

FORMULATION OF IMMEDIATE RELEASE (IR) ATORVASTATIN CALCIUM PELLETS AND SUSTAINED RELEASE (SR) GLIBENCLAMIDE FOR FIXED-DOSE COMBINATION DOSAGE FORM

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ABSTRACT

Objective: The objective of the present research was to develop fixed-dose combinations for the treatment of dyslipidemia, associated with type-II diabetes mellitus for improvement of glucose tolerance.

Methods: Multiple unit pellet systems (MUPSs) consisting immediate release atorvastatin calcium pellets and sustained release glibenclamide were formulated by spheronization technique. The characterization of formulated pellets was done by Fourier transform infrared (FT-IR) and differential scanning calorimetry (DSC) studies, and formulated pellets were evaluated for solubility, viscosity, pH, and *in vitro* studies.

Results: From FT-IR and DSC studies, it was confirmed that no chemical interaction existed between the drug and the natural polymers used. Solubility of glibenclamide was found to be 4.38 and 18.24 and atorvastatin calcium was found to be 6.84, 214.67, and 287.43 g/L. The viscosity of 1% w/v of locust bean gum, guar gum, and ghatti gum was found to be 169 cP, 124 cP, and 31 cP in distilled water. The pH of locust bean gum, guar gum, and gum ghatti solutions was found to be 5.6 ± 0.49 , 5.2 ± 0.27 , and 4.7 ± 0.51 . The *in vitro* studies suggested that glibenclamide pellets had shown a sustained release till 12 h, while atorvastatin calcium had shown immediate release of drug due to rapid disintegration of pellets.

Conclusion: Thus, MUPS can be considered as an alternative approach to treat diabetes induced dyslipidemia.

Keywords: Multiple unit pellet system, Glibenclamide, Atorvastatin calcium, Sustained release, Immediate release.

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INTRODUCTION

Diabetes mellitus is a heterogeneous group of disease characterized by elevation of glucose in the blood because of the impaired insulin secretions in body. Deficiency of insulin leads to chronic hyperglycemia with disturbances of carbohydrate, fat, and protein metabolism [1,2]. As the disease progresses, various tissues or vascular damage leads to many diabetic complications related to retinopathy, neuropathy, nephropathy, cardiovascular, and ulceration, thus diabetes covers a wide range of heterogeneous diseases. It is the most common endocrine disorder and estimated that more than 200 million people were suffering from diabetes [3]. Diabetes can be classified into two types, i.e., Type-I (insulin-dependent diabetes) and Type-II (non-insulin-dependent diabetes). Patients suffering from Type-II diabetes will not respond effectively to insulin which can be termed as insulin resistance. When the blood glucose or sugar level is high, it can be termed as hyperglycemia, whereas when the blood glucose levels are lower than normal levels, it can be termed as hypoglycemia [4].

In recent times, among various drug delivery systems to treat diabetes induced dyslipidemia, multiple unit pellet systems (MUPSs) have grabbed attention of pharmaceutical industries, as they deliver both immediate and modified release drugs from as single system. The pellets that are present in this multiple unit can be coated or uncoated. When compared with other delivery techniques, these multiple unit pellet delivery systems possess advantages such as reduced toxicity, local irritation and dose dumping, and plasma concentration fluctuations, and it is able to administer high potency drugs [5].

Glyburide [6] is another name for glibenclamide, which comes under the class known as sulfonylureas; further, it is classified as either the second generation or the third generation of sulfonylureas. It is familiarly related as sulfonamide antibiotics, pharmaceutical preparation of

glibenclamide is used only for Type-II diabetes and not for Type-I diabetes, because of insufficient production of insulin from pancreas. Glibenclamide acts by binding and inhibiting potassium channel (K_{ATP}) and sulfonylurea receptors in pancreatic beta-cell [7].

Atorvastatin comes under the family of statins; initially, it behaves as lipid-lowering agent and inhibits tasks associated with cardiovascular diseases. Atorvastatin plays a major role by inhibiting 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, an enzyme which is present in the liver tissue plays a key role in the production of cholesterol in the human body. The reduction of HMG-CoA to mevalonate is also catalyzed by HMG-CoA reductase [8].

Here, in the current research work, a basic pellet formulation consisting a drug and microcrystalline cellulose (MCC) was formulated by employing spheronization technique, to modify the drug release from various excipients which are taken in various proportions and combinations. The main objective of this formulation is to improve the glucose tolerance in Type-II diabetes.

MATERIALS AND METHODS

Materials

Glibenclamide was purchased from Sanket Pharmaceuticals Pvt., Ltd., Mumbai, and atorvastatin calcium API was purchased from Afton Pharma, Rajkot, Gujarat. All the other chemicals used in the study are of analytical grade.

