ORIGINAL ARTICLE

Development and characterization of anti-diabetic liposomal formulation

Anasuya Patil, K. Florence Rachel

Department of Pharmaceutics, KLE College of Pharmacy, Bengaluru, Karnataka, India

Abstract

Introduction: Diabetes is a chronic health condition that affects how human body turns food into energy. It is most commonly occurring disease. Liposomal technology has been employed for the flourishing encapsulation of various drug molecules and oral route of administration is much more convenient. The main aim was to develop and characterize oral antidiabetic liposomal formulation. Further, prepared liposomal formulations evaluated for particle size, % drug release, differential scanning calorimetry (DSC), Fourier transform infrared, *in vitro* release, release kinetics model, animal studies, and experimental data subjected to statistical testing using one-way analysis of variance followed by Dunnett test were carried out. **Materials and Methods:** The liposomal formulations were formulated using thin-film hydration technique. Sitagliptin liposomal formulations (F1-F6) were prepared by employing soy lecithin and cholesterol in varying concentration. **Results:** *In vitro* release of different antidiabetic liposomal formulations was performed and observed through cellophane membrane using Franz Diffusion cell. The finalized liposomal formulation (F6) showed better release. To check the antihyperglycemic activity was performed using oral glucose tolerance test using Sprague Dawley (SD) rats for the optimized formulation. Finalized formulation (F6) elicited the better in-vitro release 87.85% at 8 h in comparison with the pure drug release was initiate to be 59.57%. Particle size distribution and zeta potential were found to be 40 nm as well as 40 mV, respectively. *In vitro* release kinetic models were shown that it follows first-order kinetics and high regression coefficient r^2 value was 0.975. The DSC thermogram of sitagliptin was found 221.7°C. The DSC thermogram of physical mixture of cholesterol along with soy lecithin was found to be 42.1°C and 220.3°C. DSC analysis confirmed that there was negative interaction between the drug and excipients. **Discussion and Conclusion:** It would be concluded that antidiabetic liposomal formulation using sitagliptin as drug, soy lecithin and cholesterol as polymer can be formulated thereby can enhance oral bio-availability. The optimized formulation (F6) has shown best formulation based on the *in vitro* drug release. *In vivo* studies for the optimized formulation (F6) observed that sitagliptin was found to be more potent than sitagliptin marketed formulation and activity was persisted till 4 h.

Key words: Cholesterol, Liposomal formulation, Sitagliptin, Soy lecithin, Thin-film hydration technique

INTRODUCTION

Diabetes is a condition where the level of blood glucose is too high. Blood sugar is the main supply of energy which comes from the food. A hormone produced by the pancreas called insulin which releases glucose by consuming food and utilized as energy. When the production of insulin is inadequate and inappropriate usage of insulin stays in blood. This condition is called diabetic condition. Too much blood content in the blood causes severe health problems. Type 1 diabetes is also known as juvenile onset diabetes. People in young adult or teenage years usually develop Type 1 diabetes. Roughly, 10% of diabetes will be diabetes Type 1. These types of patients are suggested to take hormone medications. They should uphold the appropriate glucose levels. They should go for regular blood checkup and should follow a special diet. Type 2 diabetes: In this condition, the cells loose contact with the body to respond for Insulin. About 90% of all cases in the worldwide are adult onset diabetes. These Type 2 diabetes symptoms are able to control in the reduction of weight or having a healthy diet or doing work out.[1-5] The Type 2 diabetes is commonly called as non-insulin dependent diabetes disease and it gets worse so that the patient will

Address for correspondence:
K. Florence Rachel, KLE College of Pharmacy,
II Block Rajajinagar, Bengaluru, Karnataka, India.
E-mail: patilanusuy209@gmail.com

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possibly end up taking insulin in the form of tablet. An obese person has more risk of getting this type of diabetes than healthy weighted people. Gestational diabetes is mostly seen in weighty women because of their higher insulin production in their blood. Treatments of gestational diabetes will occur during pregnancy. Pregnant women are able to control this condition by performing exercise and following healthy diet. During childbirth, unrestrained gestational diabetes can lift-up the possibility of complications. Hence, they must take some category of blood glucose controlling medications.

Diabetes mellitus (DM) generally referred as group of metabolic disorders. Symptoms such as urinary disorders rise in thirst and hunger. Unprocessed diabetes can cause many complications like ketoacidosis, hyperglycemic state or death is the complications of sensitive diabetes. Serious long-term complications include cardiovascular disease, stroke, chronic renal failure, foot ulcers, and harmful to eyes.

Diabetes Analysis

Normally, diabetes is diagnosed using urine sample which helps in finding out whether glucose is inappropriate level in the urine. At the age of 45 years and above, diabetes testing was recommended for overweight patients. Under the age of 45 years, hypertension and high cholesterol levels are the risk factor.

Three Tests for Diagnosing Diabetes

In the period of fasting blood samples were collected after 8 h. This test is called fasting plasma glucose. It is determined at the level equal or above 126 mg/dL. Before 2–3 months, the average blood glucose level can be measured with the help of glycosylated hemoglobin (HbA1c). Oral glucose tolerance testing is an examination used to measures the levels before 2 h following without a sweet drink.[6-8]

Diabetes Urine Test Diagnosing

For diabetes diagnosis, urine tests are not required. Blood tests are sensitive when compared with urine tests. Hence, blood tests are rather used instead. In Type 1 diabetes, urine sample might be used for testing of ketones. Oral dosage forms for the treatment for Type 2 diabetes: An orally active dosage forms are effective in treating diabetes on regular basis in maintaining low blood glucose level, lowering glucose level to the aim of an HbA1c below 7.0%, average glucose reading is 8.3–8.9 mmol/L (around 150–160 mg/dL).

There are numerous customs to transport drugs hooked on the body such as oral, transdermal, pulmonary, mucosal lamina, and parenteral among these deliveries, the oral delivery is commonly accepted. In oral drug delivery, many scientific challenge and go through technologies are required to produce novel dosage forms raising drug delivery to higher level. A few are self-emulsifying systems, solid self-nano emulsion, polymeric micelles, spray freezing, pH controlled systems, time overdue system, osmotic pumps, and pro-drugs. Oral route of administration is best preferred when compared with the other routes.

