivalent to 60 mg of nateglinide) immediate-release layer (IRL2) containing different percentage of F-Melt type C and crospovidone and atenolol (50 mg) sustained-release layer (SRL6) using different percentage of HPMC K15, sodium bicarbonate, and MCC. Developed tablets were evaluated and found within the acceptance range as per the guidelines. The release of nateglinide and atenolol from an optimized bilayer tablet (BLT3) was 100 % within 60 min and 12 h, respectively. The floating lag time and total floating time were 2 min and 12 h, respectively. The atenolol sustained-release followed the diffusion mechanism. The combination of nateglinide and atenolol was successfully showed a biphasic release pattern. This formulation may strengthen the fixed-dose combination therapy for diabetes and hypertension at a low cost.

Keywords: Nateglinide; atenolol; biphasic; floating lag time; total floating time.

1. Introduction

High patient compliance, stability, and flexibility with formulation make the oral drug delivery most satisfactory, and it occupies 32 % of the market share [1]. Frequent administration showed fluctuation of the drug plasma concentration and toxicity [2]. Sustained-release was a delivery system to solve these problems and deliver the drugs in an extended period of time. Drugs with a narrow absorption window, alteration in emptying time of stomach, stability issues in the intestine, and drugs transported via active transport mechanism are the difficulty of this system [3]. Gastro retentive drug delivery system (GDDS) has been developed to surmount these problems. This system helps to remain the drug for a prolonged period of time in the gastric cavity. This improves solubility, bioavailability, and reduces the wastage of the drug in the gut [4]. In this system, the drug was delivered locally to the stomach

as well as the proximal small intestine [5]. The floating drug delivery system is one of the most favorable among the gastroretentive dosage forms. The motility of GIT does not affect this system [6]. The matrix bilayer floating tablet is a novel system for the delivery of the therapeutic agents. Simultaneous and desired release profile, decrease the frequency of drug administration with better patience compliance, synergistic effect leading to increase the efficacy and deliver two chemically incompatible drugs into a system are the benefits of this system [7].

In the 21st century, diabetes and cardiovascular diseases are common in society leads to global health emergencies. Globally approximately 463 million adults (20-79 years, 1 in 11 adults) have diabetes in 2019 [8, 9]. If these trends continue, by 2030, 578 million adults have diabetes (IDF, 2019) [9]. Surprisingly, 10 % of the global health budgets are spent on diabetes. According to WHO, globally estimated 1.13 billion people have hypertension [10]. The co-existence/co-occurrence of hypertension and diabetes is very high all over the world [11-13]. It escalates the risks of strokes, retinopathy, nephropathy. The commonness of hypertension with diabetes is up to threefold more than without disease in age-matched subjects [11, 12]. These patients spent 1.43 times more than diabetes without hypertension in India. It is a great challenge as the response to treatment is poor and to normalize the blood pressure in diabetes condition. The prevalence of diabetes and hypertension among young adults and old age across all geographical areas and socio-demographic groups in India was higher [11]. Therefore, diabetes and hypertension often co-exist and may require concomitant drug treatment [14, 15].

Nateglinide (BCS Class II) is an anti-diabetes agent (type 2 diabetes) and has excellent safety and tolerability profile. It shows variable bioavailability due to poor water solubility [16]. To achieve better therapeutic efficacy, nateglinide was selected to prepare an immediate drug release layer through enhancement of solubility. Atenolol (β-blockers) is a widely recommended first-line antihypertensive drug [17]. It has an absorption window in the upper GIT, whereas the poor absorption in the lower GIT. This variable absorption leads to lower bioavailability (50 %) with a half-life of 6-8 h [18]. This can be addressed by increasing gastric residence time of atenolol. Increasing the gastric residence time may promote higher absorption leading to enhanced bioavailability. Hence including atenolol in the sustained release layer may improve gastric retention and its bioavailability.

Formulating biphasic systems with immediate and sustained-release layer has been a successful strategy for developing new drug combinations. Similar formulation with metformin in the sustained-release layer and pioglitazone in the immediate-release layer has been reported to address poor absorption of metformin while reducing the risk of atherosclerosis due to pioglitazone [19]. In our earlier research, we have used a similar strategy with the immediate-release of ezetimibe [6] and simvastatin [20] and sustained-release of atenolol as bilayer gastro floating tablets. Thus the strategy for the immediate-release of nateglinide and sustained-release of atenolol may enhance the potential for enhancement in the bioavailability of these drugs. Keeping this in consideration, the investigation was carried out to develop and optimize bilayer gastro-floating tablet of nateglinide and atenolol for potential application as a fixed-dose combination against comorbidity of diabetes and hypertension.

2. Materials and Methods

Nateglinide was collected from Aurobindo Pharma Ltd., India, as a gift sample. Cremophor RH 40 was supplied by BASF, Germany. Atenolol, crospovidone, aerosol were obtained from Yarrow Chem Products (Mumbai, India). F Melt type C was received from Fuji

Chemical Industries Co., Ltd, Japan, as a gift sample. Hydroxypropyl methylcellulose (HPMC) and compritol 888 ATO were received by Piramal Health Care (Bengaluru, India) and Gattefosse, India, respectively as gift sample. Microcrystalline cellulose was obtained from Otto Chemica Biochemica Reagents, India.