Methods

Preparation of drug-loaded sustained release glibenclamide pellets

A uniform dry powder mixture (batch size 100 g) containing glibenclamide, MCC (*Avicel PH 101*), locust bean gum, and gum ghatti/guar gum is taken in polyethylene bag in different concentrations as shown in Table 1. The above mixture was then granulated using

water and isopropyl alcohol in 5:2 ratio as granulation fluid. Then, it is extruded, and the extrudates were then immediately spheronized to get the yield. The pellets were dried in hot air oven at 40°C for 10–12 h and stored in screw-capped, high-density polyethylene bottles [9].

Preparation of drug-loaded immediate release atorvastatin calcium pellets

Similar to above glibenclamide pellets formulation, uniform dry powder mixture (batch size 100 g) containing atorvastatin calcium and excipients such as MCC (Avicel PH 101), sodium starch glycolate/croscarmellose sodium, sodium lauryl sulfate (SLS), and sodium bicarbonate was taken in polyethylene bag as shown in Table 2. The mixture was then granulated using 1% w/w locust bean gum/PVP K-30 in water as granulation fluid. Then, the wet granulation mixture was extruded, and the extrudates were then immediately spheronized to yield the spherical pellets. The pellets were dried in hot air oven at 40°C for 10–12 h. After drying, pellets were then filled and stored in screw-capped, high-density polyethylene bottles [9].

Preformulation studies

The following preformulation studies were performed for glibenclamide and atorvastatin calcium pure drugs.

Fourier transform infrared (FT-IR) studies of glibenclamide and atorvastatin calcium

To determine compatibility of the drug and polymer, Fourier transform-infrared (FT-IR) spectroscopy was done. Results were recorded using FT-IR (8400S, Shimadzu Corporation, Japan). API dispersed with KBr in mortar, the material is triturated into fine powder with the help of compression gauge the powder has been compressed in a holder, and pressure of 5 ton is applied for 5 min. The pellet was placed in the light path and results were recorded [10].

Differential scanning calorimetry (DSC)

DSC studies were carried out on Shimadzu thermal analyzer. A few milligrams of sample were hermetically sealed into aluminum pans and heated under nitrogen atmosphere with the heating rate of 10°C/min [11].

Solubility

Solubility studies of glibenclamide and atorvastatin calcium were carried out using distilled water. Glibenclamide and atorvastatin calcium were taken in vials containing distilled water. The vials were subjected to stirring for 24 h on a magnetic stirrer, and the obtained solution was filtered and the filtrate was estimated spectrophotometrically at wavelengths of 242 nm for glibenclamide and 246 nm for atorvastatin calcium, respectively [12].

pH of gums

The accurate measurement of pH was done using probe pH meter. Measurement was done by submerging the probe in the liquid until a reading is registered by the meter.

Micromeritic properties

Micromeritic properties for both glibenclamide and atorvastatin calcium such as angle of repose, Carr's index, as well as tapped density were determined. The tapped density was determined using a tapped density tester in which the glass cylinder was tapped 100 times. To measure the tapped density, the process was continued until a constant value was attained. Angle of repose was the most widely used technique for measuring the flowability [by determining the shape of the powder heap]. The formula used to measure the flowability [13].

Scanning electron microscopic (SEM) studies

SEM was employed to investigate the surface morphology of the pellets which were prepared, before coating around the pellets during analysis. SEM was performed at 5 kV having different magnifications using Hitachi Noran System 7 manufactured by Thermo Fisher Scientific [14].

Evaluation of prepared pellets

Friability and pellet yield

Friability of pellets was determined by Roche friabilator. 10 g of pellets were subjected to testing at 25 rpm for 4 min. Samples were sieved and the pellets retained on the sieve were weighed, and percent friability

Table 1: Formulation chart of sustained release glibenclamide pellets

Formulation Code	Ingredient concentration (% w/w)					Wetting agent volume (Water: IPA) (5:2) (mL/g)
	Glibenclamide	MCC	Locust bean gum	Gum ghatti	Guar gum	
BD-1	2.5	96.5	1	-	-	0.80
BD-2	2.5	95.5	2	-	-	0.77
BD-3	2.5	94.5	3	-	-	0.77
BD-4	2.5	95.0	2	0.5	-	0.70
BD-5	2.5	94.5	2	1	-	0.77
BD-6	2.5	94.0	2	1.5	-	0.84
BD-7	2.5	93.5	2	2	-	0.84
BD-8	2.5	93.0	2	2.5	-	0.80
BD-9	2.5	95.0	2	-	0.5	0.74
BD-10	2.5	94.5	2	-	1	0.74

Table 2: Formulation chart of immediate release atorvastatin calcium pellets

Formulation	Ingredient concentration (% w/w)					
	Atorvastatin calcium	MCC	SSG	CCS	Sodium bicarbonate	SLS
FR-1	4	92	1	-	2	1
FR-2	4	91	2	-	2	1
FR-3	4	90	3	-	2	1
FR-4	4	89	4	-	2	1
FR-5	4	88	5	-	2	1
FR-6	4	87	6	-	2	1
FR-7	4	86	7	-	2	1
FR-8	4	92	-	1	2	1
FR-9	4	91	-	2	2	1
FR-10	4	90	-	3	2	1

was calculated from the difference in the weight of the pellets before and after friability.