As oral drug delivery is easy, mainly suitable, safest, non-invasive, and generally cost-effective, it continues to be superior route of administration and developers are seeking ways to include various technology in oral formulations; even minute improvement in drug delivery technology can make significant differences in enhancing patient compliance and drug bioavailability. Various challenge related with Oral Route Administration; difficulty in swallowing the pill, painful and indigestible drugs are not agreed by oral route, gastrointestinal damage of unstable molecules, leisurely commencement on action, very slight manage excess release of the drug; absorption of drug; non-specific delivery site and adverse effects and low level of macromolecular absorption; and absorption of drugs may be affect by foodstuff in the stomach.

Liposomes

Liposomes are imitative from two Greek words Lipos means fat and Soma means body. Liposomes are tiny artificial vesicles of sphere-shaped that can be produced from cholesterol and natural phospholipids. Liposomes property differs with lipid structure, surface charge, size, and the way of preparation. Phospholipid spontaneously forms closed structures when they are hydrated in aqueous solution. Depending on the nature of drugs, vesicles have one or more phospholipid bilyer membranes that can transfer lipid drugs. Liposomes are specific as spherical vesicles with particle sizes range from 30 nm to numerous micrometers. Liposomes are broadly used as carriers for numerous molecules in cosmetic and pharmaceutical industries. It can also entrap mutually hydrophilic and hydrophobic compounds. Liposomes have got a probable benefit to encapsulate deliquescent in addition to aqua-phobic medicament in addition to target them to the vital contaminated spot in the body.

Liposomal technology was used for the flourishing encapsulation of various drug molecules such as pacitaxel, acyclovir, tropicamide, cyclosporine, and chloroquine diphosphate. Advantages: It protects the encapsulated drug from external environment, liposomes are biocompatible, completely bio-degradable, non-hazardous, and non- immunogenic, it is suitable for the delivery of hydrophilic, hydrophobic, and amiphilic drugs, and improved efficacy, therapeutic index, and stability by encapsulation.

The main aim of the present work was to develop and characterize the liposomal formulation with antidiabetic drug which is used for the treatment of DM to acquire enhanced therapeutic effect.

The Objectives of the Current Work

The objectives of the study are as follows: To study the compatibility of drugs and the excipients, to prepare the sitagliptin liposomal suspension to improve permeability of the drug, to evaluate the liposomal suspension for: Fourier transform infrared (FT-IR) studies, determination of particle size, determination of zeta potential, estimation of entrapment efficiency, *in vitro* release profile, release kinetics, FT-IR studies, estimation of differential scanning calorimetry (DSC), statistical analysis using one-way analysis of variance (ANOVA), and to perform *in vivo* studies.

MATERIALS AND METHODS

Materials

Sitagliptin is procured from Yarrow chemical Research Private Limited. Soy lecithin is purchased from Sigma Aldrich Private Limited. Cholesterol is procured from Yarrow chemical Research Private Limited. Potassium di-hydrogen orthophosphate is purchased from Hi-Media Laboratory Private Limited. Sodium hydroxide is purchased from Hi-Media Laboratory Private Limited. Moreover, chloroform is procured from Sisco Laboratories Private Limited. All excipients and chemicals used were of pharmaceutical grade.

Methods

Determination of melting point

A lesser amount of quantity of Sitagliptin was initiated into the capillary tube and it was fixed to the stem of thermometer.[5,6] Thermometer was placed into the Thiele's tube filled with liquid paraffin. Side limb of the Thiele tube was excited and temperature was experimented at which the melting begin and complete.

Compatibility studies

FT-IR spectroscopy was passed out to ensure the compatibility among the drugs and the excipients. FT-IR spectrum of the drug and polymer was compared with the standard FT-IR spectra.

Standard calibration curve of sitagliptin

50 mg of sitagliptin was exactly weighed, dissolved in pH 7.4 phosphate buffer taken in 50 ml volumetric flask and the solution be prepared up to the spot with buffer to get the concentration (1000 µg/ml) was taken in a 10 ml volumetric flask to get the concentration (100 µg/ml) and diluted with phosphate buffer to get the concentration over the range of 2–10 µg/ml. The absorbance of dilutions was considered under the wavelength region of 267 nm using ultraviolet (UV) spectrophotometer.

Preparation of anti-diabetic liposomal suspension

A constant weight of 50 mg of cholesterol and 300 mg of phosphotidylcholine was weighed and dissolved in 20 ml of chloroform then 6.5 g of sitagliptin were added in 50 ml of phosphate buffer pH 7.4. The lipid content processed for rotary evaporator at 45°C until the lipid layer was formed around the round bottomed flask when the chloroform was evaporated without a vacuum. The buffer solution was added in round bottomed flask along with glass beads and continued again for 20 min until the lipid layer is removed. The layer was removed when the glass beads were in contact with the thin layer and the buffer solution. Finally, the suspension was formed and stored in refrigerator around at +4°C for 24 h until the maturation of liposomes. Further, evaluation studies were subjected to the anti-diabetic liposomal formulation.

Estimation of liposomal formulations

The prepared antidiabetic liposomal suspension was evaluated for the following parameters: Determination of particle size, estimation of zeta potential, estimation of entrapment efficiency, estimation of FT-IR studies, estimation of DSC, *in vitro* release, *in vitro* release kinetics, and *in vivo* studies.

Determination of Particle size

The developed formulation was subjected under microscope under ×100 magnification.

Estimation of entrapment efficiency

The prepared formulations were centrifuged for 30 min at 15000 rpm. Aliquot of 0.5–1 ml supernatant solutions was diluted with phosphate buffer pH 7.4–25 ml. By the quantity of drug in the supernatant liquid quantified by UV spectra at 267 nm.

