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DEVELOPMENT AND EVALUATION OF MUCOADHESIVE LIPOSOMES OF REPAGLINIDE FOR ORAL CONTROLLED DELIVERY SYSTEM

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ABSTRACT

The aim of the present investigation was to design a mucoadhesive liposomal system of Repaglinide for the treatment of type - 2 diabetes mellitus that is capable of delivering entrapped drug over an extended period of time. Mucoadhesive liposomal formulations were prepared by using different ratio of lecithin and cholesterol by thin film hydration technique followed by coating of liposomes by 0.1 % w/v of chitosan and were evaluated for entrapment efficiency, particle size, zeta potential, surface morphology and *in-vitro* drug release. Particle size and zeta potential of the F2 and CF2 formulation was found to be 413.5 and 830.9nm -40.9 mV and -46.8 mV respectively. Coating of liposomes resulted increase in particle size and also increases the zeta potential. Highest entrapment efficiency was observed in F1 and CF1 90% and 95%. The percent drug release from F1-F3 and CF1-CF3 was observed as follows F1- 79.04%, F2- 76.77%, F3- 64.32% and CF1-66.65%, CF2- 62.12%, CF3- 56.54% which follows first order drug release and non-Fickian diffusion mechanism.

KEYWARDS: Repaglinide, Diabetes mellitus, mucoadhesive liposome, thin film hydration method, stability studies, *in-vitro* release

INTRODUCTION

Mucoadhesive dosage forms have received substantial attention as novel drug delivery systems able to improve the bioavailability of drugs by prolonging their residence time and controlling the drug release characteristics. Mucoadhesive nanoparticulate systems such as

polymer coated liposomes were found to be useful carriers for improved oral delivery because of their prolonged retention in the GI tract and excellent penetration into the mucus layer.[1]

The oral route remains to be the most convenient and comfortable way of drug administration. However, the success of liposomal formulating through oral route of administration is limited due to their short residence time at the site of absorption. It would, therefore, be advantageous to have means for providing an intimate contact of the drug delivery system with the absorbing membrane. It can be achieved by coupling bioadhesion characteristics to liposomes and developing mucoadhesive liposomal delivery system.[2]

Development of mucoadhesive liposomal drug delivery system have advantages such as improved bioavailability of poorly absorbed oral drugs by prolonging their gastric and intestinal residence time, through facilitating the intimate contact of the delivery system with the absorption membrane and specific targeting of drug to the absorption site.

One of the most promising strategies in developing mucoadhesive particulate system is surface modification or coating of the drug carrier particles with mucoadhesive polymers.[3]

Diabetes Mellitus is a major and growing health problem in most countries and an important cause of prolong ill health and early death. It is a chronic metabolic disorder characterized by high blood glucose concentration-hyperglycemia; glycosuria, negative nitrogen balance and sometimes ketonemia.[3] In recent years, developing nations have witnessed an explosive increase in the prevalence of diabetes mellitus predominantly related to life science changes and the resulting surge in obesity. The centers for disease control and prevention predicts the national incidence of diabetes will rise by 37.5% by the year 2025.[4]

Rapid intestinal absorption of oral hypoglycemic drugs is required to avoid rapid increase in the concentration of blood glucose after meals. Repaglinide is an oral blood glucose lowering drug of Meglitinide class used in the management of type-II diabetes mellitus (NIDDM).[5]

Repaglinide has an extremely short half-life of 1hr. In addition, the oral bioavailability of Repaglinide is low (56%) due to poor absorption in the upper intestinal tract and extensive hepatic first-pass effect after either an IV or oral dose. Moreover, it produces hypoglycemia after oral administration. Dosage frequency of Repaglinide is 0.5 to 4 mg in 3 to 4 times in a day. It belongs to class BCS class-II compound with poor solubility. Hence, the

enhancement of repaglinide dissolution can be taken as a tool to improve the bioavailability of this drug.[6]

The benefit becomes even greater if the selected technique and/or additive can reduce the first pass effect while increasing the dissolution rate of the drug. Lipid based systems can provide another alternative. These systems can improve the bioavailability of poorly soluble drug candidates. Lipid based formulations offer a variety of options like solution, suspension, solid dispersion and self-emulsifying drug delivery system.

Hence, in the present investigation mucoadhesive based liposomal approaches have been proposed for improved oral delivery because of their prolonged retention in the GI tract and excellent penetration into the mucus layer.