Drug loading and encapsulation efficiency

To the freshly prepared 100 ml phosphate buffer of pH 6.8 with 0.5% w/v SLS, a specific amount of crushed pellets were suspended with constant agitation at room temperature for 24h. The final solution was filtered via Whatman filter paper, and drug content was determined separately for each pellet formulation by spectrophotometric method at the specific wavelengths.

Pellets disintegration time

Special transparent tubes having the diameter of 10-mm and 15-mm length were employed in pellet disintegration tester. The sieves of 710- μ m mesh size were at bottom and as well as top of the tubes. 100 mg of pellets is filled in the tubes and they were placed in the standard tablet disintegration tester. The disintegration time of six dried samples at 37°C was determined at a speed of 30 dips. Disintegration test was carried out 3 times for each formulation, and results were expressed [14].

In vitro dissolution studies

Electrolab USP dissolution testing apparatus I (basket type) was used to evaluate the rate of drug release from formulated pellets. A weighed quantity equivalent to 100 mg of pellets was taken in 500 mL of 7.4 pH phosphate buffer. The test was carried out for 12 h at 37 \pm 1°C and 100 rpm. 2 mL of

sample was withdrawn at preset time intervals and replaced with equal volume of dissolution medium. Withdrawn sample was filtered and analyzed spectrophotometrically at wavelength of 242 nm and 246 nm [14].

RESULTS AND DISCUSSION

Solubility

Solubility of glibenclamide was found to be 4.38, 18.24, and 24.09 g/L in pH 1.2, 6.8, and 7.2. Similarly, the solubility of atorvastatin calcium was found to be 6.84, 214.67, and 287.43 g/L in pH 1.2, 6.8, and 7.2 phosphate buffers, respectively. From the results, it can be inferred that both glibenclamide and atorvastatin calcium were more soluble in alkaline pH, i.e., >7.

pH of gums

The locust bean gum is a non-ionic neutral polysaccharide and it reduces syneresis at a pH range of 5.6 \pm 0.49, guar gum had shown results to treat diabetes, and pH of guar gum obtained was 5.2 \pm 0.27. Ghatti gum having a pH range of 4.7 \pm 0.51 was favorable for formulation of pellets.

Characterization of sustained release glibenclamide pellets

FT-IR studies of glibenclamide

The position of peak in FT-IR spectra of pure glibenclamide is compared with those in FT-IR spectra of glibenclamide with excipients as shown in Fig. 1. FT-IR spectral analysis indicates that the characteristic absorption peaks present in the IR spectra of glibenclamide drug were also found in the physical mixtures of the polymers used (locust bean

