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Research Article

Formulation and Evaluation of Sitagliptin Liposomes

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ABSTRACT

Sitagliptin is a (BCS) III drug which has low permeability and high solubility. The major purpose of the study is to develop & differentiate the Anti-diabetic liposomal formulation and to evaluate the prepared formulations. The Liposomes were formulated by 'Thin Film hydration technique'. Six liposomal formulations of 'sitagliptin' were prepared. Soy lecithin and cholesterol in the concentration range of 300:100mg were used as an polymer. The *In-vitro* release data of different formulations was studied and observed through cellophane membrane using Franz Diffusion cell. The formulation (F6) showed best 'drug release'. Finalised formulation (F6) gave the best *In-Vitro* drug release 87.85% at 8 hours in comparision with the 'pure drug release. The DSC thermogram of physical mixture of cholesterol along with soy lecithin was found to be 42.1°C and 220.3°C.DSC confirmed that there is negative interaction between the drug and excipients. To check the Anti-hyperglycaemic activity, Oral glucose tolerance test and Anti-diabetic action *In-vivo* anti-diabetic activities were performed by using SD rats for the optimised formulation. *In-Vivo* studies revealed that optimised formulation (F6) of sitagliptin was found to be more potent than sitagliptin marketed formulation and activity was persisted till 4 hour.

Key words: Sitagliptin, soy lecithin, cholesterol, Thin film hydration technique, liposomal formulation, Statistical analysis

INTRODUCTION

Diabetes have been prescribed by doctors as Diabetes, which are detailed by cluster of metabolic in-born blunder, which a person have high-minded level of glucose in blood since the secretion of Insulin is inadequate or eventually the human body does not respond suitably to the Insulin or both. Patients with glucose in the bloodstream will normally experience renal disorder termed as polyuria. People will become excessive thirsty called as polydipsia and eating disorder named as polyphagia.

Objectives of diabetes complications: Through Atherosclerosis, blood vessels are damaged by high blood glucose levels that raise the possibility of them narrowing and also this break leads to

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deprived supply of blood to nerves. By affecting microvascular (small vessels), macrovascular (large vessels) or both blood vessels leads to complications that are persisting for years through poor controlled hyperglycemia.

Problems caused by diabetes

Complications which cause damage to small blood vessels are known as Micro-vascular complications.

1. RETINOPATHY- It is a state in which it causes spoil to the retina of the eye.
2. NEPHROPATHY- It is a condition which causes damage to kidneys.
3. NEUROPATHY- It is a condition which causes damage to nerves.

Complications which cause damage to large blood vessels are known as Macro- vascular complications.

1. Angina pectoris and congestive heart failure.
2. Transient ischemic stroke.
3. Peripheral atrial disease.

Precautions taken for Diabetes Complications:

- By maintaining HbA1C levels below 7% these complications can be prevented.
- By controlling the blood pressure and lipid levels these complications can be prohibited.

Treatment for type 2 diabetes oral dosage forms: 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 8.3-8.9mmol/L (around150- 160mg/dL).

Treatment for Type1diabetes and Type 2 diabetes:

1. The foremost target of diabetes treatment is to maintain blood sugar (glucose) level.
2. Type 1 diabetes patients are managed with insulin as well as dietary changes and exercises.
3. Type 2 diabetes patients might be managed with non-insulin medication, insulin, weight decline or dietary changes.
4. The selection of medication for type 2 diabetes is particular:
    1) Efficacy and consequences of each medication,
    2) The patients basic health status,
    3) Any medications fulfillment issue, and
    4) Expenditure to the tolerant or health-care system.
    5) Medications for Type 2 diabetes can work in diverse ways to diminish blood glucose level. They are:
        a. Raise in insulin sensitivity,
        b. Increase in glucose secretion,
        c. Decrease incorporation of carbohydrates from the digestive tract, or
        d. Work from side to side further mechanisms.
    6) Medications for Type 2 diabetes are frequently used in combination.
    7) Dissimilar methods for production of insulin include:
        a. Syringes,
        b. Pre-filled pens, and
        c. The insulin inject.
    8) Suitable diet is a part of any diabetes arrangement there is no one exact “diabetic diet” that is not compulsory for all individuals.

There are numerous customs to transport drugs hooked on the body, such as **oral**, **transdermal**, **pulmonary, mucosal lamina** and **parenteral** etc., 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, pro-drugs etc. 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.
- Low level of macromolecular absorption; 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 form 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 30nm 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.

ADVANTAGES:

- 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.
- Improved efficacy, therapeutic index, stability by encapsulation.

DISADVANTAGES:

- Manufacturing cost is high.
- Small half-life.
- Less stability.
- Little solubility.
- Sensitive to allergic reactions might occur to liposomal constituents

MATERIALS AND METHODS

Materials: Sitagliptin was purchased from Yarrow chem. Research Pvt. Ltd, Soy lecithin was purchased from Sigma Aldrich Pvt. Ltd, Cholesterol was purchased from Yarrow chem. Research Pvt. Ltd, Potassium di-hydrogen Orthophosphate, Sodium hydroxide was purchased from Hi-Media lab Pvt. Ltd, Chloroform was purchased from Sisco laboratories Pvt. Ltd

Preformulation Studies Determination of λ_{max} of Sitagliptin: The solution contain concentration of 10µg/ml of 'Sitagliptin' was all set and scan over the wavelength of 200-400nm in Shimadzu UV Spectrophotometer to conclude the wavelength of maximum absorbance.

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. Thermometer was placed into the Thiele 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 were compared with the standard FT-IR spectra.

Standard Calibration Curve of Sitagliptin: 50mg of sitagliptin was exactly weighed, dissolved in pH7.4 phosphate buffer taken in 50ml volumetric flask and the solution be prepared upto the spot with buffer to get the concentration(1000µg/ml) were taken in a 10ml volumetric flask to get the concentration (100µg/ml) and diluted with phosphate buffer to get the conc over the range from 2 Aliquots of 2-10µg/ml. The absorbance of dilutions was considered under the wavelength region of 267nm using UV spectrophotometer.

Preparation of Sitagliptin Liposomal Suspension:[4] A constant weight of 50mg of cholesterol and 300mg of phosphotidylcholine were weighed and dissolved in 20mlmof chloroform. 6.5gm of sitagliptin were added in 50ml of phosphate buffer (pH 7.4). The lipid content processed for rotary evaporator at 45°C until the lipid layer is 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 minutes until the lipid layer is removed. The layer is removed when the glass beads are 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 hours until the maturation of liposomes. Further evaluation studies are proceeded with the liposomal formulation.

Evaluation of Liposomal Formulation

The prepared liposomal suspension is evaluated for the following parameters:

Determination of Particle size:[2] The developed formulation was subjected under microscope under 100X magnification.