MATERIALS AND METHOD

Repaglinide was gifted from Biocon Ltd. Karnataka, Soya lecithin was purchased from Pharma Sonic Biochem Extractions Ltd. Indore, Cholesterol, and other solvent like Chloroform and Methanol purchased from S d fine chem Ltd. Mumbai. 1.2 N of HCL were prepared as described in the indian pharmacopoeia (1996)

Methods

PREPARATION OF REPAGLINIDE LIPOSOME

A. Preparation of liposomes.[7]

Cationic multilamellar liposomes can be prepared by hydration of lipid film. The lipid mixture is dissolved in a small amount of chloroform and placed in a rotary evaporator at 40°C until a thin film is obtained, and allowed to stand overnight in a vacuum chamber to ensure complete solvent removal. Phosphate buffer pH 6.8 is used to hydrate the thin film. The hydrated thin film is melted in water bath at 70°C for 1 min and blended to obtain multilamellar liposomes. Then prepared liposome will be sonicated to reduce particle size.

B. Coating of liposomes [8]

A volume of 2.0 mL of chitosan solution was added drop-wise to the 2.0 mL of liposomes under magnetic stirring at room temperature for 1 hr. followed by incubation in refrigerator overnight.

Table 1: Formulation design for the preparation Repaglinide liposomes

Formulation Code	Drug (mg)	Soya Lecithin (mg)	Cholesterol (mg)	Chitosan % w/v
F1	50	500	100	-
F2	50	500	200	-
F3	50	500	300	-
CF1	50	500	100	0.1
CF2	50	500	200	0.1
CF3	50	500	300	0.1

EVALUATION PARAMETER OF MUCOADHESIVE LIPOSOMES.[9,10]

The prepared liposomes and coated liposomal formulation were evaluated for different parameters like Drug-Excipients compatibility, Surface morphology, Vesicle size analysis, Entrapment efficiency determination, Zeta potential determination, *Invitro* diffusion study, *In vitro* wash-off test for mucoadhesive test and Stability studies as per ICH guidelines.

Invitro diffusion study

In-vitro release pattern of liposomal suspension was carried out in dialysis bag method. Repaglinide liposomal suspension equivalent to 10 mg was taken in the dialysis bag and the bag was placed in a beaker containing 100ml of 1.2N HCL. The beaker was placed over magnetic stirrer having stirring speed of 100 RPM and the temperature was maintained at 37±0.5°C. 1ml sample were withdrawn periodically and were replaced by freash buffer. The sample were assayed by UV spectrophotometer at 242 nm using 1.2N HCL as blank and cumulative % of drug released was calculated and plotted against time

In vitro wash-off test for mucoadhesive testing.[11, 12]

The mucoadhesive property of the polymer-coated liposomes was evaluated by an *in vitro* adhesion test. The method used was the modified *in-vitro* wash-off test. The mucoadhesion of the polymer-coated liposomes was compared with that of a non mucoadhesive material, uncoated liposomes containing Repaglinide. Freshly excised pieces of sheep intestinal mucosa (2 × 2 cm) were tightened onto glass slides (3 × 1 inches) with thread. A volume of 0.5 ml of the liposomes, 0.1% and 0.3% (w /v) chitosan-coated liposomes, liposomes were spread onto each wet-rinsed tissue specimen and immediately incubated at 37 ℃. The tissue specimens were taken out at 1 and 3 hrs. The samples were washed with 10.0 ml of PBS at each time interval.

Determination of mucoadhesive strength

From the 10.0 ml of the eluted buffer containing nonadhered drug, 500 µl aliquots were taken and liposomal lipids were dissolved by methanol. It was measured by a UV spectrophotometer. The concentration of repaglinide eluted in the 1.2N HCL was measured and the remaining drug was assumed to be present in liposomes adhered to the intestinal mucosa. Hence, the percentage of mucoadhesive strength can be calculated by Eq

$$\text{Mucoadhesion (\%)} \quad \frac{\text{Amount of drug remaining in mucosa}}{\text{Amount of drug taken in tests}} \text{ X } 100$$

Stability studies as per ICH guidelines [13]

Accelerated stability testing studies was performed for 6 months as per ICH guidelines. The optimized formulation was kept at 4 ± 2 ℃ and 75 ± 5 % RH in stability chamber. Regular tested for % entrapment, vesicle size and drug release were fixed as physical parameters for stability testing.