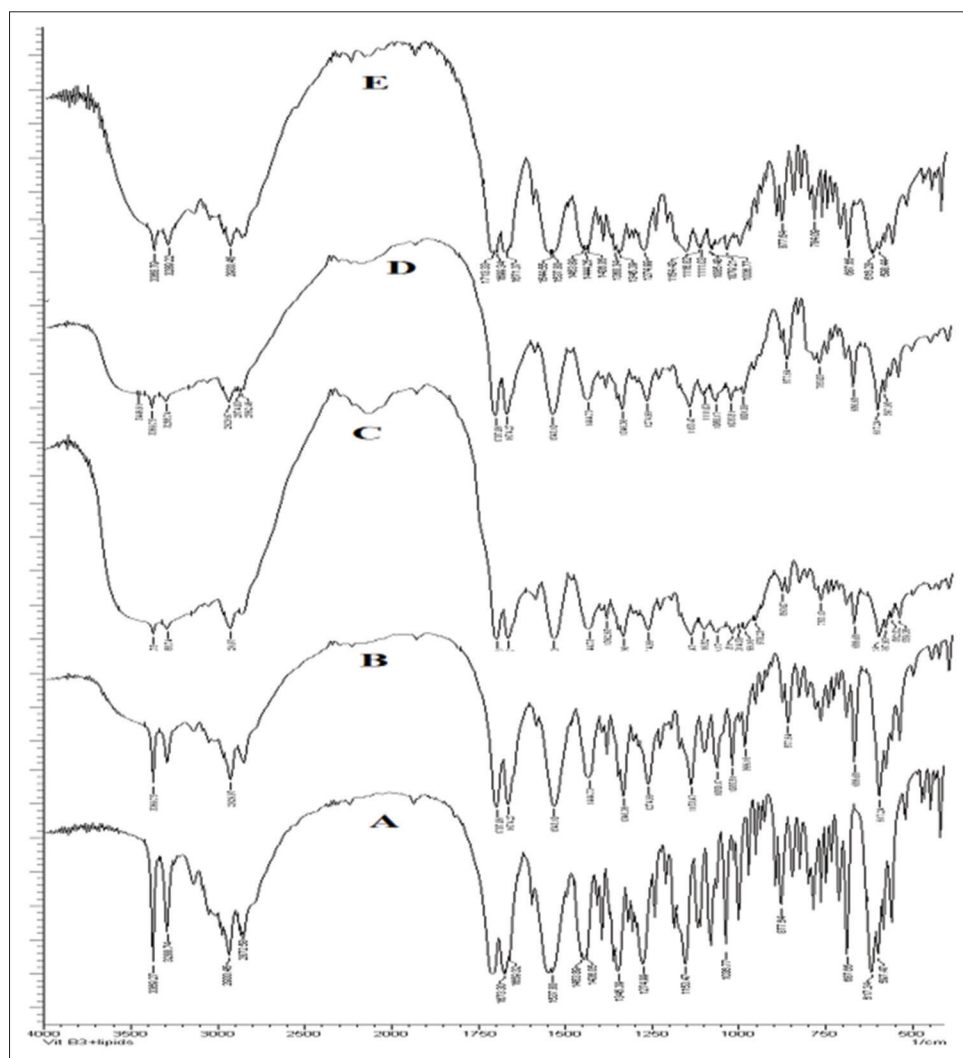


Fig. 1: Fourier transform infrared spectra of (a) glibenclamide pure drug, (b) glibenclamide + locust bean gum, (c) glibenclamide + gum ghatti; (d) glibenclamide + guar gum; (e) glibenclamide + MCC

gum/gum ghatti/guar gum/MCC) without any appreciable change in the position, attributing to the compatibility of drug-polymer. Hence, it can be inferred that drug can be used with the selected polymer without causing any instability in the formulation.

FT-IR studies of atorvastatin calcium

The position of peak in FT-IR spectra of pure atorvastatin calcium is compared with those in FT-IR spectra of atorvastatin calcium with excipients as shown in Fig. 2. FT-IR spectral analysis indicates that the absorption peaks present in the IR spectra of atorvastatin calcium were also found in the physical mixtures of the excipients used (MCC/locust bean gum/sodium starch glycolate/croscarmellose sodium/SLS/sodium bicarbonate/PVP K-30) without any appreciable change in the position, attributing to the compatibility of drug-polymer. From the obtained results, it can be inferred that drug can be used with the selected polymer without causing instability in the formulation.

DSC studies of glibenclamide

From Fig. 3, it was evident that glibenclamide showing a sharp endothermic peak at its melting point, i.e., at 213.83°C, indicating its crystalline nature of the drug. On the other hand, the DSC thermographs of formulation BD-2, BD-8, and BD-13 showed identical peaks, to that of pure drug indicating the absence of chemical interaction between the drug and polymers.

DSC studies of atorvastatin calcium pellets

The thermal analysis of pure atorvastatin calcium API showed a sharp endothermic peak at its melting point, i.e., at 156.87°C, demonstrating its crystalline nature. On the other hand identical, but broad peaks were found on DSC analysis of formulations FR-7, FR-14, and FR-15, indicating no interaction of atorvastatin calcium with its polymers and excipients. DSC thermographs of atorvastatin calcium API, formulation FR-7, FR-14, and FR-15 are presented in below Fig. 4.