In vitro release study

In vitro release was passed out for liposomal preparations (F1-F6) and the standard drug. Franz diffusion cell containing cellophane membrane was used. The donor compartment was full with 1 ml of the formulation soaking in phosphate buffer pH 7.4 at room temperature beneath slow magnetic stirring. At normal time intervals,

Table 1: FT-IR studies of sitagliptin

Functional group	Reported (cm^{-1})	Observed (cm^{-1})
C=O	1600–1700	1644.02
N-H	3480–3520	3322.75
C=N	1020–1250	1131.05
C-F	800–1276	1212.04
C-H	2950–3100	2961.16
		2976.59
		3044.09

FT-IR: Fourier transform infrared

Table 2: Reported and observed IR frequencies of formulation (F6)

Sl. No	Functional groups	Observed Peak (cm^{-1})	Standard peak (cm^{-1})
1	C-N	1026.87	1020–1030
2	C-C	1075.36	1070–1083
3	C=O	1158.08	1150–1158
4	N-O	1520.34	1500–1550
5	C=C	1635.86	1630–1658
6	N-H	3313.09	3300–3320

IR: Infrared

Table 3: FT-IR frequencies for drug and polymer

Sl. No.	cm^{-1}	%T
	3366.14	21.59
	3322.75	21.43
	2958.27	21.07
	2922.59	17.32
	2854.13	18.11
	1736.58	21.07
	1637.27	22.34
	1522.52	21.11
	1465.63	21.04
	1371.14	22.75
	1272.79	21.54
	1215.90	20.66
	1168.65	21.20
	1137.80	21.27
	1044.26	21.98
	1010.52	21.73
	844.66	21.27
	808.02	23.63
	727.03	24.81

FT-IR: Fourier transform infrared

Table 4: Entrapment efficiency, pH value, and clarity of the prepared sitagliptin liposomal suspension

Formulation	Entrapment efficiency (%)	pH	Clarity
F1	92.55	7.02.0	Clear
F2	94.20	7.01.0	clear
F3	85.93	7.03.0	Clear
F4	89.35	6.09.0	Clear
F5	91.35	7.04	Clear
F6	95.22	7.50	Clear

1 ml of aliquot was withdrawn from receptor chamber throughout the sampling port and immediately replace with same volume of fresh buffer solution. Aliquot was diluted and it was determined using UV spectrometer at 267 nm.

Table 5: Kinetic values obtained from *in vitro* release data of finalized formulation F6

Kinetic models	Slope (n)	Rate constant	Regression coefficient
Zero order	2.673	+2.265	0.961
First order	10.13	+1.278	0.975
Korsmeyer-Peppas	0.687	–0.363	0.957
Higuchi	0.086	–6.352	0.908

Table 6: Formulae for calculating ANOVA

Analysis of Variance(ANOVA)

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Squares (MS)	F
Within	$SS_w = \sum_{j=1}^{k}\sum_{j=1}^{l}(X-\bar{X}_j)^2$	$df_w = k-1$	$MS_w = \frac{SS_w}{df_w}$	$F = \frac{MS_b}{MS_w}$
Between	$SS_b = \sum_{j=1}^{k}(\bar{X}_j-\bar{X})^2$	$df_b = n-k$	$MS_b = \frac{SS_b}{df_b}$	
Total	$SS_t = \sum_{j=1}^{n}(\bar{X}_j-\bar{X})^2$	$df_t = n-1$		

Release kinetics

The release data were subjected to a choice of kinetic models to determine its mechanism and its model.

FT-IR spectroscopy

FT-IR range of the drug, individual polymer, and physical blend of the drug and polymer were recorded by FT-IR spectrophotometer using Jasco 460 plus model.

Estimation of DSC

DSC of the sitagliptin and physical combination of soy lecithin plus cholesterol was performed using the instrument called Perkin Elmer 4000.

Statistical analysis using one-way ANOVA

Statistical analysis was performed using one-way ANOVA followed by Dunnett test through GraphPad Prism5 software by taking the particle size range for all the formulations F1-F6.[3,5,20]

In vivo studies

To determine the antidiabetic activity of test sample for streptozotocin-induced diabetes followed by oral glucose tolerance test (OGTT) in rats using SD rats given orally in a dose of 2 g/kg dose for 1 day.[18,19,21,22] In this study, animals were kept fasting overnight and given dose of 25 mg/kg by single injection IP. To inhibition

Table 7: Observed blood glucose levels at different time intervals

Group	Animal No.	Body weight in g	Blood glucose in mg/Dl					
			At 0 h	At 0.5 h	At 1 h	At 1.5 h	At 2 h	At 4 h
Glucose control	1	230	144	174	170	175	160	148
	2	225	141	162	163	171	172	165
	3	232	135	170	178	177	160	151
	4	218	146	168	194	184	190	178
	5	213	140	178	188	178	161	152
	6	221	138	172	178	169	188	176
Mean		223.2	140.7	170.7	178.5	175.7	171.8	161.7
SEM		3.0	1.6	2.2	4.6	2.2	5.7	5.4
Sitagliptin Marketed formulation	7	230	135	136	167	166	172	171
	8	220	144	162	168	160	167	165
	9	196	146	144	152	154	157	155
	10	223	145	158	162	165	161	163
	11	228	141	130	155	150	158	155
	12	220	138	131	155	158	163	165
Mean		219.5	141.5	143.5	159.8	158.8	163.0	162.3
SEM		5.0	1.8	5.6	2.8	2.5	2.3	2.6
Sitagliptin test sample	13	228	141	158	152	147	147	145
	14	230	138	145	144	140	144	146
	15	204	139	166	155	155	141	138
	16	214	141	153	151	147	148	140
	17	227	138	140	155	145	140	138
	18	217	143	141	138	140	138	136
Mean		220.0	140.0	150.5	149.2	145.7	143.0	140.5
SEM		4.1	0.8	4.2	2.8	2.3	1.6	1.7

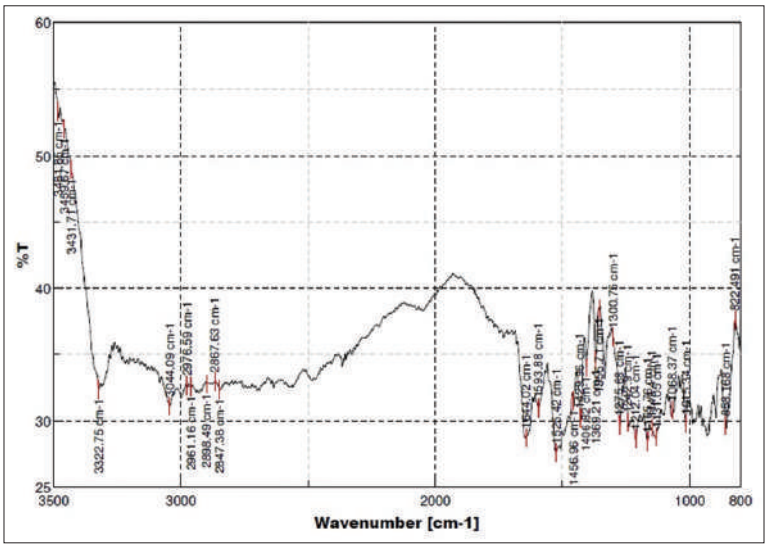

Figure 1: Infrared spectrum of sitagliptin

of dipeptidyl peptidase 4 inhibition, the rats were given glucose load to check for OGTT and finally glucose was measured.