Estimation of Entrapment efficiency:[2,12] The prepared formulations were centrifuged for 30 minutes at 15000rpm. 0.5- 1 mL aliquot of supernatant solutions was diluted with phosphate buffer pH 7.4 to 25mL. By the quantity of drug in the supernatant liquid quantified by UV spectra at 267nm.

***Invitro* drug release:**[7,9] The *Invitro* drug release were passed out for all the preparations and the standard drug. Franz diffusion cell containing cellophane membrane was used. The donar compartment was full with 1ml of the formulation soaking in phosphate buffer pH7.4 at room temperature beneath slow magnetic stirring. At normal time intervals, 1ml 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 by UV spectrometer at 267nm.

R**elease kinetics:**[6] The release data were subjected to a choice of kinetic models to determine its mechanism and its model.

Fourier Transform-Infrared Spectroscopy:[14,15] FTIR 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.

Differential Scanning Calorimetry:[2] DSC of the 'Sitagliptin' and physical combination of soy lecithin plus cholesterol was performed by using the instrument called Perkin Elmer 4000.

Statistical Analysis using one-way ANOVA:[3,5] Statistical analysis was performed by using 'One-Way ANOVA' followed by 'Dunnett test' through Graphpad prism5 software by taking the particle size range for all the formulations F1 to F6.

***In-Vivo* studies:**[16,17,18,19] To determine the antidiabetic activity of test sample for Streptozotocin (STZ) induced diabetes followed by Oral glucose tolerance test (OGTT)in rats by using SD rats given orally in a dose of 2g/kg dose for 1 day. In this study, animals were kept fasting overnight and given dose of 25mg/kg by single injection IP. In order to inhibition of DPP4 the rats were given glucose load to check for OGTT and finally glucose was measured. By administering the test samples at the dose of 10mg/kg body weight were given till 60 minutes. After administration, the rats of all groups were orally treated with 2g/kg body weight dose of glucose. The blood samples were collected through orbital route at o-120 mins and blood-glucose level were measured.

RESULTS

Preformulation studies

Determination of λ_{max} of Sitagliptin: from the Figure 1 it was evident that the UV-Spectra of Sitagliptin in pH7.4 phosphate buffer showed the greatest absorbance at 267nm using the instrument Shimadzu UV-1700 PC that is comparable to the value reported.

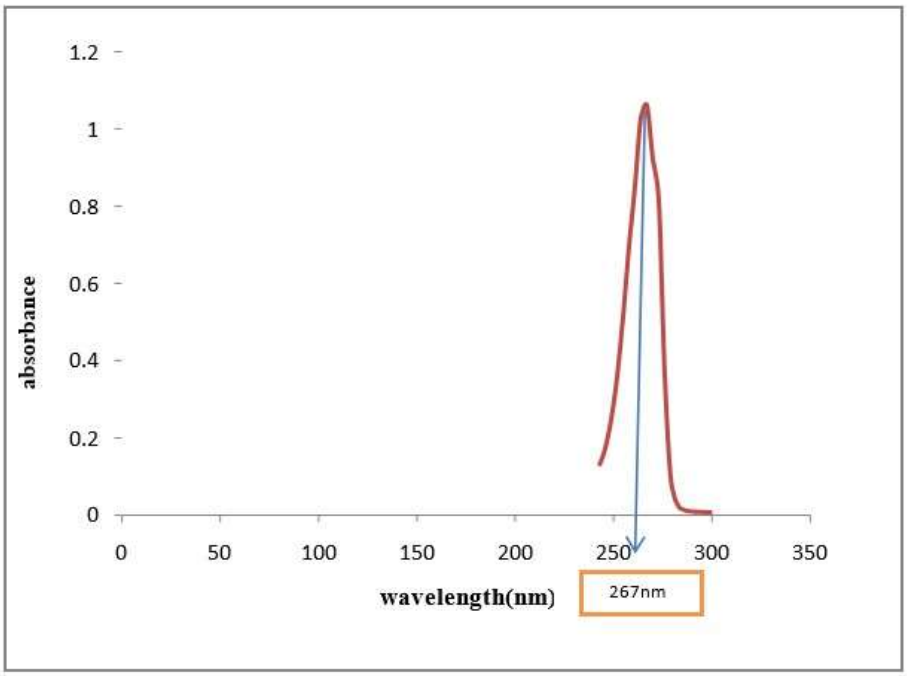

Figure 1: UV-Spectra of Sitagliptin

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 literature, thus indicate the purity of the drug.

Saturation solubility of Sitagliptin: The saturation solubility of Sitagliptin be evaluate in different solvents such as water, chloroform, ethanol and buffer pH 7.4. Solubility of Sitagliptin in water, choloroform, ethanol and phosphate buffer was establish to be 9.99±0.20mg/ml, 2.4±0.37mg/ml, 9.77±0.21mg/ml, 3.13±0.26mg/ml correspondingly which was shown in Table 1.

Calibration curve of sitagliptin: Above table shows the absorbance values of sitagliptin calculated at 267nm with respect to the linear concentration range of 2-10µg/ml of the drug in pH 7.4 phosphate buffer and its fig shown linear standard curve with slope 0.9995 and regression co-efficient value of 0.0887 which was shown in Table 2 and Figure 2.

Table 1: Saturation Solubility of sitagliptin in different solvents

Solvents	Trial 1	Trial 2	Trial 3	Average(mg/ml)±STDEV
Water	10.12	10.15	9.7	9.99±0.20
Chloroform	2.5	2.8	1.9	2.4±0.37
Ethanol	9.8	10.02	9.5	9.77±0.21
PBS pH 7.4	3.5	3.02	2.87	3.13±0.26

Table 2: Standard calibration curve for sitagliptin in pH7.4

Sl.No.	Concentration(µg/ml)	Absorbance at 267nm±STDEV
0	0	0
1	2	0.17±0.025
2	4	0.34±0.063
3	6	0.54±0.051
4	8	0.69±0.015
5	10	0.89±0.049