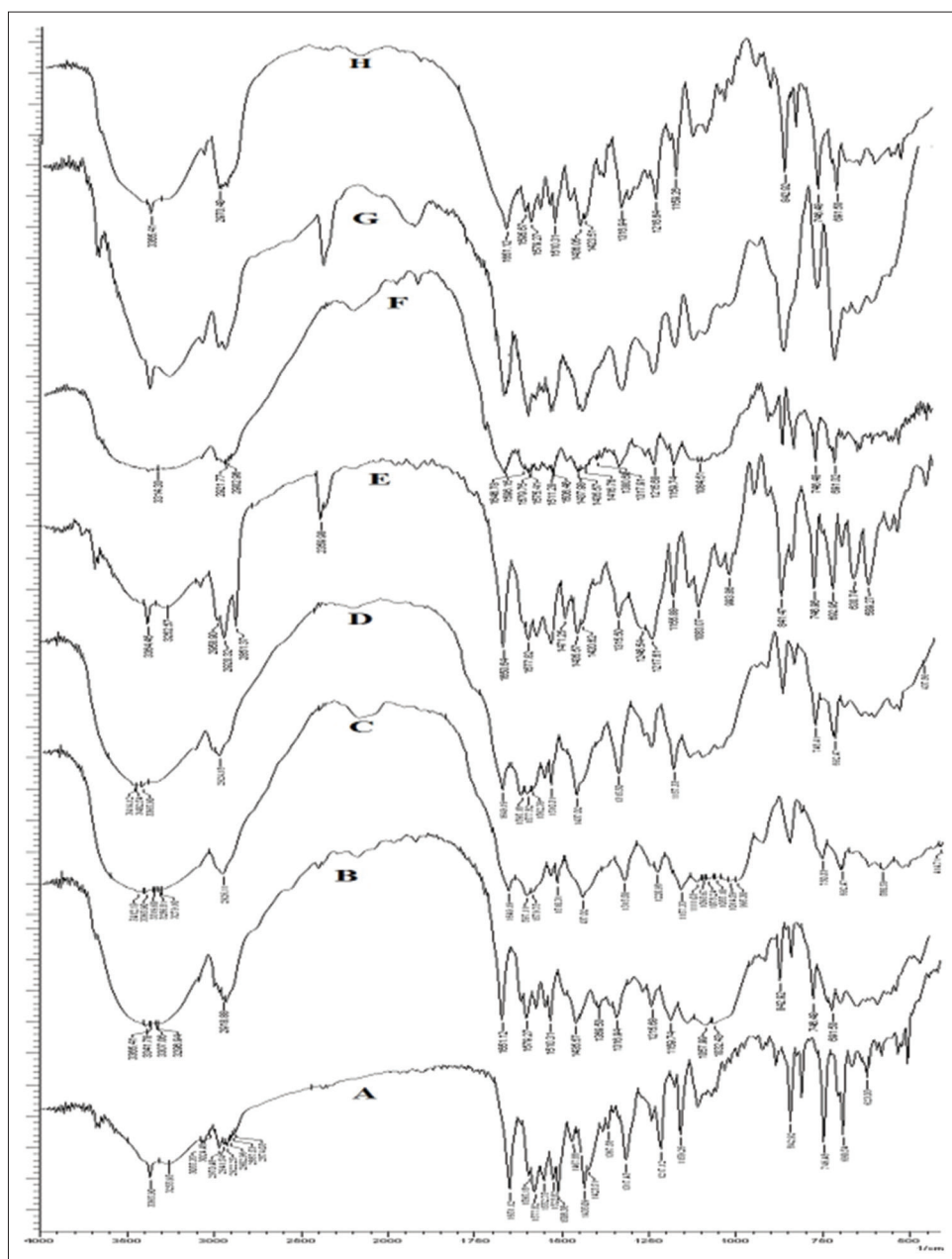


Fig. 2: Fourier transform infrared spectra of (a) atorvastatin calcium pure drug, (b) atorvastatin calcium + microcrystalline cellulose; (c) atorvastatin calcium + sodium starch glycolate; (d) atorvastatin calcium + croscarmellose sodium; (e) atorvastatin calcium + sodium lauryl sulfate; (f) atorvastatin calcium + locust bean gum; (g) atorvastatin calcium + sodium bicarbonate; (h) atorvastatin calcium + PVP K-30

Micromeritic properties of glibenclamide

The micromeritic properties of different batches of sustained release glibenclamide pellets are summarized in Table 3. The properties such as average size (mm), angle of repose, Carr's index, and tapped density explain that no respective change differences among all batches. Angle of repose having a range of 23.20 ± 1.35 – $27.35 \pm 1.58^\circ$ which indicates good flow properties of pellets. The bulk density results of different batches of sustained release glibenclamide pellets indicated closely packed and settled nature because of narrow particle size distribution.

Micromeritic properties of atorvastatin calcium

The micromeritic properties of different batches of immediate release atorvastatin calcium pellets are summarized in below Table 4. The properties such as average size (mm), angle of repose, Carr's index, and tapped density did not show any significant differences among

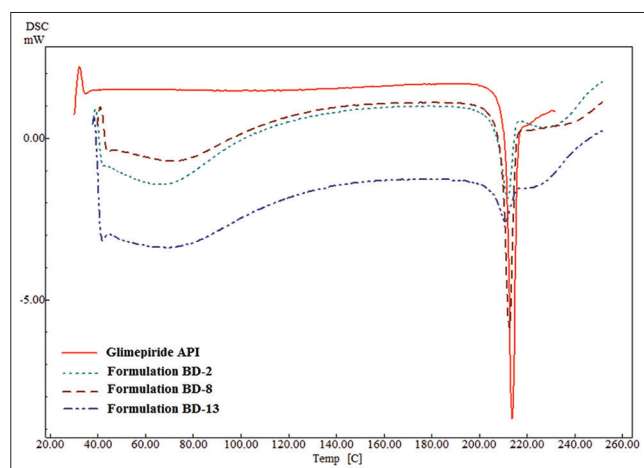


Fig. 3: Differential scanning calorimetry thermogram of glibenclamide API, formulation BD-2, BD-8, and BD-13

the batches. Angle of repose was found to be within the range of 24.19 ± 1.33 – $27.21 \pm 1.69^\circ$ indicating good flow properties of pellets.

SEM studies of glibenclamide pellets

From the SEM photographs (Fig. 5), it was observed that the sustained release glibenclamide pellets formulated using locust bean gum and gum ghatti/guar gum polymers exhibited spherical shape. The pellets consisting of locust bean gum alone and locust bean gum with gum ghatti polymer were of smoother surface, while pellets prepared using guar gum along with locust bean gum showed chapped surface.