2.1. Preparation of the immediate-release layer.

The feasible industrial technique, solid dispersion (SD) was chosen to enhance the solubility of model anti-diabetes agent nateglinide with cremophor RH 40 and aerosol. SDs at various weight ratios were formulated by the suitable solvent evaporation method. Different weight ratios of 1:0.25, 1:0.5, 1:0.75, 1:1 drug, and carrier (cremophor RH 40) were weighed individually and were dissolved in 10 mL ethanol to get a clear solution. The polymer solution was added to the drug solution with continuous stirring. Ethanol was completely removed by continuous heating on a heating plate at 40-50 ºC, which was carried out until a semisolid mass was obtained. The aerosil (0.02 % w/w) was added to make solid in nature and free-flowing. The powder was kept in a desiccator for further study [21]. The formulations were named F1-F8.

2.2. Percentage yield.

The practical yield was calculated in an appropriate method that signifies the efficiency of any method [6].

2.3. Drug content test.

The percentage of drug content in SD's, was estimated by dissolving the SDs equivalent to 10 mg of nateglinide in 5 mL of ethanol. Each of these solutions was further diluted with 0.01 N HCl buffer, and nateglinide was analyzed at a wavelength of 245 nm by U.V. Visible spectrophotometer (Model: cary 60, Agilent, USA) [6, 18].

2.4. Fourier transforms in infrared spectroscopy (FTIR).

The prepared formulations, pure drugs, polymers/excipients were scanned at a resolution of 2 cm^{-1}, from 4000 to 400 cm^{-1} using FTIR (Cary 60, Agilent Technologies, USA). The FTIR spectra were obtained for the characterization of functional groups.

2.5. Differential scanning calorimetry (DSC).

DSC (Pyris Diamond, Singapore) thermograms of nateglinide, cremophor RH 40, and SDs were recorded. Operating conditions: heating rate (10°/min), temperature 30-280 °C, alumina powder as reference.

2.6. X-ray diffraction analysis (XRD).

XRD diffraction pattern of nateglinide, cremophor RH 40, and SDs were recorded using ULTIMA III, Japan (Cu target slit 10 mm).

2.7. Scanning electron microscopy (SEM).

Nateglinide and SDs were subjected to SEM analysis using JSM6360, Jeol SEM (UK), to investigate morphological characteristics. Operating conditions: probe current 45 nA, accelerating voltage 20 kV, counting time 60 sec.

2.8. In vitro dissolution studies.

The dissolution was performed using USP II (paddle) dissolution apparatus (Electrolab, Mumbai, Model: TDT-08 L) in triplicate. The operation conditions of dissolution: temperature 37 ± 0.5 ºC and paddle rotation speed 50 rpm. Nateglinide and developed SDs equivalent to 60 mg nateglinide were individually placed in 1000 mL of 0.01 N HCl with 0.5 % w/v sodium lauryl sulfate (SLS). At predetermined time intervals 5, 10, 15, 30, 45, and 60 min, 5 mL of aliquots were withdrawn. The equal quantity was replaced with a preheated medium after each sampling. The aliquots were filtered using a 0.45 µm syringe filter and analyzed at a wavelength of 245 nm using a UV-Visible spectrophotometer (Agilent, Cary 60).

2.9. Compression of immediate-release and sustained-release layer tablets.

Based on physicochemical characterizations, SDs containing nateglinide and cremophor RH 40 in the weight ratio of 1:0.25 and 0.02 % w/w aerosol (F5) (of drug equivalent to 60 mg of nateglinide) was selected to formulate into immediate-release tablets. All the ingredients of the immediate-release layer (IRL1-IRL3) (Table 1) and sustained- release layer (SRL1-SRL12) (Table 2) were weighed separately and passed through sieve 44#. Two layers of ingredients were taken in a mortar separately and mixed using a pestle. Magnesium stearate was added before punching tablets by the direct compression method.

Table 1. Formulation of immediate-release layer tablets by direct compression method.

Formulation code	SD equivalent 60 mg of nateglinide (F5)	F Melt type-C (mg)	Crospovidone (mg)	Magnesium stearate (mg)	Total (mg)
IRL1	75	74	-	1	150
IRL2	75	72	3	1	150
IRL3	75	68	6	1	150

Each batch contains 50 tablets

Table 2. Formulation of sustained-release layer by direct compression method.

Formulation code	Drug (mg)	HPMC K100 (mg)	HPMC K15 (mg)	Eudragit RS 100 (mg)	Compritol 888 ATO (mg)	MCC (mg)	Magnesium Stearate (mg)	Total (mg)
SRL1	50	50	-	-	-	192	8	300
SRL2	50	100	-	-	-	142	8	300
SRL3	50	150	-	-	-	92	8	300
SRL4	50	-	50	-	-	192	8	300
SRL5	50	-	100	-	-	142	8	300
SRL6	50	-	150	-	-	92	8	300
SRL7	50	-	-	50	-	192	8	300
SRL8	50	-	-	100	-	142	8	300
SRL9	50	-	-	150	-	92	8	300
SRL10	50	-	-	-	50	192	8	300
SRL11	50	-	-	-	100	142	8	300
SRL12	50	-	-	-	150	92	8	300

Each batch contains 50 tablets

2.10. Preparation of gastro-bilayer floating tablets (GBFTs).

Based on the dissolution profiles and solid-state characterizations, formulation IRL2 (immediate-release) and SRL6 (sustained-release) were taken for the preparation of GBFTs. A varying concentration of sodium bicarbonate was added in the sustained-release layer (Table 3). GBFTs were fabricated by feeding the sustained-release followed by immediate-release layer power to the die cavity. Tablets were compressed using a 12 mm flat punch with an acceptable hardness of 5.5-6.5 kg/cm^2.