Administering the test samples at the dose of 10 mg/kg body weight were given till 60 min. After administration, the rats of all groups were orally treated with 2 g/kg body weight dose

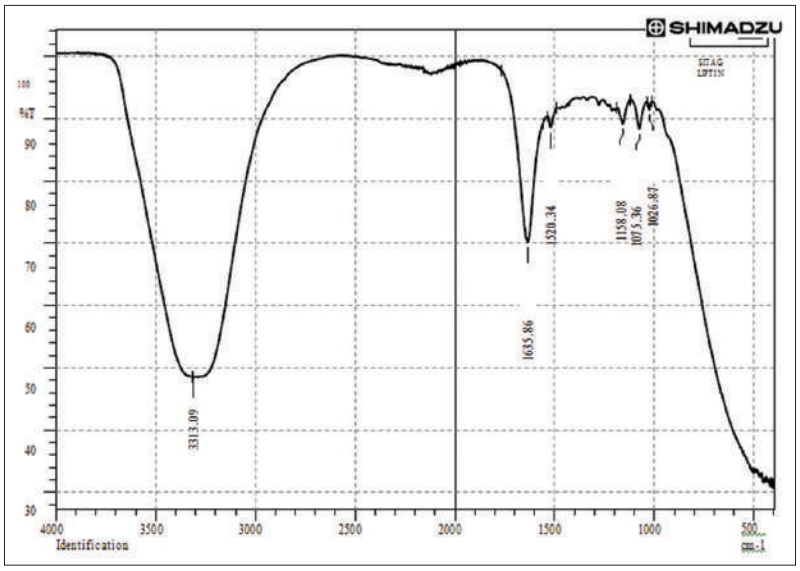

Figure 2: Infrared spectrum of formulation (F6)

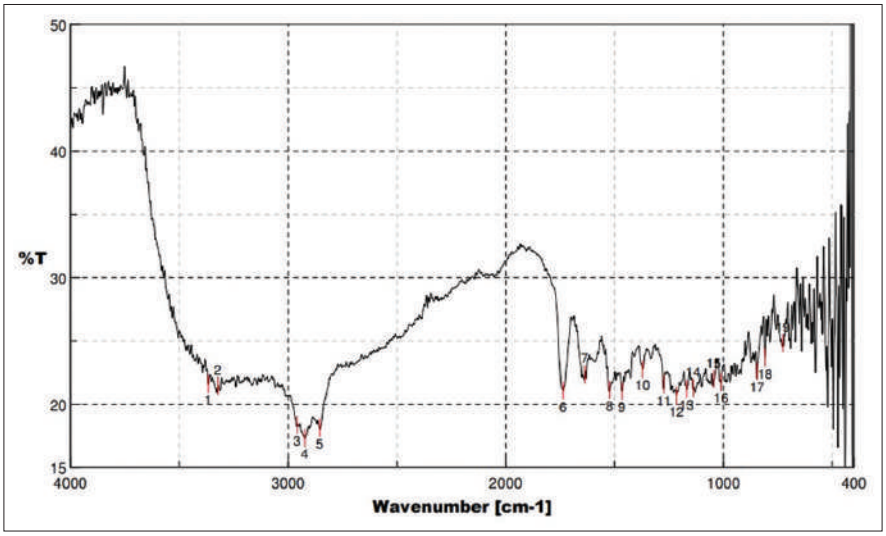

Figure 3: Fourier transform infrared spectrum of drug and polymer

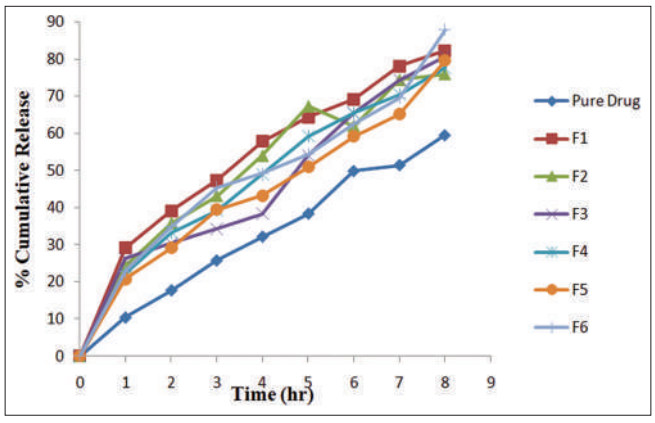

Figure 4: Cumulative percentage release of sitagliptin liposomal suspension (F1-F6) and pure drug

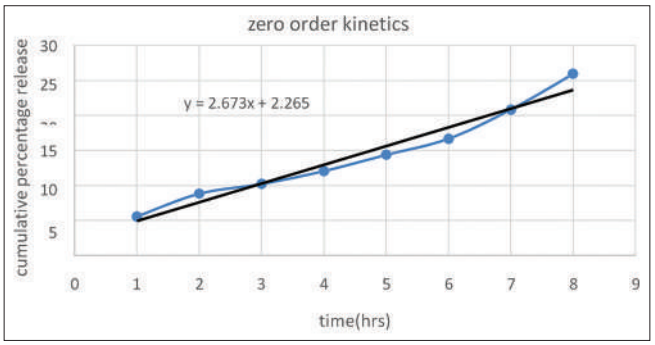

Figure 5: Plot of cumulative percentage release versus time for finalized formulation F6 (Zero-order plot)

of glucose. The blood samples were collected through orbital route at 0–120 min and blood-glucose level was measured.