RESULT AND DISCUSSION

FTIR spectra of pure Repaglinide showed sharp characteristic peaks at 3309.96, 2931.90, 2800.73, 1774.57, 1566.25, 1381.08, 1296.21, and 1087.89. Physical mixture showed all the characteristic peaks of pure drug, confirmed no interaction between the drug and excipients. Comparative studies of FTIR graphs are showed in Fig.1-2. The surface morphology was studied by Scanning electron microscopy (SEM). The SEM photographs of optimized liposomes formulation F2 and CF2 as shown in Fig. 3-4. The porous structure in the images of Fconfirmed the formation liposomes that are confirmed the incorporation of lipids and drug. SEM photographs of coated liposomal formulation showed the smooth coating of chitosan over the liposomes as shown in Fig. 4. The size analysis of prepared liposome formulation was done by optical microscope. It was shown in the Table 2. We observed that, increase in the concentration cholesterol in the formulation F1 to F3, the vesicle size was increased. The optimized coating of liposomes in concentration of 0.1% CF2 is 830.9 nm. Shown in the fig. 5-6. The % entrapment efficiency was found to decrease with increasing the cholesterol concentration. It is shown in the Table 2. Zeta potential of optimized formulation F2 and CF2 of Repaglinide liposomes shown in fig. 7-8. and it was found to be -40.9 mV and -46.8 mV, respectively which indicate that they are sufficient to be stable. *In vitro* release behavior of all formulations is summarized in Table .3-4. *In vitro* drug release of Repaglinide

liposomes in 1.2N HCL was performed using dialysis tube diffusion technique. The *in vitro* drug release profile of Repaglinide liposomes formulations obtained from dialysis experiment was shown in Fig.9-10. The release of Repaglinide containing liposomes Chitosan coated liposomes was varied according to concentration of soya lecithin and cholesterol. The progressive decrease in the amount of drug diffused through cellophane membrane from formulations F1 to F3, CF1 to CF3 attributed to gradual increase in soya lecithin and cholesterol content. It has been concluded that, if we increase the concentration of soya lecithin and cholesterol, the diffusion of drug also decreases. The amount of drug diffused from formulation F3 was showed 64.32 % which was lower among the formulations F1 to F3, CF3 was showed 56.54 % which was lower among the formulation CF1 to CF3. The Mucoadhesive strength was measured by a UV spectrophotometer. Mucoadhesive strength optimized formulation CF2. The amount of drug released in the formulation CF2 60 %. Percent mucoadhesion was calculated and found the mucoadhesive strength was 65% which showed sufficient mucoadhesive property. Stability studies of mucoadhesive liposome formulation CF2 as shown in Table. 5, respectively, that negligible change in % Entrapment efficiency and % CDR revealed that the formulations are stable on storage.