Table 3. Compression of gastro-bilayer floating tablets.

Ingredients (mg)	**BLT1**	**BLT2**	**BLT3**
Immediate-release layer			
SD equivalent to 60 mg of nateglinide	75	75	75
F-Melt type C	72	72	72
Crospovidone	3	3	3
Sustained-release layer			
Atenolol	50	50	50
HPMC K15	150	150	150
Sodium bicarbonate	12.5	25	50
MCC	129.5	117	92
Magnesium stearate	8	8	8
Total	500	500	500

2.11. Post compression parameters of GBFTs.

The thickness of the ten randomly selected tablets from each batch was determined with the Vernier caliper scale (Model: 530-312, Japan).

2.12. Weight variation.

Randomly twenty tablets were selected from each batch and calculated the percentage deviation of individual tablet weight from the average weight of tablets.

2.13. Crushing strength.

Crushing strength was determined using Monsanto type hardness tester by selecting randomly six tablets from each batch.

2.14. Friability.

As the weight of the formulation was 500 mg, 6.5 g of the tablet was used for the friability test as per IP (2010). The tablets were put into the Roche friabilator apparatus (Model: 40 FT A01). With a rotation speed of 25 rpm, it was rotated for 4 min while allowing the tablets to fall from a height of 6 inches in each turn. Following this, the tablets were weighed again, and the loss in weight was expressed in percentage as a parameter of friability following the official method of IP 2010.

2.15. Drug content.

The average drug content was analyzed after verifying the UV-Visible spectrophotometric method for the simultaneous estimation of nateglinide and atenolol from bulk (Fig. S1 and Table S1-S6). Briefly, 20 tablets were crushed, and powder equivalent to 50 mg of drug combination was transferred to a 50 mL volumetric flask and dissolved in 0.1 M

HCl. This was sonicated to ensure a homogeneous solution. This was further filtered 1 mL of the filtrate was used for estimation of drug content at a wavelength of 232.7 nm.

2.16. In vitro floating ability.

The floating or buoyancy ability to float tablets was determined following established protocols [22]. Briefly, the tablet was placed in a beaker containing 250 mL of 0.1 M HCl. Floating lag time (FLT) was noted as the time taken by the tablet to appear on the surface of the medium. Similarly, the time to constantly float on the medium was considered as the total float time (TFT).

2.17. Dissolution studies of GBFTs.

The dissolution study was carried out by operating the USP type II apparatus (paddle method) at 50 rpm and 37 ± 0.5 ºC temperature. The developed GBFT was placed in 900 mL of 0.1 N HCl (pH 1.2 simulating gastric pH) dissolution medium. Aliquots of 5 mL from each were withdrawn at specified time intervals of 5, 10, 15 min, 0.5, 0.75, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12 h. Equal volume was replaced with fresh preheated medium after each sampling. The aliquots were filtered using 0.45 µm Millipore syringe filters. The filtrates were analyzed at a wavelength of 232.7 nm for the estimation of nateglinide and atenolol simultaneously.

2.18. Release kinetics.

In vitro, drug release data were subjected to mathematical models like zero order, first order, Higuchi, Hixson Crowell, Korsmeyer, and Peppas in order to investigate the release pattern of formulated batches [23, 24].

3. Results and Discussion

Nateglinide (BCS Class II) is practically insoluble in water (8.8 mg/L) [25]. In order to meet the aim of the study, an attempt was made to improve the solubility of nateglinide. There are eight different formulations (F1-F8) that were prepared with cremophor RH 40 and with and without aerosol (0.02% w/v) by solvent evaporation technique in a weight ratio of 1:0.25, 1:0.5, 1:0.75, 1:1. The formulations were subjected to physicochemical characterization like FTIR, DSC, XRD, SEM, and *in vitro* dissolution studies.

3.1. FTIR.

The FTIR study provides evidence of the chemical/physical interaction and the shifting of the bonds. The FTIR spectrum of the nateglinide, cremophor RH 40, and its developed SDs are shown in Figure 1. Nateglinide has a C=O group, which may participate in intermolecular interactions. Solutol HS 15 and cremophor RH 40 has functional groups which could favor the interaction with nateglinide by hydrogen bonding. The characteristic peaks of nateglinide [26] were observed at 3307.92 cm^{-1} for N-H stretching and 3068.75 cm^{-1} for aromatic C-H stretching. C-H symmetric and asymmetric stretching were observed at 2964.59 and 2918.30 $cm^{-1,}$ respectively (Figure 1A). C=O stretching was assigned to 1687.71 cm^{-1}. SDs showed a slight shifting of the peaks corresponding to N-H and carbonyl stretching groups (Figure 1D). This may occur due to hydrogen bond formations (non-covalent interactions) with polymeric groups, which might contribute to the enhancement of solubility of nateglinide in the SDs.

There was no chemical interaction between the nateglinide and cremophor RH 40. The optimized formulation showed characteristic peaks of the drugs and excipients (Figure 1E) that indicate the compatibility between them.