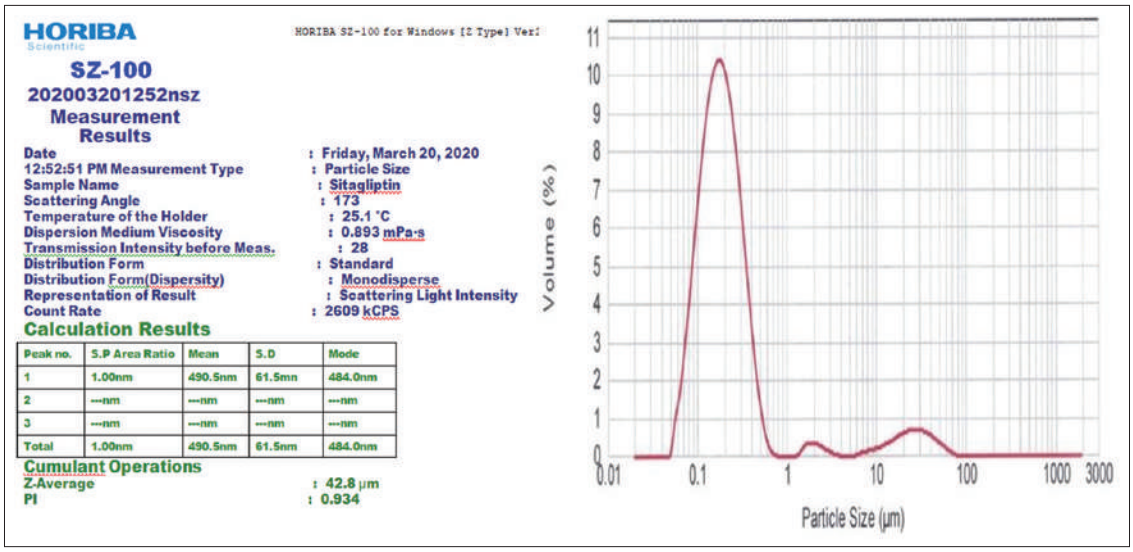

Figure 6: Particle size for optimized formulation

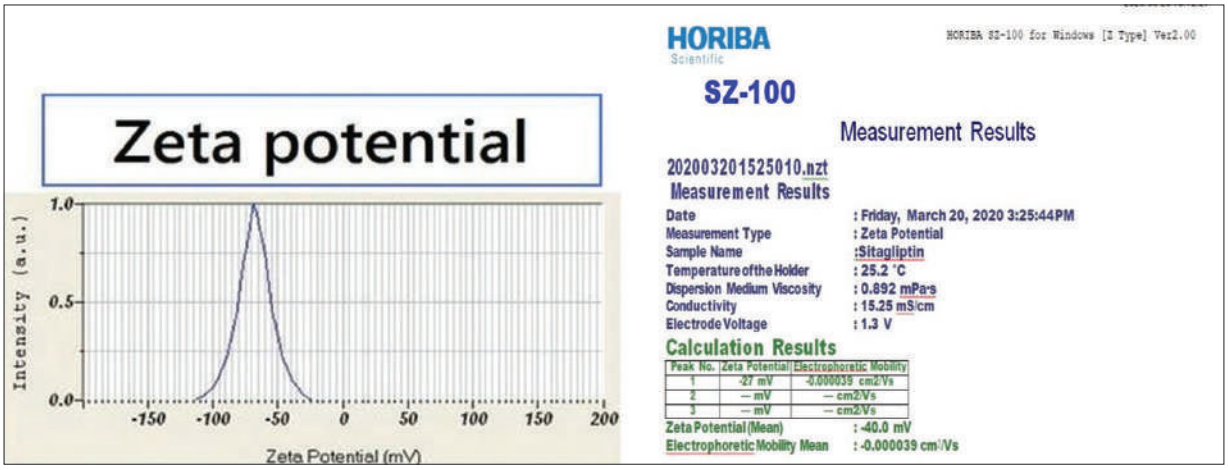

Figure 7: Zeta potential for the optimized formulation

RESULTS

Pre-formulation Studies

Determination of λ_{max} of Sitagliptin

UV-spectra of sitagliptin in pH 7.4 phosphate buffer showed the greatest absorbance at 267 nm using the instrument Shimadzu UV-1700 PC that is comparable to the value reported.

Determination of melting point

Melting point is prepared to categorize the drug and also to check the cleanliness. From the test, melting point of sitagliptin was established to be 212.66°C as reported in the literature, thus indicates the purity of the drug.

Saturation solubility of sitagliptin

The saturation solubility of sitagliptin performed in different solvents such as water, chloroform, and buffer pH 7.4. Solubility of sitagliptin in water, chloroform, ethanol, and phosphate buffer was established to be 9.99 ± 0.20 mg/ml, 2.4 ± 0.37 mg/ml, 9.77 ± 0.21 mg/ml, and 3.13 ± 0.26 mg/ml correspondingly.

Calibration curve of sitagliptin

Table shows the absorbance values of sitagliptin calculated at 267 nm with respect to the linear concentration range of 2–10 µg/ml of the drug in pH 7.4 phosphate buffer and plot shown linear standard curve with slope 0.9995 and regression coefficient value of 0.0887.

Compatibility study

The spectrum of the pure drug sitagliptin, individual polymer soy lecithin, and physical combination of the drug and polymer recorded by FT-IR spectrophotometer using Jasco 460 Plus are shown in Figures 1-3, which was compare with standard functional group frequencies of sitagliptin as shown in Tables 1-3.

Evaluation of antidiabetic liposomal formulations

The equipped formulations were subjected to different evaluation parameters such as appearance, pH, and entrapment efficiency and the results are as shown in Table 4.

Appearance

Transparency of the formulations was found to be cleared and acceptable.

Determination of particle size

Particle size for the optimized formulation was found to be 42.8 µm using Horiba S 100.

Measurement of pH

The pH of the formulation was found to be pH value 7.2–7.5 using the instrument Digisum electronics.

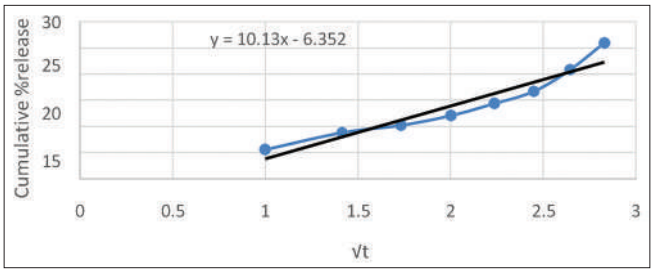

Figure 10: Plot of cumulative percentage release verses √t for finalized formulation F6 (Higuchi plot)

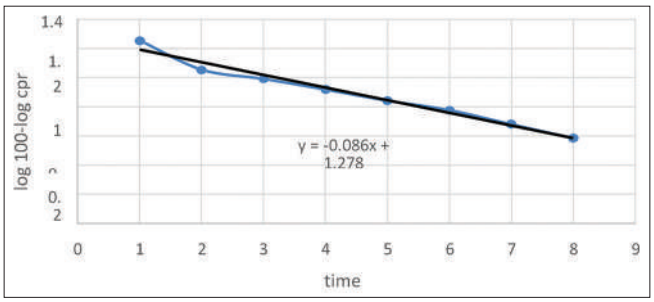

Figure 11: Plot of log cumulative percentage retained verses time for finalized formulation F6 (First-order plot)