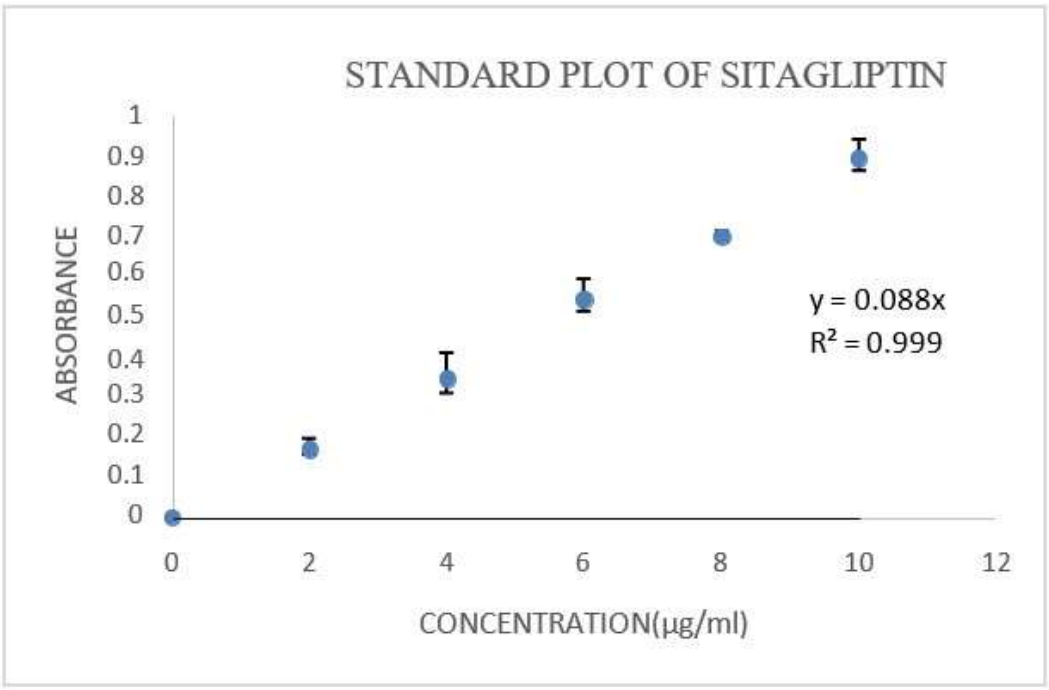

Figure 2: Standard calibration curve for sitagliptin in pH 7.4 at 267nm

Compatibility Study:[14,15] 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 figure, which was compare with standard functional group frequencies of sitagliptin as shown in Table 3,4,5 and Figure 3,4,5.

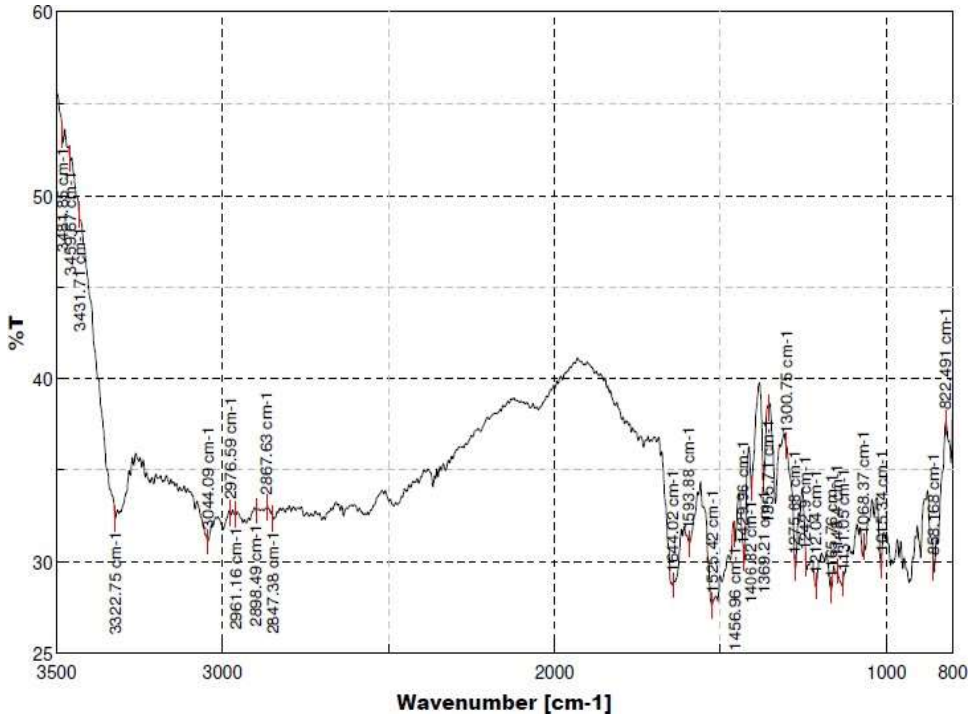

Figure 3: Infrared spectrum of sitagliptin

Table 3: 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

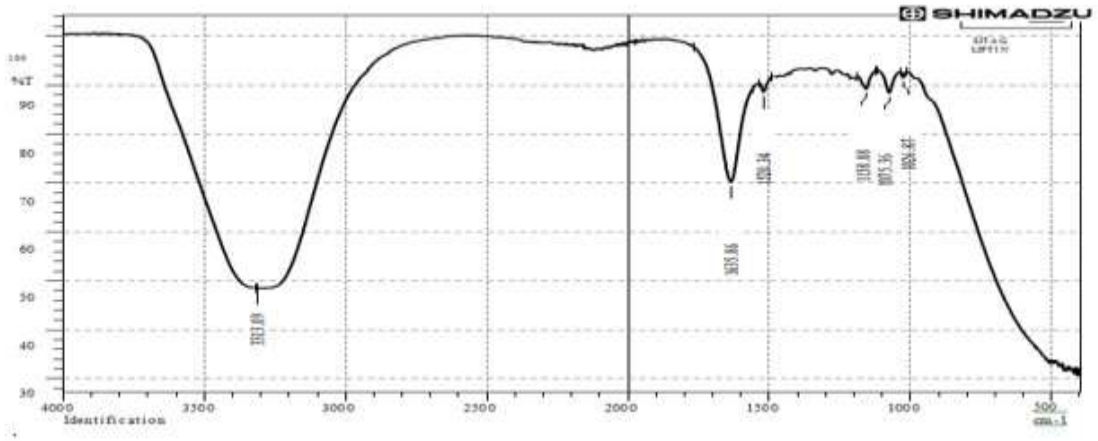

Figure 4: Infrared spectrum of formulation (F6)