SEM studies of atorvastatin calcium pellets

From the SEM photographs (Fig. 6), it was observed that the immediate release atorvastatin calcium pellets formulated using 1% w/w locust bean gum as binder and sodium starch glycolate/croscarmellose sodium exhibited spherical shape. The pellets consisting of locust bean gum and sodium starch glycolate were of smoother surface, while pellets prepared using locust bean gum and croscarmellose sodium showed chapped surface.

Evaluation of glibenclamide pellets and atorvastatin calcium pellets

Friability and pellet yield of glibenclamide

The results of friability test and pellet yield of the sustained release glibenclamide pellet formulations were summarized. The friability of the sustained release glibenclamide pellet formulations was found to be in the range of 0.32 ± 0.08 – $0.53 \pm 0.07\%$ results were in acceptable limits. The pellet yield was in the range of 76.9 ± 2.31 – $83.3 \pm 3.16\%$ in successive unit operations when 100 g of bulk material is being used.

Friability and pellet yield of atorvastatin calcium

The results of friability test and pellet yield of the immediate release atorvastatin calcium pellet formulations were summarized. The friability of the immediate release atorvastatin calcium pellet formulations was found to be in the range of 0.44 ± 0.02 – $0.54 \pm 0.05\%$ results were in acceptable limits. The pellet yield was in the range of

Table 3: Characteristics of sustained release glibenclamide pellets formulation

Formulation code	Average size (mm)*	Angle of repose** θ°	Granule density (g/cm^3) **	Tapped density (g/cm^3) **	Carr's index (%)**
BD-1	1135±51	25.14±1.24	1.05±0.03	0.84±0.04	9.12±0.32
BD-2	1189±25	26.42±1.18	1.03±0.04	0.86±0.07	8.79±0.24
BD-3	1245±23	25.45±1.26	1.08±0.03	0.90±0.03	9.39±0.21
BD-4	1224±35	23.20±1.35	1.05±0.02	0.89±0.06	8.93±0.34
BD-5	1318±27	27.21±1.30	1.12±0.06	0.83±0.05	8.76±0.30
BD-6	1336±32	26.48±1.44	1.04±0.08	0.83±0.05	8.69±0.37
BD-7	1361±37	25.16±1.15	1.06±0.07	0.84±0.07	8.85±0.24
BD-8	1380±21	26.65±1.88	1.09±0.05	0.90±0.03	9.39±0.36
BD-9	1262±73	26.83±1.28	1.08±0.06	0.89±0.04	8.93±0.29
BD-10	1294±42	26.25±1.36	1.16±0.04	0.83±0.05	8.76±0.38

*Standard deviation n=3

Table 4: Characteristics of immediate release atorvastatin calcium pellet formulations

Formulation code	Average size* (mm)	Angle of repose** θ°	Granule density** (g/cm^3)	Tapped density** (g/cm^3)	Carr's index** (%)
FR-1	1127±41	25.14±1.52	1.09±0.04	0.79±0.05	8.11±0.42
FR-2	1177±30	26.42±1.24	1.04±0.02	0.81±0.04	8.19±0.49
FR-3	1255±43	25.25±1.46	1.07±0.08	0.80±0.05	9.21±0.44
FR-4	1274±45	24.19±1.33	1.03±0.06	0.75±0.05	8.01±0.18
FR-5	1371±50	27.21±1.69	1.05±0.04	0.76±0.04	8.24±0.80
FR-6	1204±57	26.18±1.27	1.05±0.06	0.83±0.03	9.09±0.41
FR-7	1140±27	25.06±1.29	1.08±0.03	0.80±0.05	8.21±0.61
FR-8	1208±32	26.65±1.47	1.06±0.05	0.84±0.02	8.54±0.54
FR-9	1219±44	26.55±1.24	1.02±0.04	0.81±0.04	8.05±0.87
FR-10	1244±37	26.04±1.35	1.07±0.06	0.85±0.05	8.46±1.03

*Standard deviation n=3

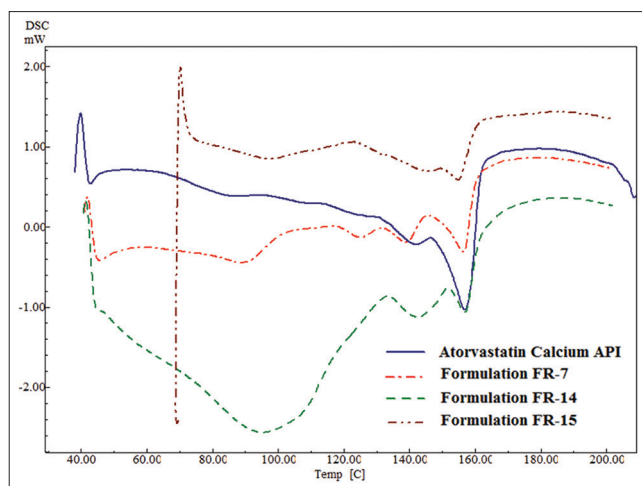


Fig. 4: Differential scanning calorimetry thermogram of atorvastatin calcium API, formulation FR-7, FR-14, and FR-15