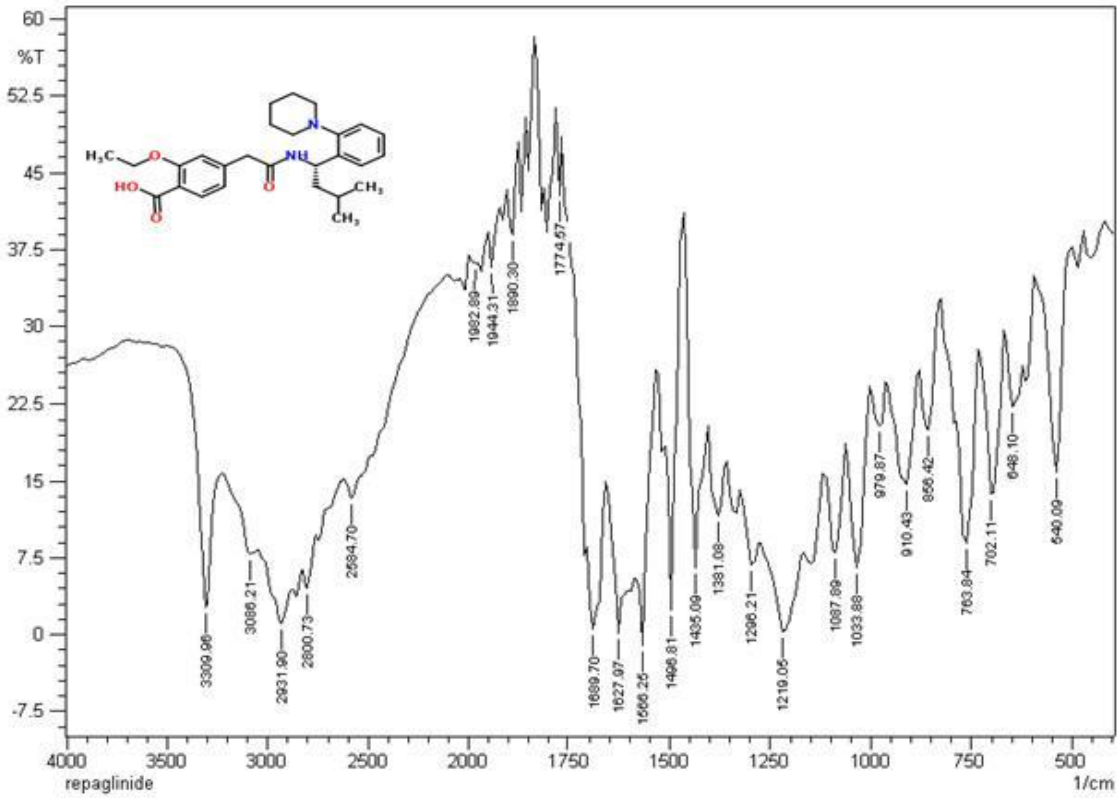

Fig.1: FT-IR Spectroscopy of Repaglinide

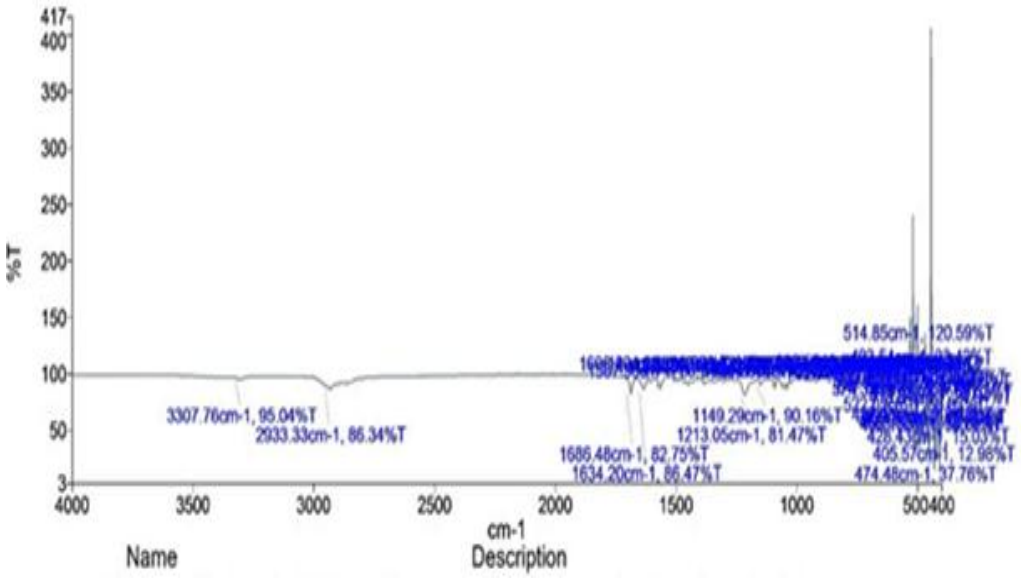

Fig.2: FT-IR Spectroscopy physical mixture of Repaglinide+Soya Lecithin+Cholesterol