Table 4: 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

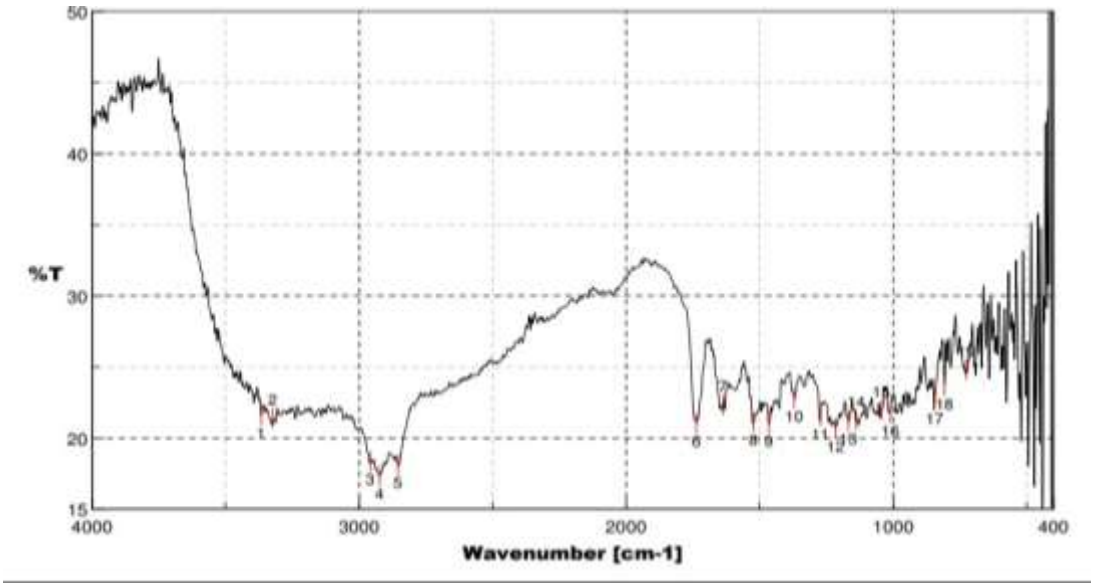

Figure 5: FT-IR spectrum of drug and polymer

Table 5: FT-IR frequencies for drug and polymer

No.	cm-1	%T	No.	cm-1	%T	No.	cm-1	%T
1	3366.14	21.5911	2	3322.75	21.4375	3	2958.27	18.3673
4	2922.59	17.323	5	2854.13	18.1166	6	1736.58	21.0769
7	1637.27	22.3492	8	1522.52	21.1164	9	1465.63	21.0498
10	1371.14	22.759	11	1272.79	21.541	12	1215.9	20.6649
13	1168.65	21.2038	14	1137.8	21.277	15	1044.26	21.9824
16	1010.52	21.7362	17	844.669	22.6164	18	808.028	23.6926
19	727.032	24.8108						

Evaluation: The equipped formulation were subjected to different evaluation parameters like appearance, pH, entrapment efficiency etc.

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

Determination of Particle size: From the Figure 6 it was evident that the particle size for the optimized formulation was found to be 42.8µm by using Horiba S 100.

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

Determination of Zeta potential: From the figure 7 it shows that the zeta potential for the optimised formulation was found to be -40mV by using Horiba S-100.

Estimation of Entrapment Efficiency:[2,12] From the data given in table 6, it shows that the entrapment efficiency of all the formulation was found in the range of 85.93% to 95.22%.

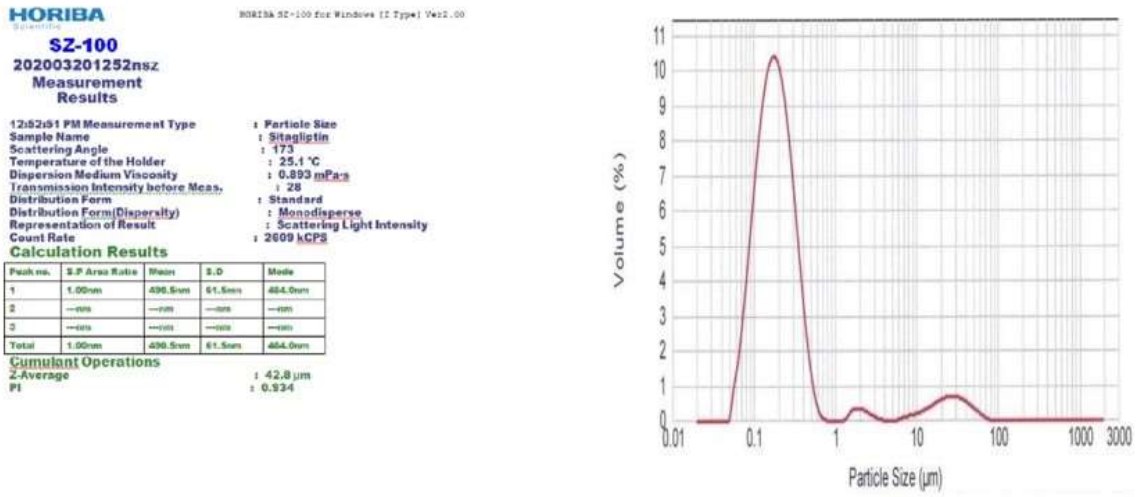

Figure 6: Particle size for optimized formulation

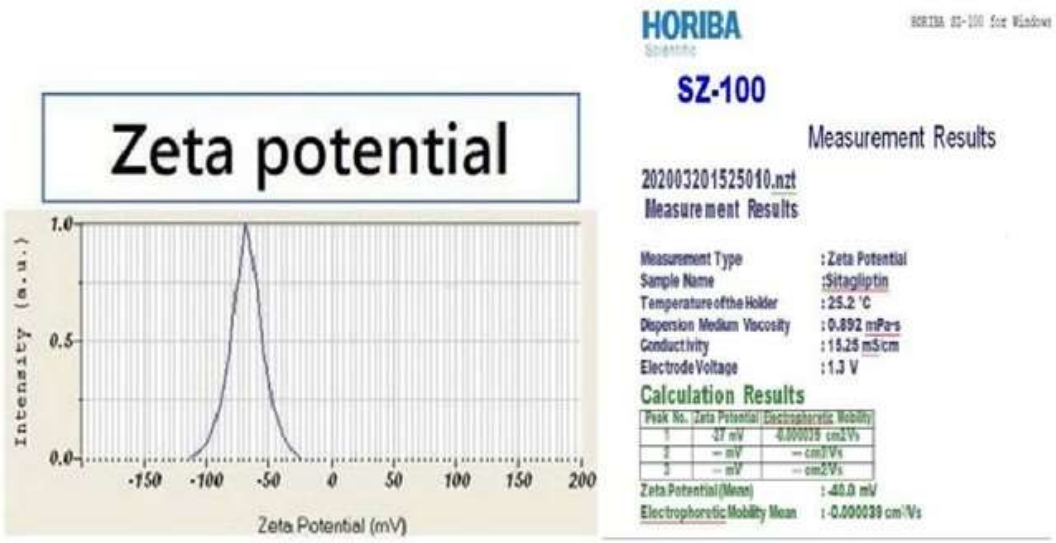

Figure 7: Zeta potential for the optimized formulation

Table 6: Entrapment Efficiency, pH and clarity of the prepared Sitagliptin Liposomal suspension

Formulation	Entrapment Efficiency (%)	pH	Clarity
F1	92.55%	7.02	Clear
F2	94.20%	7.01	Clear
F3	85.93%	7.03	Clear
F4	89.35%	6.09	Clear
F5	91.35%	7.04	Clear
F6	95.22%	7.5	Clear

***In-vitro* release studies:**[7,9] The drug release information obtained for the formulations F1 to F6 and pure drug 'sitagliptin' is tabulate in table 7 and figure 8 shows the plot of %CDR verses time for all formulations. The sitagliptin liposomal suspension is observed in *In-Vitro* release studies and carried out using pH 7.4 PBS as the dissolution media.