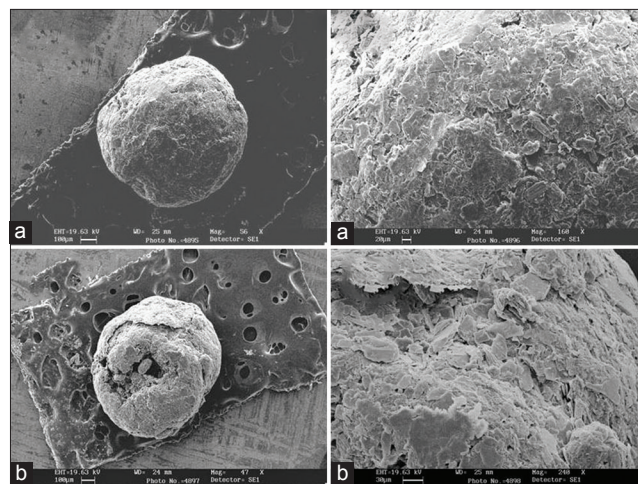


Fig. 6: Scanning electron microscopic of immediate release atorvastatin calcium pellets: (a) formulation FR-7 and (b) formulation FR-14 at different magnifications

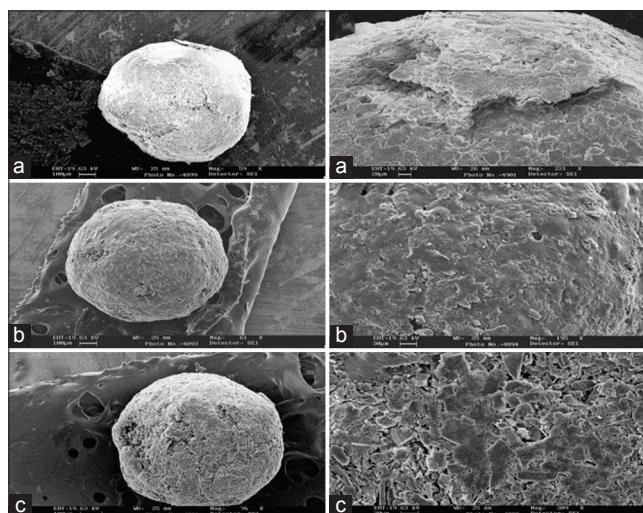


Fig. 5: Scanning electron microscopic of sustained release glibenclamide pellets: (a) formulation BD-2, (b) formulation BD-8, and (c) formulation BD-13 at different magnifications

74.8±1.21–82.4±1.49% in successive unit operations when 100 g of bulk material is being used.

Drug loading and encapsulation efficiency glibenclamide

Percentage of drug loaded and percentage of drug encapsulated were found to be within the ranges of 2.28±0.23–2.55±0.15% and 85.40±1.21–95.51±1.23%. Results suggested that as the concentration of polymer increased there was an increase in drug loading and encapsulation efficiency. Thus, from the results, it can be inferred that glibenclamide was distributed uniformly in the pellets with acceptable deviations.

Drug loading and encapsulation efficiency of atorvastatin calcium pellets

Percentage of drug loaded and percentage of drug encapsulated were found to be within the ranges of 3.06±0.18–3.80±0.31% and 87.15±0.96–95.19±1.76%. Thus, from the results, it can be inferred that atorvastatin calcium was distributed uniformly in the pellets with acceptable deviations.

Pellets disintegration time atorvastatin calcium

Atorvastatin calcium pellets formulated using sodium starch glycolate as superdisintegrant had shown high disintegration when compared

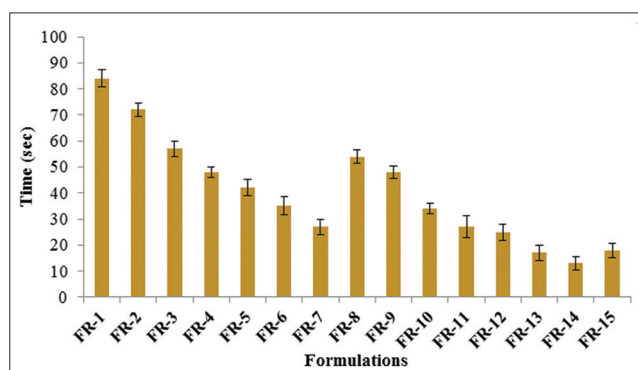


Fig. 7: Disintegration time of different formulations of immediate release atorvastatin calcium pellets

to croscarmellose sodium. As depicted in Fig. 7, results suggested that higher disintegration time is due to swelling of pellets when it comes to contact with aqueous medium and forming a gel, forming pores in the pellets prolonging the disintegration. While pellets formulated by using croscarmellose had shown enhanced disintegration by rapid swelling caused by capillary action.