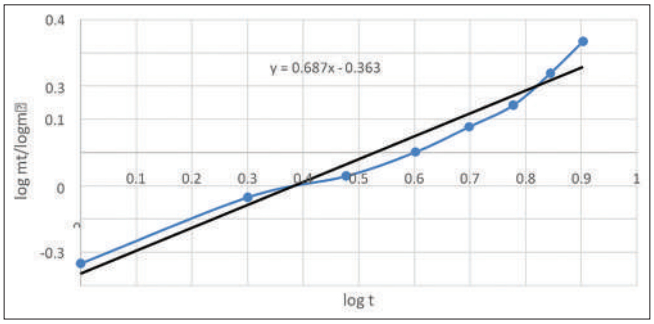

Figure 12: Plot of logMt/logM verses time for finalized formulation F6 (Korsmeyer-Peppas plot)

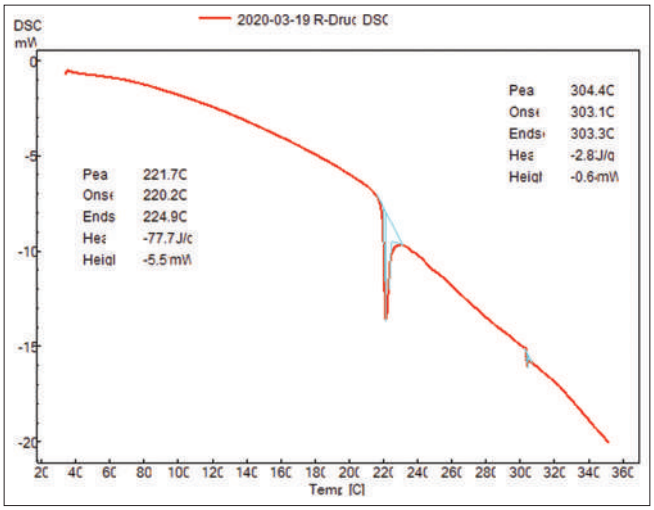

Figure 13: Differential scanning calorimetry thermogram of sitagliptin

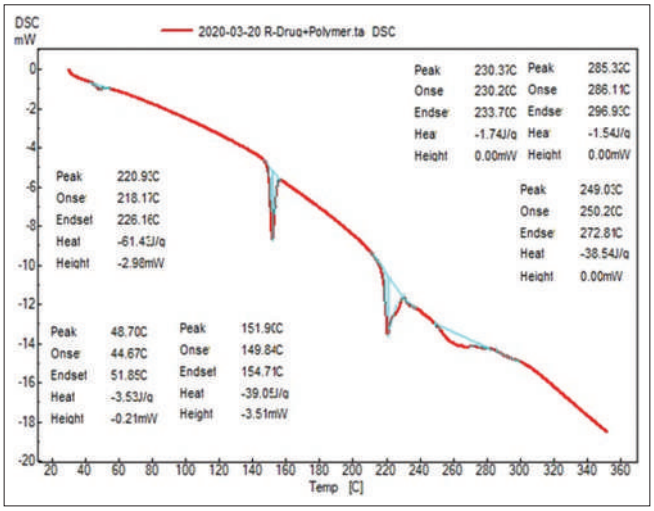

Figure 14: Differential scanning calorimetry thermogram of sitagliptin with polymer (soy lecithin)

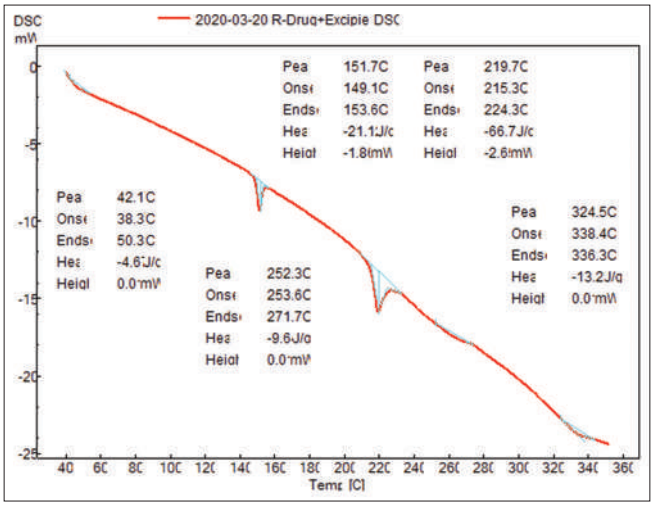

Figure 15: Differential scanning calorimetry thermogram of sitagliptin with cholesterol and phosphotidylcholine

PARTICLE SIZE

	F1	F2	F3	F4	F5	F6
	6.1	6.3	7.2	6.7	7.4	6.9
	7.2	6.8	6.7	6.4	6.1	7
	6.8	6.4	6.5	6.9	7.2	7.7
	6.3	6.7	6.8	7.4	6.4	7.9
	6.9	7.4	6.3	7.8	5.9	7.5
	6.7	7.1	6.1	7.2	6.3	7.3

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
F1	6	40	6.666667	0.162667
F2	6	40.7	6.783333	0.173667
F3	6	39.6	6.6	0.152
F4	6	42.4	7.066667	0.254667
F5	6	39.3	6.55	0.371
F6	6	44.3	7.383333	0.153667

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	3.129167	5	0.625833	2.962135	0.027341	2.533555
Within Groups	6.338333	30	0.211278			
Total	9.4675	35				