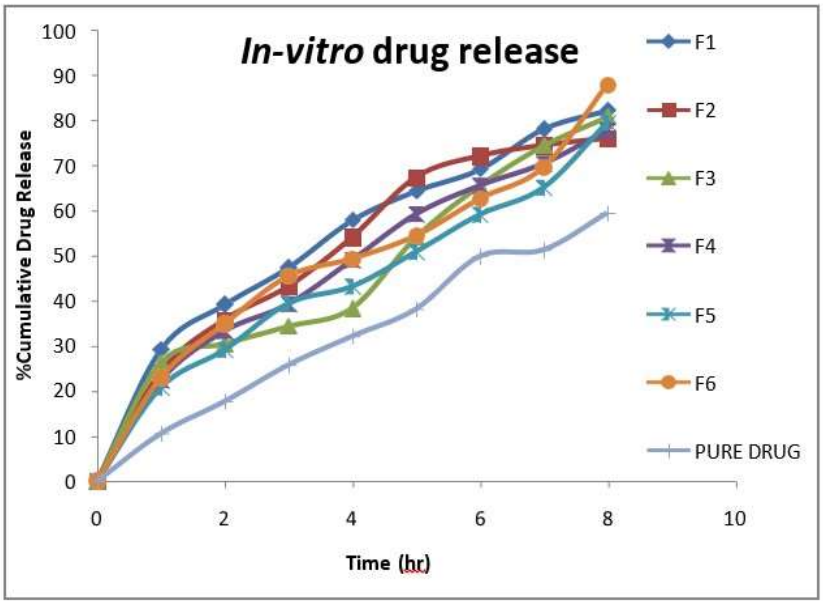

Figure 8: Cumulative percentage release of sitagliptin Liposomal suspension (F1-F6) and pure drug

Table 7: Percentage Cumulative drug release of formulation (F1-F6) and pure drug

Time(hrs)	Pure drug	F1	F2	F3	F4	F5	F6
1	10.51	29.16	24.19	26.29	22.32	20.76	22.90
2	17.71	39.15	35.78	30.45	33.25	29.16	34.85
3	25.81	47.39	43.19	34.34	39.22	39.47	45.42
4	32.19	57.89	54.09	38.39	49.02	43.24	49.29
5	38.29	64.39	67.37	54.09	59.17	50.95	54.38
6	49.95	69.22	22.01	65.52	65.52	59.21	62.57
7	51.38	78.18	74.46	74.41	70.42	65.23	69.57
8	59.57	82.31	76.00	80.79	77.74	79.60	87.85

Selection of kinetic model:[6] *In-Vitro* release data were subjected for several kinetic model.

1. Zero-order kinetics
2. First-order kinetics
3. Higuchi diffusion
4. Peppa's equation

The regression co-efficient (r) and 'n' values of all the mathematical models are tabulated in table for formulation F6. Plots are shown in figure 9,10,11,12 and Table 8.

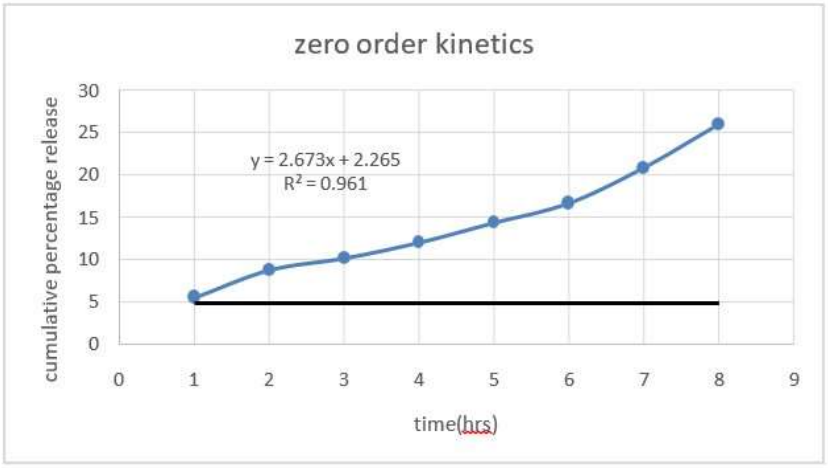

Figure 9: Plot of cumulative percentage release Vs time for finalised formulation F6 **(Zero order plot)**

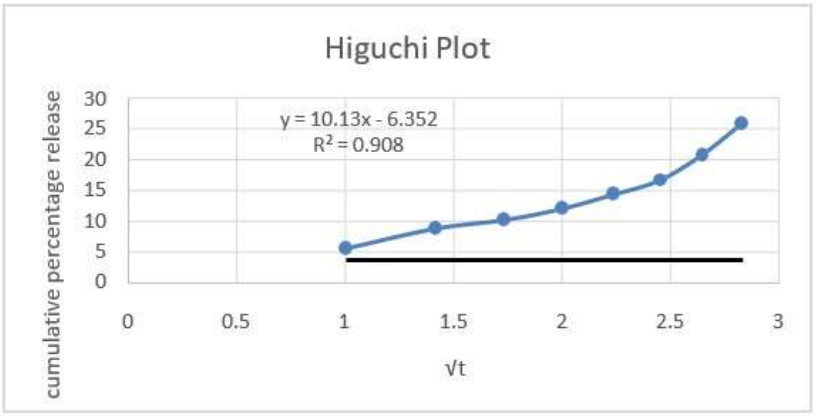

Figure 10: Plot of cumulative percentage release Vs √t for finalised formulation F6 (Higuchi plot)