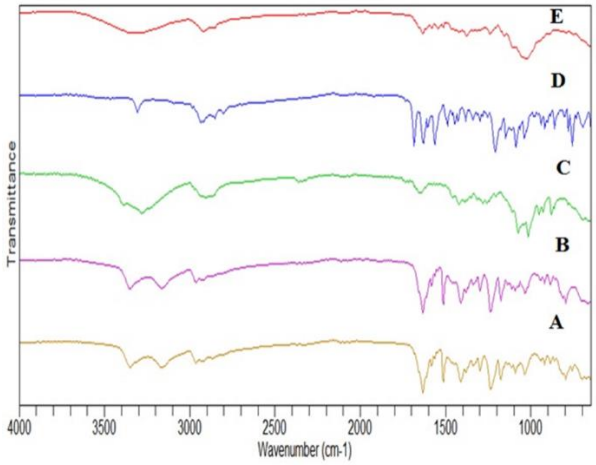

Figure 1. FTIR absorption spectra of A) nateglinide, B) atenolol, C) cremophor RH 40, D) nateglinide SD (F5), and E) optimized bilayer tablet (BLT3).

3.2. DSC.

The DSC thermographs of nateglinide, cremophor RH 40, and SDs are depicted in Figure 2. Nateglinide recorded a melting point at 136.67 ºC (ΔH = 89.83 J/g) suggesting its crystallinity (Figure 2A) [23, 26]. The endotherm of cremophor RH 40 displayed a melting point at around 32.2 ºC (Figure 2B). The nateglinide melting peak disappeared in SD when prepared with cremophor RH 40 (Figure 2C). This indicates the amorphous state of the drug and that the amount of cremophor RH 40 used was sufficient to solubilize nateglinide.

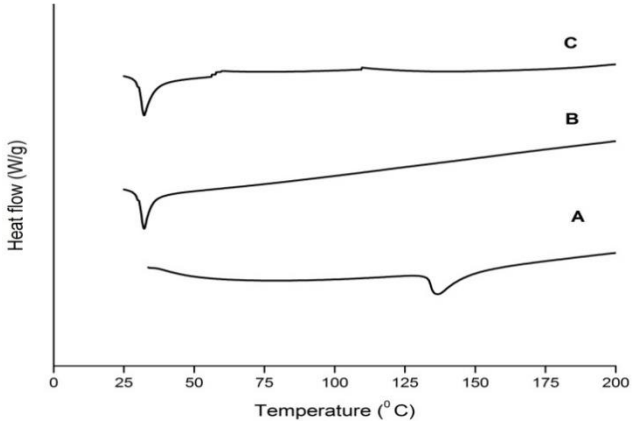

Figure 2. DSC thermograms of A) nateglinide, B) cremophor RH 40 and C) nateglinide SD (F5).

3.3. XRD.

The peak heights of nateglinide, cremophor RH 40, and their SDs are exhibited in Figure 3. Pure drug nateglinide exhibited sharpener and intense peak at diffraction angles (2θº) equivalent to 7.44º, 9.94º, 12.28º, 12.88º, 13.10º, 13.64º, 14.48º, 16.56º, 17.42º, 18.5º, 20.22º, 20.42º, 21.4º, 22.44º, 22.86º, 23.92º, 30.87º. Maggi *et al.*[25] observed the same pattern of the nateglinide indicates the strong crystal habit of the pure drug. Cremophor RH 40 (Figure 3B) exhibited no characteristics peaks indicating their amorphous nature. The XRD diffractogram of nateglinide in cremophor RH 40 showed the disappearance of drug peaks (Figure 3C). This

suggests conversion to an amorphous state. The amount of cremophor RH 40 was sufficient to solubilize the drug in solvent evaporation techniques. This is in agreement with the DSC data.

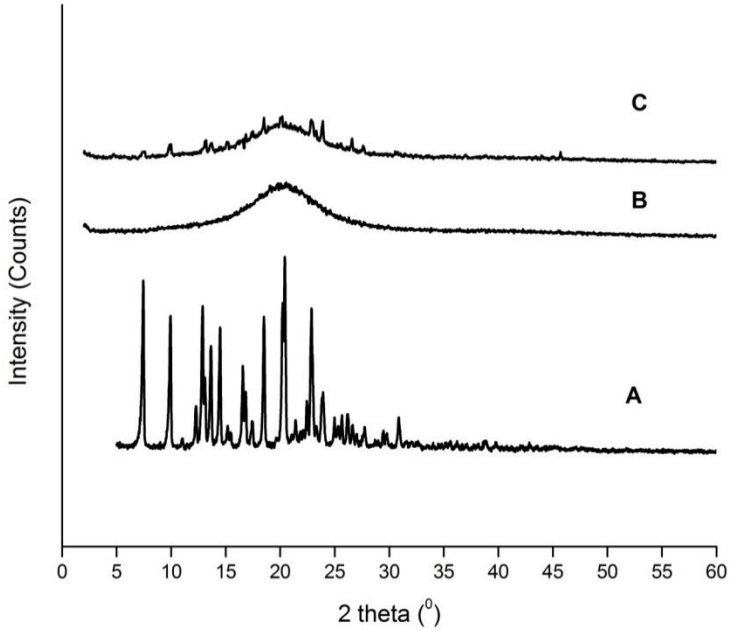

Figure 3. XRD pattern of A) nateglinide, B) cremophor RH 40 and C) nateglinide SD (F5).

3.4. SEM.

SEM photomicrographs of nateglinide and its selected SD formulations are shown in Figure 4. Pure drug nateglinide demonstrated rod-shaped crystallites with various sizes (Figure 4A). This is in agreement with the DSC and XRD data. Photographs of SD (Figure 4B) indicated a loss of crystallinity, which might be the reason for the faster dissolution of the nateglinide.