Figure 8: Statistical analysis using one-way analysis of variance

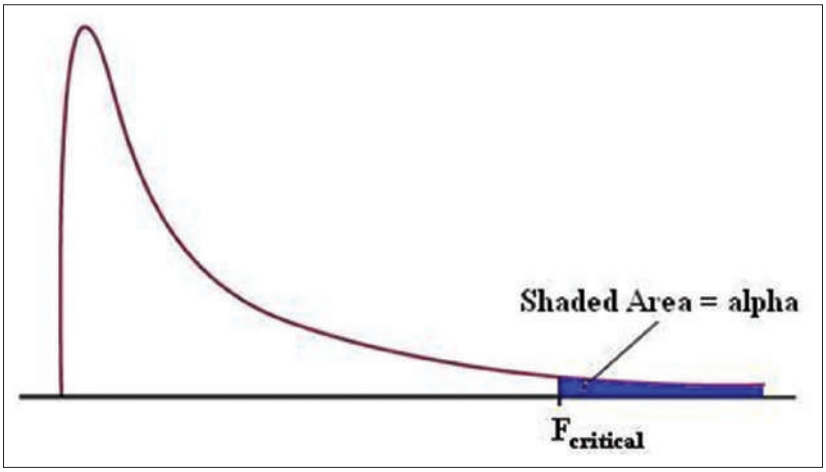

Figure 9: Logp value using one-way analysis of variance

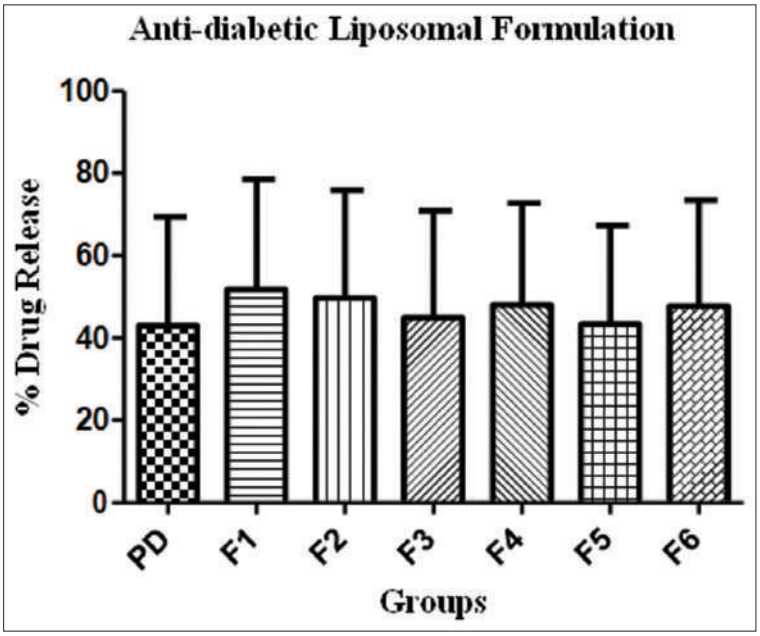

Figure 16: Statistical analysis of *in-vitro* release data using GraphPad Prism5

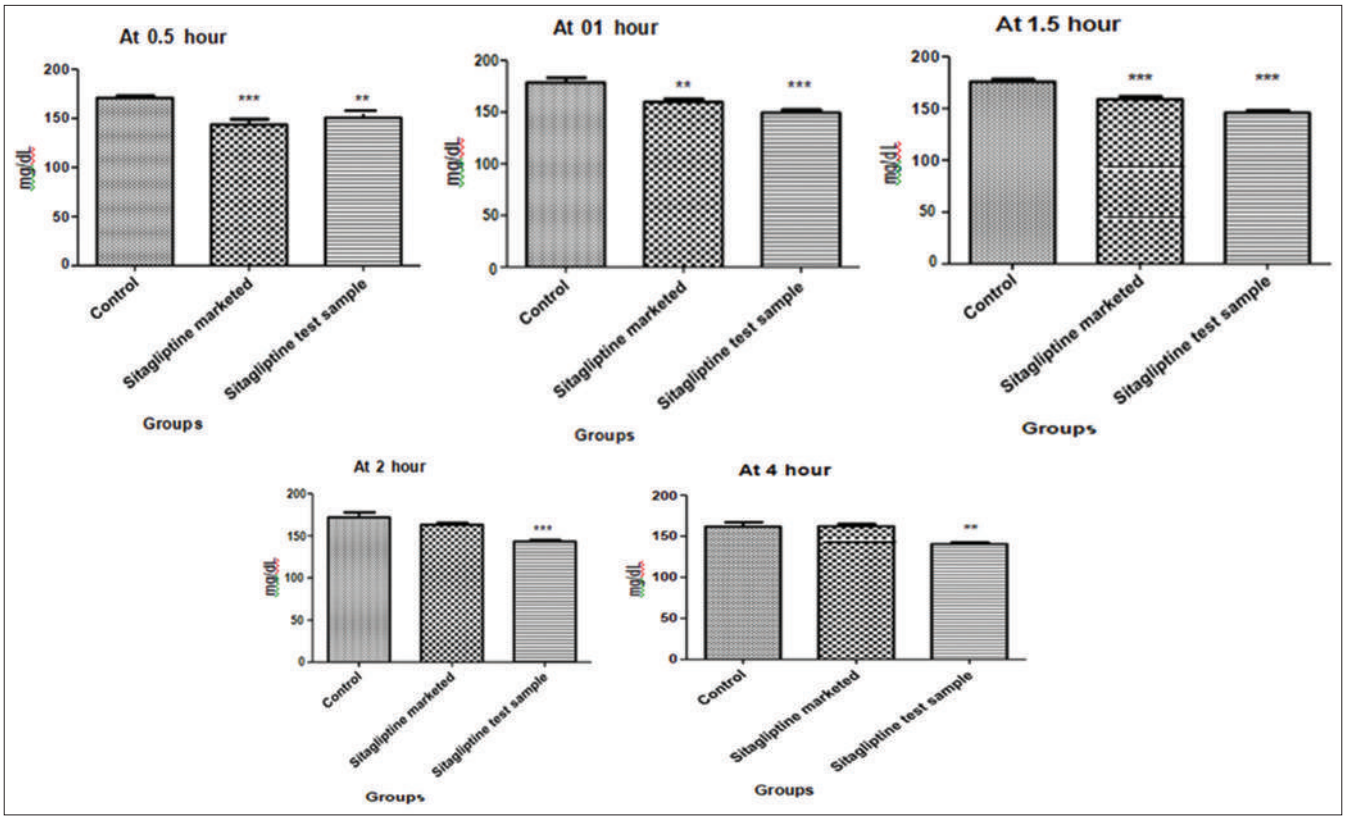

Figure 17: Graphs of sitagliptin test and control sample at different time intervals

Determination of zeta potential

Zeta potential for the optimized formulation was found to be −40mV using Horiba S-100.

Estimation of entrapment efficiency

The entrapment efficiency of all the formulation was found in the range of 85.93–95.22%.

In vitro release studies

The drug release information obtained for the formulations F1 to F6 and pure drug sitagliptin is tabulate in Table 5 and Figures 4 and 5 shows the plot of % Cumulative Drug Release verses time for all formulations. The sitagliptin liposomal suspension is observed in *in vitro* release studies and carried out using pH PBS as the dissolution media.