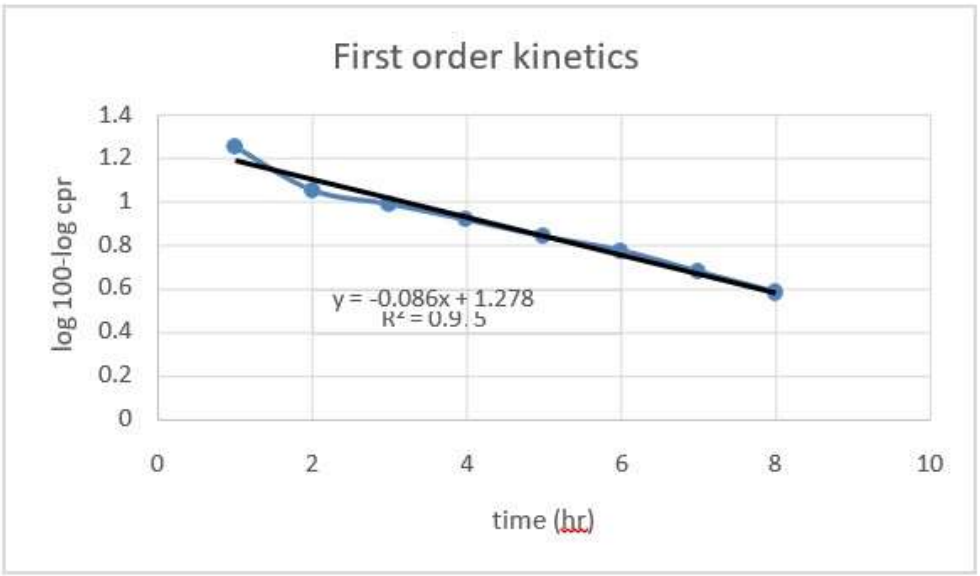

Figure 11: Plot of log cumulative percentage retained Vs time for finalized formulation F6 **(First order plot)**

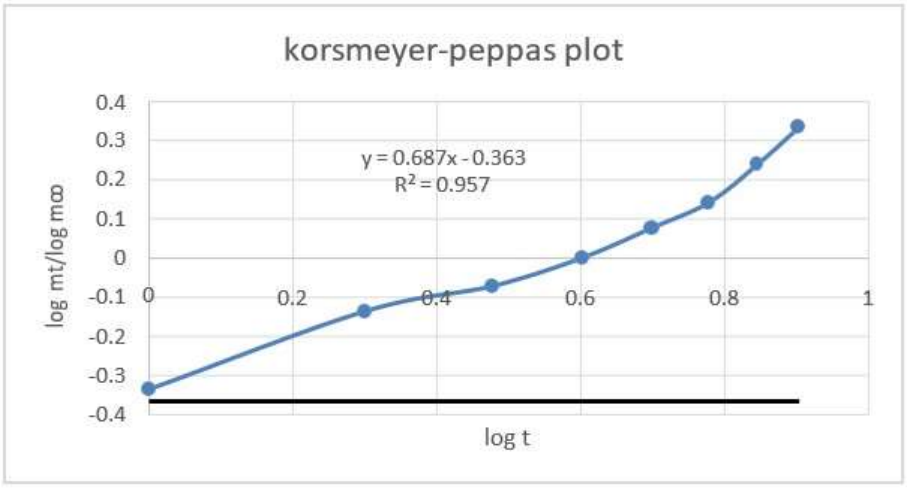

Figure 12: Plot of logMt/logMœ Vs time forfinalised formulation F6 (Korsmeyer-Peppas plot)

Table 8: Kinetic values obtained from *in-vitro* release data of finalised 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

'*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 above 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 the table 8.

Differential Scanning Calorimetry:[2] Differential scanning calorimetry for the sitagliptin was performed by using the instrument called Perkin Elmer 4000. The DSC thermogram of sitagliptin showed an endotherm at 221.7°C and 304.4°C (figure1). The DSC thermogram of the physical mixture showed the peak of cholesterol at 42.1°C (figure2) and the integrated peak of soy lecithin was showed at 220.03°C which was given in figure 13 (a),13(b) and 13 (c).

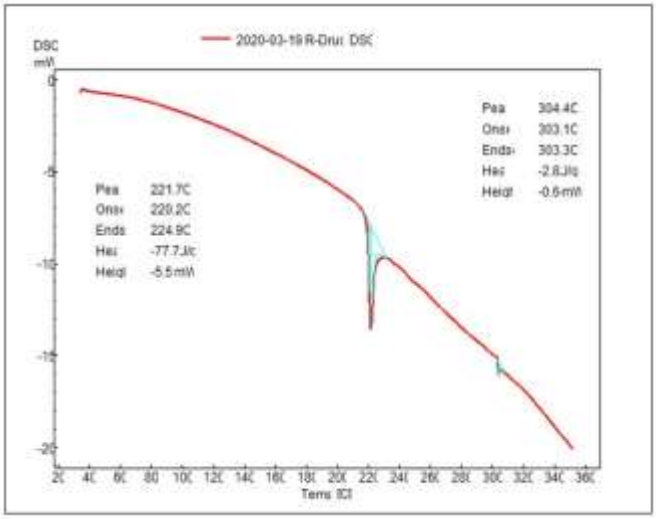

Figure 13(a): DSC thermogram of sitagliptin

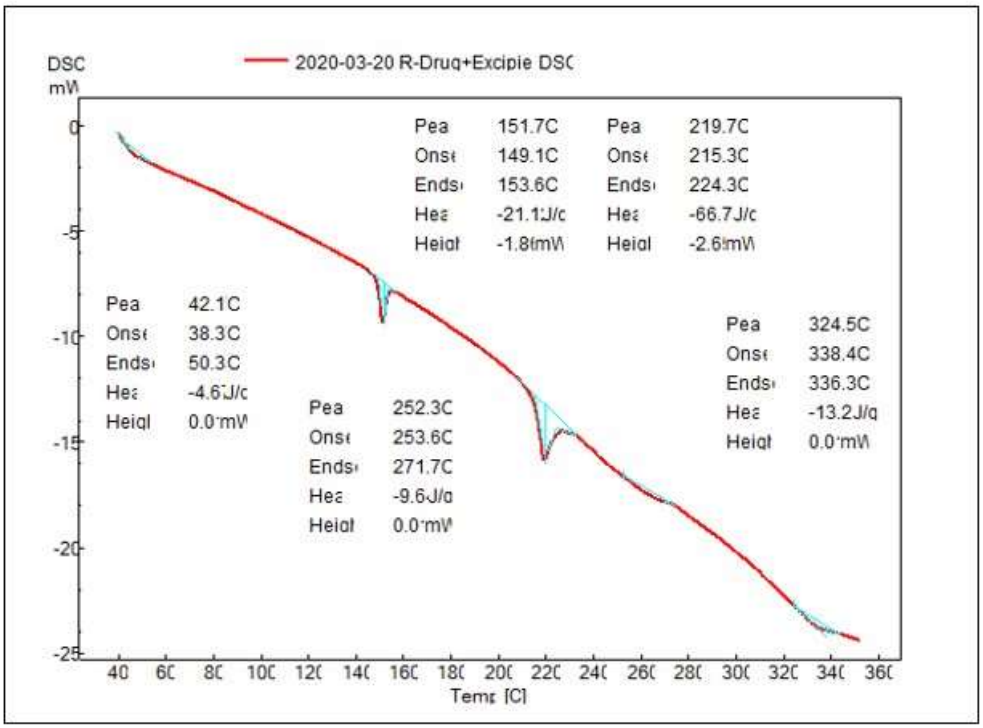

Figure 13(b): DSC thermogram of sitagliptin with Cholesterol and Phosphotidylcholine