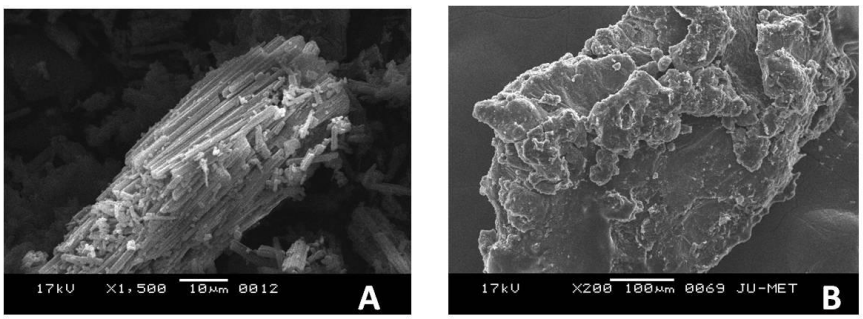

Figure 4. SEM image of A) nateglinide and B) nateglinide SD.

3.5. In-vitro dissolution studies.

The dissolution profiles of pure drug nateglinide and prepared SD are shown in Figure 5. A small amount of SLS in the dissolution medium was more similar to physiologically bile salt, which was a natural surfactant useful for the dissolution of BCS class II drugs [28]. So, SLS will satisfy their needs. Therefore the dissolution was performed in 0.1N HCl with a 0.5 % SLS solution. The pure drug nateglinide showed an incomplete drug release (23.01 ± 1.99) within 60 min of dissolution. Incorporation of carriers showed enhancement of solubility. There was a 7.57 fold enhancement of dissolution observed for SD as compared with pure drug. The quick enhancement (99.92 ± 1.21) of dissolution at 30 min may be due to the interaction

of surfactant carrier cremophor RH 40 and drug. In 60 min of dissolution, SD showed complete drug release (99.92 ± 1.21). This was approximately 4.34 fold more than pure drug. This was supported by DSC, XRD, and SEM analysis. This formulation F5 was further used for the preparation of the immediate-release layer.

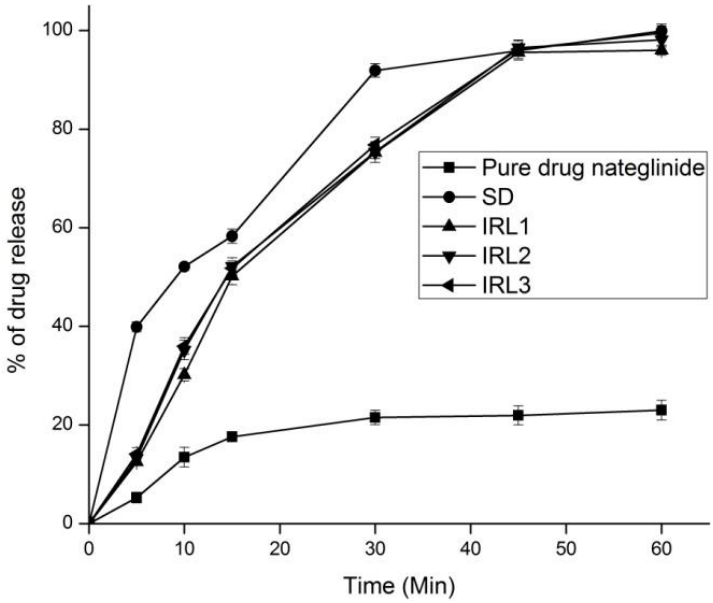

Figure 5. *In-vitro* release profile of solubility enhancement of nateglinide and *in-vitro* release profile of immediate-release layer.

3.6. Compression of an immediate-release layer and a sustained-release layer.

SD equivalent to 60 mg of nateglinide was taken and formulated into immediate-release tablet by F Melt type C and varying the concentrations of super disintegrant crospovidone (Table 1). Sustained-release tablets were formulated by varying the concentration of release retardants (HPMC K100, HPMC K15, Eudragit RS 100, and Compritol 888 ATO) (Table 2). The powder blends were compressed individually by using a direct compression method. *In-vitro* dissolution studies were performed to optimize the formulations for the development of bilayer tablets.

3.7. In vitro dissolution.

The in-vitro release profile of immediate-release formulations (IRL1-IRL3) is shown in Figure 5. The formulations IRL1 and IRL2 showed 96 % and 98 % drug release within 1 h, which was less than the IRL3 formulation (99.5 %). This shows a nearly 5 fold increase in dissolution efficiency when compared to a pure drug over a period of 1 h. This may be due to the crospovidone in the immediate-release layer that swells by absorbing the liquid medium by wicking and help to liberate nateglinide with fine dispersion. Therefore, the IRL3 formulation was selected as an immediate-release in the preparation of GBFTs.

The release profiles of atenolol formulations (SRL1-SRL12) are illustrated in Figure 6. The formulations with HPMC K15 (SRL4, SRL5, SRL6) showed 100 % of drug release within 9-12 h whereas HPMC K100 (SRL1, SRL2, SRL3), eudragit RS 100 (SRL7, SRL8, SRL9) and compritol 888 ATO (SRL10, SRL11, SRL12) showed 100 % drug release in 5-7h respectively. This showed that HPMC K100, eudragit RS 100, and compritol 888 ATO polymers unable to maintain their integrity for a longer period when compared to HPMC K15. Therefore, HPMC

K15 showed a better retardant effect when compared to the other three polymers over a period of 12 h. Among three different concentrations of HPMC K15, the formulation with 50 % polymer (SRL6) showed a good release profile over a period of 12 h. This may be due to the increase in the concentration of polymer that may increase the thickness of the gel barrier and tortuosity. With 30-35 % of drug release in 1 h, 60- 65 % release in 6 h and the remaining drug release after 12 h, the release is proposed to follow release profile as per Robinson and Eriksen equation [29]. Therefore, SRL6 formulation was optimized and selected as a sustained-release layer in the preparation of GBFTs.