Particle size and zeta potential

The mean particle range of the F6 formulation was initiated to be 40 nm and the zeta potential of the same was found to be 40 mV in the Figures 6,7,8,9 which indicates that the positive charges on the particles prevents aggregation and, therefore, the stability of the formulation is maintained.

Selection of kinetic model

In vitro release data were subjected for several kinetic models: Zero-order kinetics, first-order kinetics, Higuchi diffusion, and Peppa's equation. The regression coefficient (r) and n values of all the mathematical models are tabulated in table for formulation F6. Plots are shown in Figures 10-12.

In vitro release data were attached in mathematical models which was interpreted in the structure of graphical presentation and valuated by correlation coefficient (R^2) shown in table. The higher degree of the correlation coefficient determines the appropriate kinetic model that follows drug release kinetics. It was found that first-order kinetics showed the highest correlation coefficient than another models from table.

DSC

DSC for the sitagliptin was performed using the instrument called Perkin Elmer 4000. The DSC thermogram of sitagliptin showed an endotherm at 221.7°C and 304.4°C. The DSC thermogram of the physical mixture showed the peak of cholesterol at 42.1°C and the integrated peak of soy lecithin were showed at 220.03°C [Figure 13-15].

Statistical Analysis using One-way ANOVA

Statistical analysis was explained using one-way ANOVA by taking the particle size range for all the formulations F1 to F6.[3,5,10] Using data analysis, it was observed that F value – 2.96 was greater than F critical value −2.55. *P*-value is also less than the value of 0.05 and hence it rejects null hypothesis

(H_0). By accepting alternative hypothesis, we concluded that all the formulations are not same.

Statistical investigation was performed using one-way ANOVA which was followed by Dunnett test through GraphPad Prism5 software.

In vivo studies of an oral antidiabetic liposomal formulation-F6

Sitagliptin test sample treated groups were measure and at 0 h it was found that there was no significant difference in any groups, at 30 min there was a significant decrease in blood glucose in test and as well as marketed product. At 1 h, test sample was shown more reduction in blood glucose when compared with control group. At 1.5 h, both samples were shown significant reduction in glucose level. At 2 h and 4 h, test sample was shown significant reduction compared with control group but observed that no significant changes observed in control group shown in the Table 7 and Figures 16 and 17.

DISCUSSION

Sitagliptin has been employed as oral antidiabetic drug which belong to BCS class III. In the present work, liposomal formulations containing sitagliptin were prepared using thin film hydration method. The liposomal suspension containing sitagliptin was done. The liposomal suspension equipped using a mixture of carriers in different ratios. Sitagliptin concentration in every sample is analyzed by means of UV spectrophotometer at 267 nm. The calibration curve of sitagliptin was found to be linear over a concentration range 2–10 µg per ml and regression value $r^2 = 0.995$.

The FT-IR spectrum of sitagliptin and all other excipients was recorded by FT-IR spectrophotometer and was compared with standard functional frequencies and excipients. The frequencies of functional group of the obtained sample of sitagliptin and excipients were in the range which indicated that the obtained samples of drug and other excipients used were pure. FT-IR spectrum obtained for drug with physical mixture showed characteristic peaks of the drug due to C-N, C-O, C=O, N-O, C=C, and N-H appeared at their respective wave numbers 1026.87 cm^{-1}, 1075.36 cm^{-1}, 1158.08 cm^{-1}, 1520.34 cm^{-1}, 1635.86 cm^{-1}, and 3313.09 cm^{-1} with no major shifts indicating compatibility of drug and excipients used. The FT-IR study was performed and it almost showed the similar characteristic peaks of sitagliptin indicating negative interaction and it would be concluded that sitagliptin was completely compatible with soy lecithin used in the finalized formulation (F6).

Sitagliptin and the optimized preparation were subjected to differential scanning calorimetric studied in DSC to identify the melting point of drug and soy lecithin. The DSC spectra of sitagliptin and finalized formulation had shown in the Figures 11 and 12. The DSC thermogram of sitagliptin shown sharp endotherm at 221.70°C which is near to the actual melting point of sitagliptin. The soy lecithin liposomal suspension showed sharp endothermic peak at 220.9°C which is near to actual melting point of phosphotidylcholine.

In vitro release studies in different ratios for sitagliptin and polymers performed at 8 h by Franz diffusion cell apparatus. The experimental data of *in vitro* releases were evaluated by cumulative % drug release and time. Liposomal formulation was compared with sitagliptin in *in vitro* data. Liposomal suspension of sitagliptin with phosphotidylcholine and cholesterol prepared using thin film hydration technique showed release in formulations such as F1, F2, F3, F4, F5, and F6 that were 82.31%, 76%, 80.79%, 77.74%, 79.66%, and 87.85%, respectively, at the end of 8 h. In *in vitro* drug release, liposomal suspension of formulation (F6) was found 87.85% of drug release at 8 h. Drug release was highest in the best formulations F6.

In an animal study, it was found that both test and control sample have shown reduction in blood glucose level at various time intervals and promotes glucose tolerance. It was observed that sitagliptin test sample was found to be more potent than sitagliptin marketed formulation.

CONCLUSION

Sitagliptin is an antidiabetic drug with low permeability as well as high solubility belongs to BCS class III. Liposomal formulation using sitagliptin was successfully prepared using soy lecithin and cholesterol as a polymer using thin-film hydration technique. Based on the *in vitro* release studies, statistical study using one-way ANOVA followed by Dunnett test through GraphPad Prism5 software was selected and to review the Log P value. Through *in vitro* drug release data, as observed its appropriate composition of final formulation (F6) was obtained with elevated percentage entrapment efficiency and % drug release. The formulation (F6) was considered as the best formulation. Particle range was found to be 40 nm and zeta potential was found to be 40 Mv in the best formulation (F6). FT-IR study and DSC analysis shown that there was a denial interaction between drug and the excipient in the finalized formulation-F6. It was observed that the release kinetics profiles for liposomal preparation were explained by first-order kinetics with highest regression coefficient $R^2 = 0.957$, and it states that the drug transport mechanism follow non-Fickian diffusion in the finalized formulation (F6). In *in vivo* study, it was observed that sitagliptin test sample was more potent than the sitagliptin marketed product. Hence, it could be concluded that oral antidiabetic liposomal formulations give prolonged release of the drug and to improve its permeability as well as bioavailability of the drug.

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