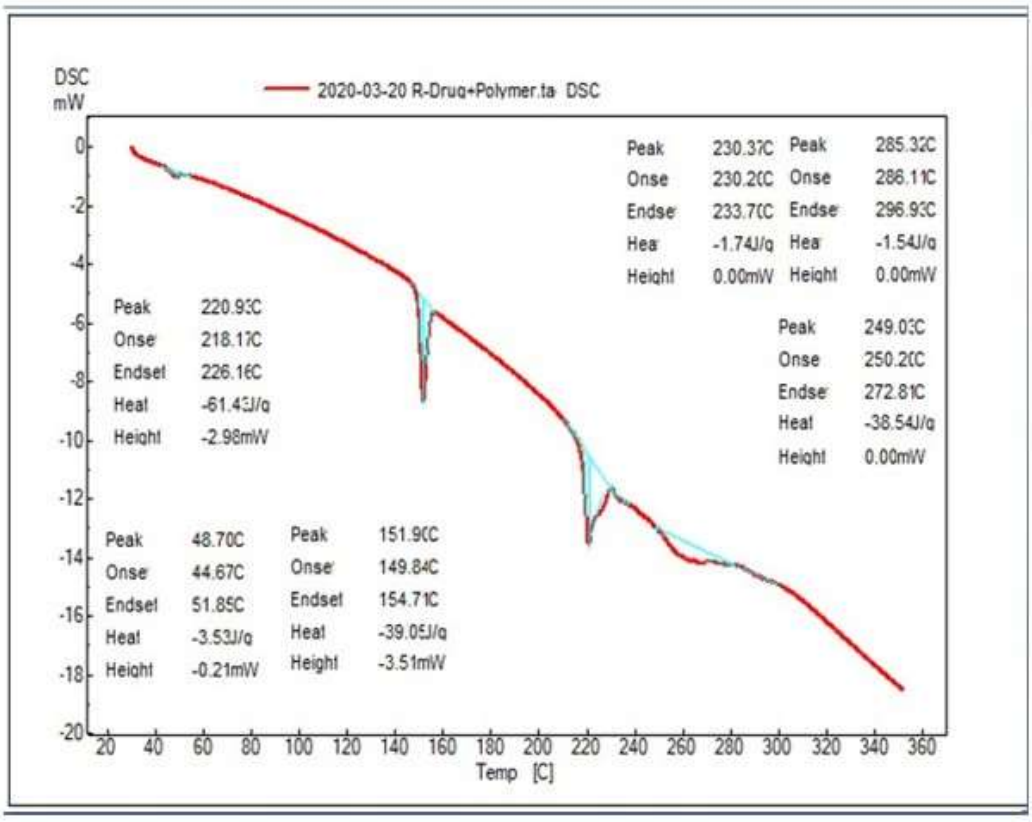

Figure 13(c): DSC thermogram of sitagliptin with polymer (soy lecithin)

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 14: Statistical Analysis using One-Way ANOVA

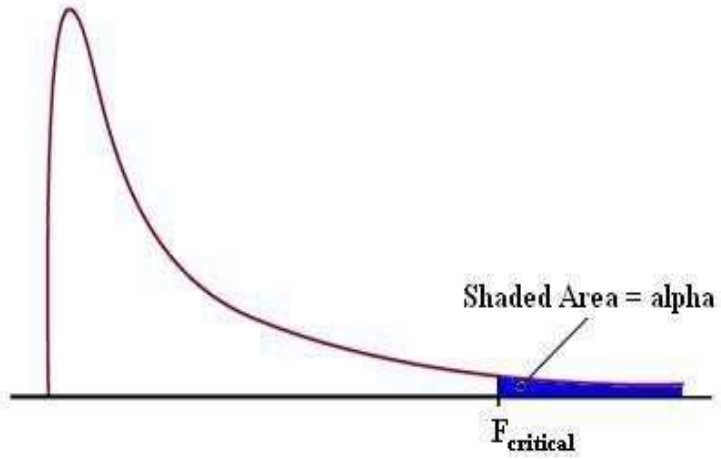

Figure 15: Log p value using One-Way ANOVA

Table 9: Formula 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	$SS_t = \sum_{j=1}^{n}(\bar{X}_j - \bar{X})^2$	$df_t = n-1$		

Statistical investigation was performed using One-Way ANOVA which was followed by 'Dunnett test' through GraphPadPprism5 software and shown in Figure 16.

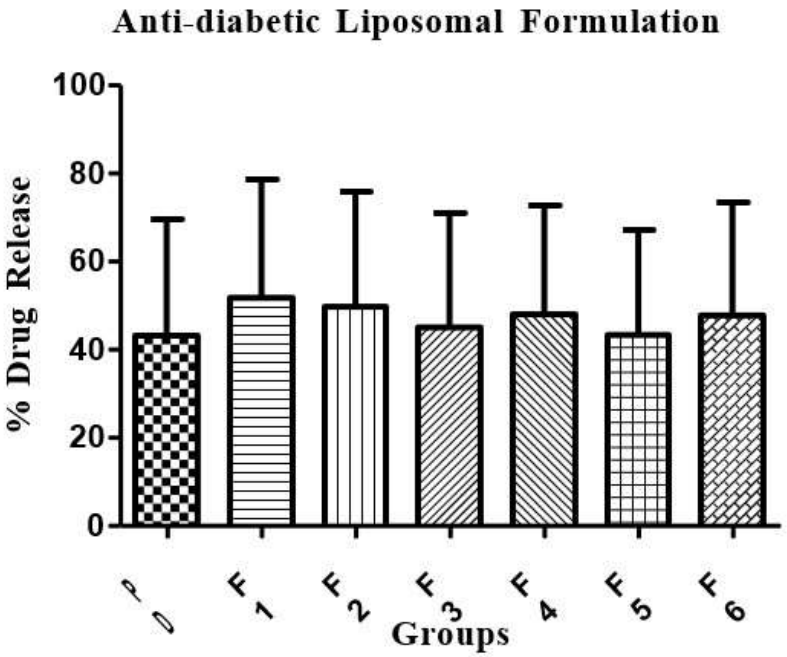

Figure 16: Statistical analysis of *in-vitro* release data using GraphPad Prism[5]

***In-vivo* studies of an oral anti-daibetic liposomal formulation-F6:**[16,17,18,19] Sitagliptin test sample treated groups were measure and at 0 hour it was found that there was no significant difference in any groups, at 30min there was significant decrease in blood glucose in test and as well as marketed product. At 1 hr, test sample was shown more reduction in blood glucose when compared with control group. At 1.5hr., both samples were shown significant reduction in glucose level. At 2 hr and 4 hr, test sample was shown significant reduction compared with control group but observed that no significant changes observed in control group which was shown in Table 10 and Figure 17.