GBFTs were directly compacted using the optimized formulations (IRL3 and SRL6) by varying the concentration of sodium bicarbonate (Table 3) to optimize the FLT and TFT. The developed tablets were characterized for post-compression parameters, drug release profile, and *in-vitro* floating ability.

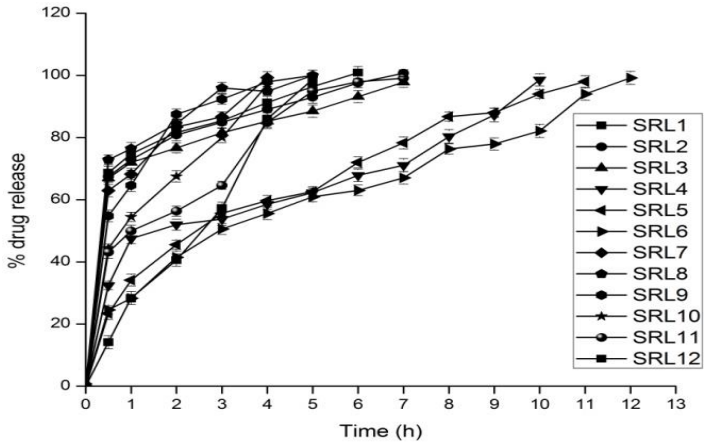

Figure 6. *In-vitro* release profile of sustained-release layer tablets.

3.8. Post compression Parameters.

Post compression parameters of the prepared GBFTs are shown in Table 4.

3.9. General appearance.

The general appearance of prepared bilayer tablets was elegant. As shown in Figure 7, pink color was added to distinguish the immediate-release layer from the sustained-release layer (white color).

3.10. Weight variation.

A weight variation test was performed to check whether uniform weight was maintained among all the formulation batches. It is affected by the flow properties of the powder blend. As the weight of the prepared tablet was 500 mg, the percentage deviation allowed was 5 % (25 mg) as per IP. The prepared GBFTs were in the range of 498-500 mg (Table 4). So, these results suggested that weight variation was within the range, and there was no significant variation in weight between different batches of tablets. This showed uniform die filling during tablet compression.

Figure 7. The general appearance of GBFTs with a pink immediate-release layer (front view) and white sustained-release layer (back view).

Table 4. Post-compression parameters of the prepared gastro-bilayer floating tablets.

Formulation code	Weight variation[a] (mg)	Hardness[b]	Friability[c]	Drug content (%)[d]		FLT (min)[e]	TFT (h)[f]
				Nateglinide	Atenolol		
BLT1	498 ± 1.17	5.6 ± 0.17	0.638	99.85 ± 1.18	99.56 ± 1.23	8	8
BLT2	500 ± 1.24	5.5 ± 0.19	0.459	100.12 ± 0.99	100.08 ± 1.24	5	12
BLT3	499.9 ± 1.11	5.4 ± 0.22	0.329	99 ± 0.20	100.21 ± 0.95	2	12

(a) Avg ± % deviation, n = 20; (b) mean ± S.D n = 5; (c) mean ± SD, n=3 (6.5 g of tablet); (d) mean ± SD, n=10; (e) mean ± SD, n=3; (f) mean ± SD, n=3.

Table 5. Kinetics of release from formulated tablets.

Formulation code	Zero-order		First-order		Higuchi		Koresmeyer and Peppas		Hixon Crowell	
	r^2	K_0	r^2	K_1	r^2	K_H	r^2	n	r^2	K
BLT1	0.886	6.563	0.884	0.110	0.977	25.63	0.971	0.372	0.926	-0.220
BLT2	0.856	7.274	0.873	0.106	0.948	28.47	0.675	1.07	0.933	-0.277
BLT3	0.886	6.402	0.701	0.121	0.969	24.9	0.971	0.378	0.834	-0.223

3.11. Hardness.

Hardness gives an idea regarding how far the tablets resist capping, aberration, or breakage under conditions of handling and transportation. It affects drug dissolution and releases to some extent. The hardness of GBFTs was within the range of 5.4-5.6 kg/cm^2 (Table 4). Therefore, all formulations were within the range. Therefore, all the batches were found to have a good thickness and had the ability to withstand the handling abrasion.

3.12. Friability.

The ability of the tablet formulation to withstand mechanical stress is evaluated by friability testing. Friability of GBFTs was in the range of 0.325-0.638 (Table 4), i.e., below 1% of the tablet. This indicates the developed tablets can withstand the mechanical shocks during handling and are suitable for further processing.

3.13. Drug content.

Assay of drug content uniformity is essential to assess the consistency of dosage forms. A verified UV-Visible Spectrophotometric method was used for the simultaneous estimation of the nateglinide and atenolol content. Drug content for all the prepared formulation batches was found in the range of 99.85– 100.12 % for nateglinide and 99.56–100.21 % for atenolol (Table 4). This was acceptable as per IP.