In vitro dissolution studies

The sustained release glibenclamide and immediate release atorvastatin calcium pellets were mixed together in fixed-dose combination (FDC) and filled in a single hard gelatin capsule and evaluated for *in vitro* drug release study. As depicted in Figs. 8a-c and 9a and b, results suggested that as the concentration of polymer increases in glibenclamide pellets led to a significant decrease in dissolution of drug up to 12 h. While for atorvastatin calcium pellets, containing superdisintegrant had shown the complete release of drug within 45 min.

CONCLUSION

There is a strong prophylactic and clinical need to develop novel fixed combination delivery systems for the patients suffering from dyslipidemia in type-II diabetes mellitus for improvement of glucose tolerance with desired characteristics such as better therapeutic efficacy, convenience, reduced pill burden, and simplified administration of regimens with cost-effective medication. The developed FDC dosage forms, namely MUPSs, have demonstrated their superiority in both retarding the drug release of glibenclamide and immediate release of atorvastatin calcium. Thus, the study demonstrates that the developed FDC systems have a great appeal for the convenient treatment of

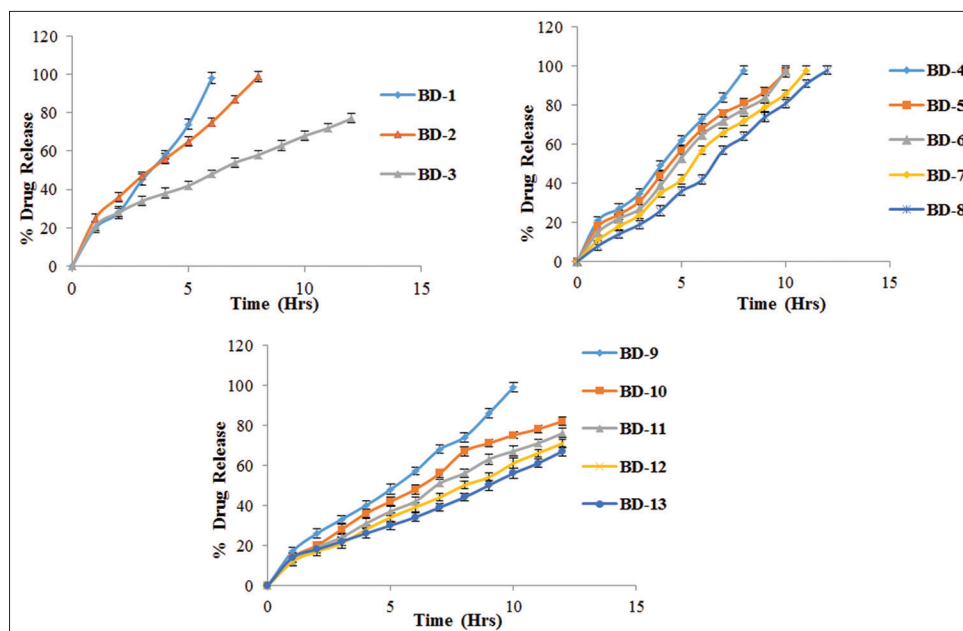


Fig. 8: (a-c) *In-vitro* dissolution profile of sustained release glibenclamide pellets (BD1-BD13)

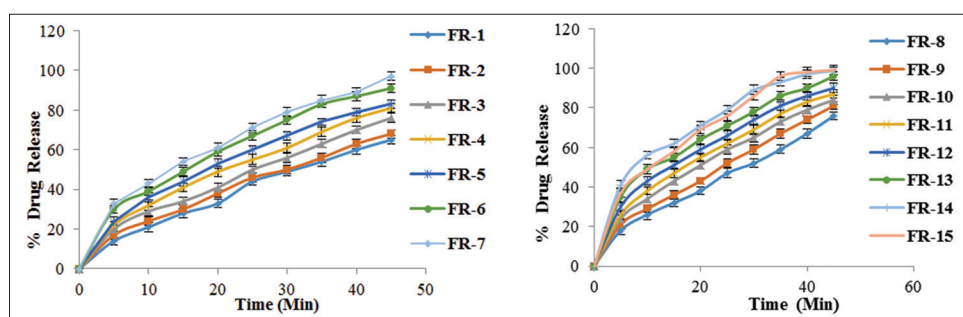


Fig. 9: (a and b) *In vitro* dissolution profile of atorvastatin calcium pellets (FR1-FR13)

diabetic dyslipidemia that may be explored in improving the limitations of existing monotherapy drug delivery systems.

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AUTHORS' CONTRIBUTIONS

The authors are a faculty in division of pharmaceuticals and the work contributed on faculty development programme in the institution.

CONFLICTS OF INTEREST

The author confirms that this article content has no conflicts of interest.

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