Table 10: 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**

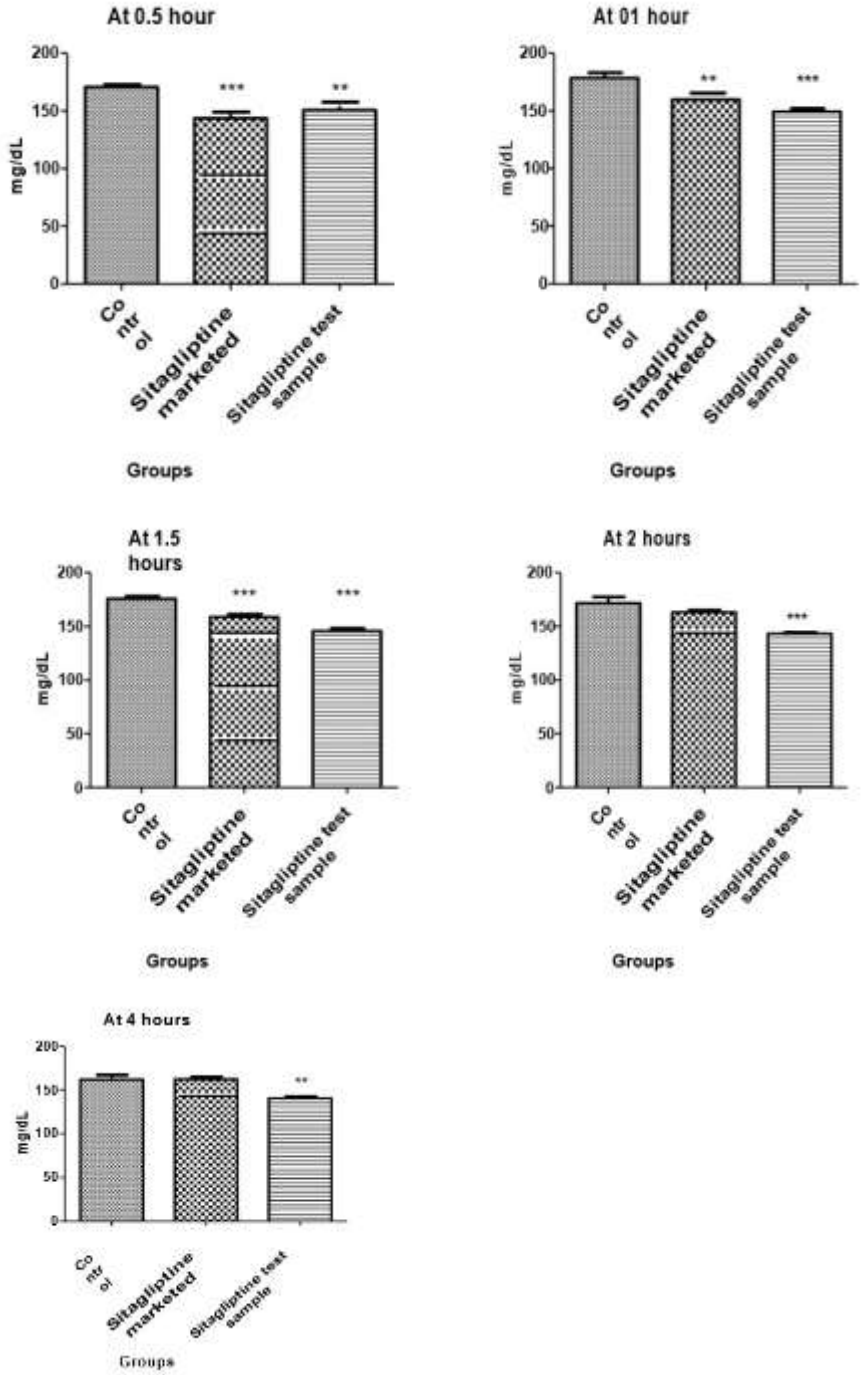

Figure 17: Graphs of sitagliptin test and control sample at different time intervals

DISCUSSION

Sitagliptin is used as an anti-diabetic drug,nwhich belong to BCS class III. In the present work, liposomal formulations of sitagliptin were prepared using thin film hydration method. The UV spectra for sitagliptin showed at 267nm. The calibration curve of sitagliptin was found to be linear over a concentration range 2-10µg per ml and regression value r^2 =0.995.

The FT-IR spectrum of sitagliptin and all other excipients were recorded by FT-IR spectrophotometer and were compared with standard functional frequencies and excipients. The frequencies of functional group of the obtained sample of siatgliptin 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 1026.87cm^{-1},1075.36cm^{-1},1158.08cm^{-1},1520.34cm^{-1},1635.86cm^{-1} and 3313.09cm^{-1} with no major shifts indicating compatibility of drug and excipients used.

Sitagliptin and the optimised preparations were subjected to differential scanning calorimetric studied in DSC to identify the melting point of drug and soy lecithin. The DSC spectrum of sitagliptin and the finalised formulation is shown in the figure. The DSC thermogram of sitagliptin shows sharp endotherm at 221.7°C which is near to the actual melting point of sitagiptin. The soy lecithin liposomal suspension showed sharp endothermic peak at 220.9°C which is near to actual melting point of phosphotidylcholine.

The *in-vitro* release studies in different ratios for sitagliptin and polymers performed at 8 hours by Franz Diffusion Cell apparatus. The data 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 a release in formulations such as F1,F2,F3,F4,F5 and F6 were 82.31%, 76%, 80.79%, 77.74%, 79.66% and 87.85% respectively at the end of 8 hours. Drug release was highest in the best formulations F6.

In *In-vivo* studies 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 'Anti-diabetic drug' with low permeability as well as high solubility belongs to BCS class III. Liposomal formulation using sitagliptin were successfully prepared using soy lecithin and cholesterol as a polymer by 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, % drug release. Since the finalised formulation, liposomal formulation was considered. Particle range was found to be 40nm and zeta potential was found to be 40Mv. By the FTIR studies and Differential Scanning Calorimetry study proved that, there is a denial interaction between drug and the excipient. It was observed that the release kinetics profiles for liposomal preparation was 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. Hence we will bring to a close that the liposomal formulations give prolonged release of the drug and to improve its permeability as well as bioavailability of the drug. In *In-vivo* studies it was observed that the sitagliptin test sample was more potent than the sitagliptin marketed product.

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