3.14. In vitro floating ability.

Ideally, a low FLT and high TLT is desirable for the continuous floating of the dosage form in the upper GIT to prevent escaping to the lower GIT. The optimum concentration of

sodium bicarbonate is a critical factor in achieving the shortest lag time and highest TLT. This is because sodium bicarbonate evolves CO_2 bubbles that react with HCl to form pores in the swollen polymer matrices of HPMC due to entrapment of bubbles. This helps the floating ability of the formulation. Thus optimum buoyancy was achieved by optimization of combinations of sodium bicarbonate and HPMC. *In-vitro* floating ability was determined by considering the sodium bicarbonate in three different concentrations 2.5 %, 5 %, 10 % w/w. An increase in concentration decreased the FLT from 25 min to 2 min. The stages of the floating of the optimized formulation are shown in Figure 8. Formulation (BLT1 and BLT2) containing 2.5 % and 5 % w/w of sodium carbonate took more time to appear on the surface of the dissolution medium and floated up to 12 h. The formulation containing 10 % w/w sodium bicarbonate showed FLT of 2 min and TFT up to 12 h. So, the formulation (BLT3) was considered as the optimized formulation as it has good FLT (2 min) along with TFT (up to 12 h).

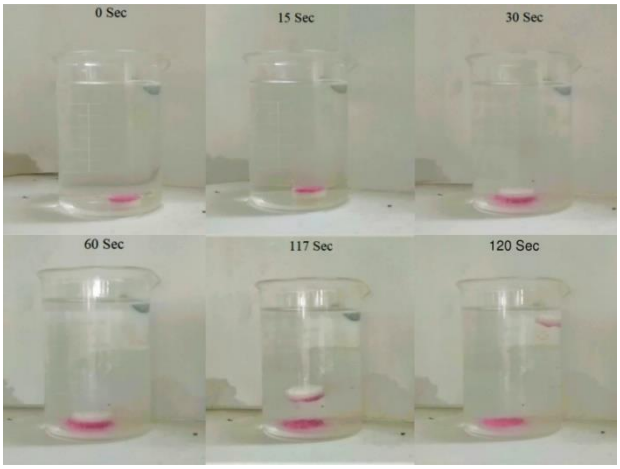

Figure 8. Stages of floating of prepared GBFT (BLT3).

3.15. In-vitro dissolution studies.

The nateglinide and atenolol release profiles of the GBFTs are depicted in Figure 9. Increasing concentrations of sodium bicarbonate may produce a higher level of effervescence that may result in an increased rate of pore generation. This ultimately results in rapid hydration of the matrices, and consequently, faster drug release may occur. The formulation BLT1 showed approximately 98 % of atenolol and nateglinide over a period of 12 h and 60 min, respectively (Figure 9).

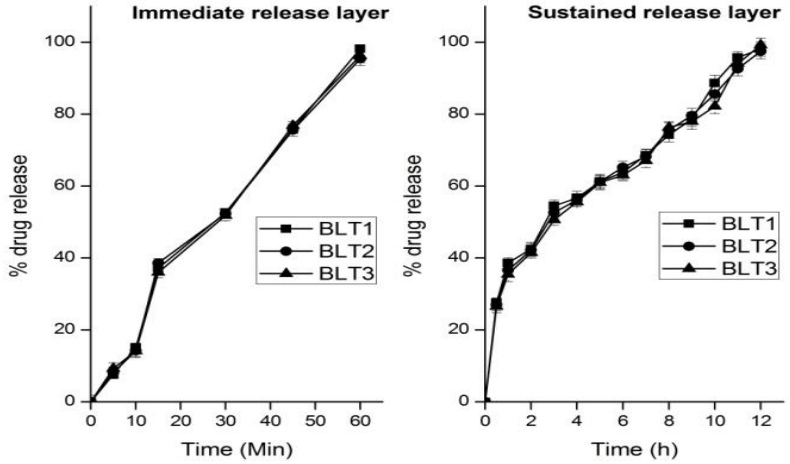

Figure 9. *In-vitro* release profile of nateglinide and atenolol from layers of the GBFTs.

This may be due to a decreased rate of pore generation, which results in a slower rate of drug release. The BLT2 showed 97.5 % atenolol and 95.4 % of nateglinide release with 12 h and 60 min, respectively. The formulation BLT3 showed a 100 % drug release over a period of 12 h (Figure 9) without losing the integrity of the tablet. The immediate-release layer containing nateglinide released 100 % within 60 min of dissolution. Therefore, BLT3 was selected as an optimized formulation.

3.16. Kinetics of drug release.

Optimized formulation showed a biphasic release pattern, i.e., the burst effect is followed by sustained-release. Burst effect may be due to a combination of factors, including the hydrophilic nature of drug, polymer, and method of preparation. The direct compression method allows the drug to be on the surface of the tablet. Therefore, when the tablet comes in contact with the dissolution medium, the drug present on the surface of the tablet immediately enters into the medium. Subsequently, drugs are released from the matrix. So, the burst effect is considered as the additive effect of the free drug present at the surface and the initial release of drug from the instantly swelled gel barrier. The presence of sodium bicarbonate may also facilitate drug release through the formation of bubbles and pores in the matrix.

Following analysis in zero and first-order model, a higher r^2 value was obtained for zero-order compared (0.886) to first-order (0.701) (Table 5). This suggested that the formulation followed the zero-order release. The optimized formulation was studied for Higuchi and Hixon Crowell models, where Higuchi model r^2 value (0.969) was found to be higher than the Hixon Crowell value (0.834) (Table 5), which indicated that the release might follow diffusion mechanism. This may be due to the hydrophilic nature of drugs incorporated in a semisolid matrix or sodium bicarbonate that forms gas bubbles on reaction with the dissolution medium, which creates a porous system that allows the drug diffuses through the tortuous pathway created by the porous system.

4. Conclusions

The solubility of nateglinide was enhanced successfully using cremophor RH 40 by the solvent evaporation method. GBFTs were successfully developed using these optimized immediate and sustained-release layer. The gastro retention of the formulations was demonstrated with low FLT and 12 h TFT. This may result in higher bioavailability. However, this needs to be validated by a pharmacokinetic study. Further, pharmacodynamics may be studied to justify its application to manage the co-existing disease conditions of hypertension and diabetes.

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Conflicts of Interest

The authors declare no conflict of interest.

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Supplementary data

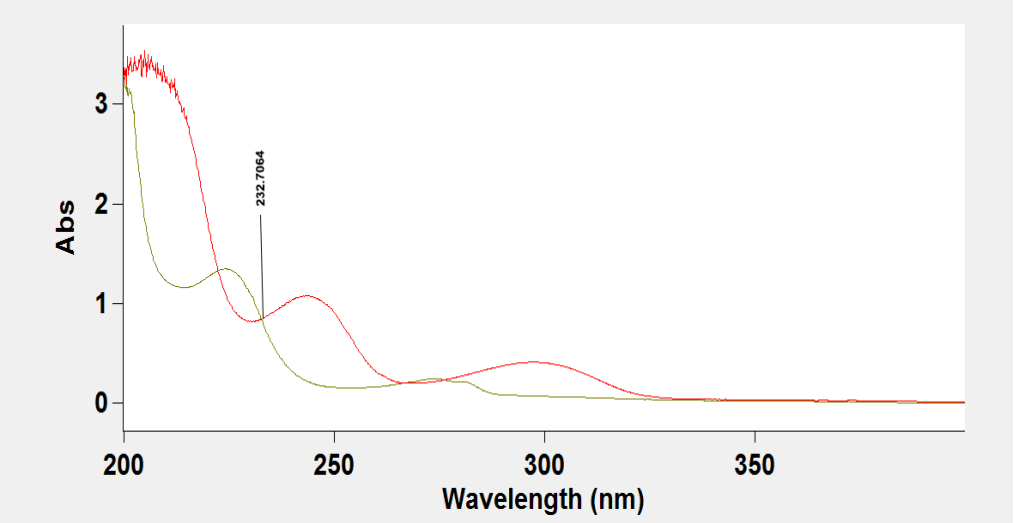

Figure S1. Simultaneous estimation of nateglinide and atenolol by U.V.-Visible Spectrophotometer.

Linearity

Table S1. Statistical Parameters of linearity.

Statistical Parameters	UV	
	First Order	
	Nateglinide	Atenolol
Linearity(µg/ml)	5-25	5-25
Correlation coefficient(R^2)	0.999	0.998
Regression Equation y=mx+c	y = 0.023x + 0.013	y = 0.033x + 0.024
Slope(m)	0.023	0.033
Intercept(c)	0.013	0.024

Table S2. Concentration and absorbance values obtained for linearity curve determination.

S. no	Nateglinide		Atenolol	
	Concentration (µg/ml)	Absorbance at 245 nm	Concentration (µg/ml)	Absorbance at 225 nm
1	5	0.1309	5	0.1992
2	10	0.2489	10	0.3525
3	15	0.3597	15	0.5103
4	20	0.4762	20	0.684
5	25	0.5992	25	0.8622

Accuracy

Table S3. Accuracy table of nateglinide.

S. no	Level of addition (%)	Amount added (µg)	Amount found (µg)	% Recovery	Average
1	50	10	9.56	95.6	97.1 ± 1.26
		10	9.87	98.7	
		10	9.70	97.0	
2	100	20	19.55	97.7	98.53 ± 063
		20	19.88	99.4	
		20	19.70	98.5	
3	150	30	29.88	99.6	98.96 ± 0.57
		30	29.75	99.1	
		30	29.46	98.2	

Table S4. Accuracy table of atenolol.

S. no	Level of addition (%)	Amount added (µg)	Amount found (µg)	% Recovery	Average
1	50	10	9.46	94.6	95.76 ± 1.37
		10	9.77	97.7	
		10	9.50	95.0	
2	100	20	19.05	95.25	95.33 ± 0.06
		20	19.08	95.4	
		20	19.07	95.35	
3	150	30	29.15	97.16	97.76 ± 0.69
		30	29.62	98.73	
		30	29.22	97.4	

Precision

Table S5. Precision table of nateglinide.

S. no.	Concentration (µg/ml)	Nateglinide Absorbance at245 nm	
		Intra- Day	Inter-Day
1	20	0.6820	0.6832
2	20	0.6799	0.6795
3	20	0.6834	0.6809
4	20	0.6825	0.6854
5	20	0.6856	0.6845
Average		0.6826 ± 0.02	0.68 ± 0.024

Table S6. Precision table of atenolol.

S. no	Concentration (µg/ml)	Atenolol Absorbance at225 nm	
		Intra- Day	Inter-Day
1	20	0.4608	0.4611
2	20	0.4633	0.4625
3	20	0.4659	0.4658
4	20	0.4622	0.4677
5	20	0.4593	0.4628
Average		0.4623± 0.02	0.4693 ±0.02

Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD and LOQ were calculated based on the standard deviation of the analytical response and the slope of the calibration curve. The LOD and LOQ were 0.015, 0.050, 0.025 and 0.075 µg/mL respectively for nateglinide and atenolol with acceptable precision and accuracy under the stated conditions.