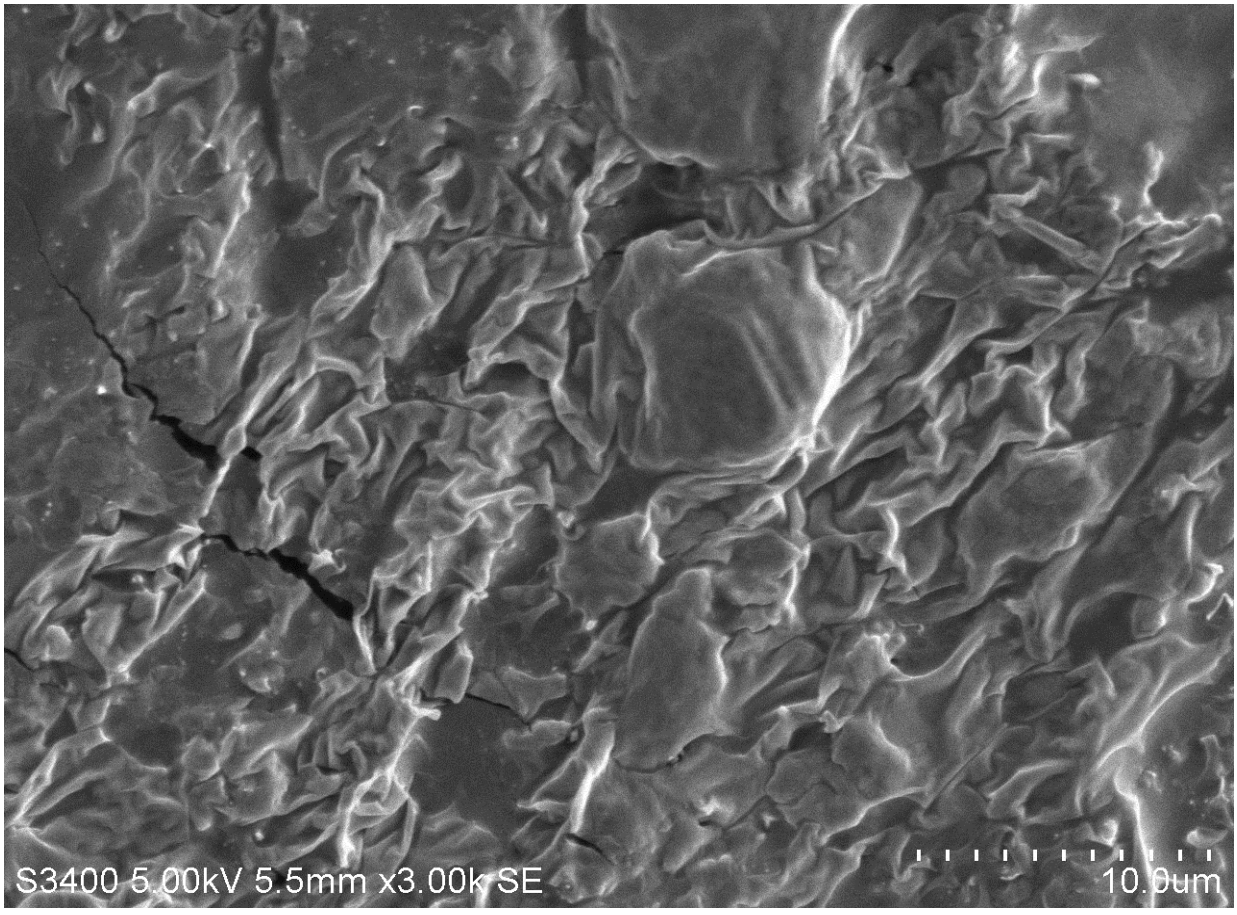

Fig.3: Scanning Electron micrograph of liposomes formulation F2

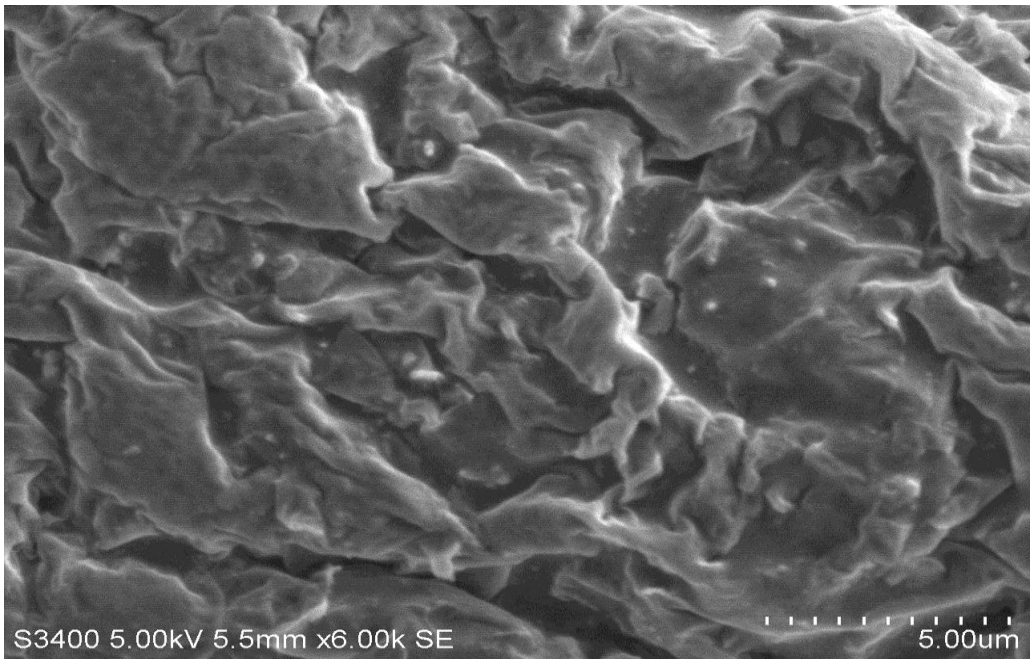

Fig.4: Scanning Electron micrograph of liposomes formulation CF2

Results

			Size (d.nm):	% Intensity:	St Dev (d.n...
Z-Average (d.nm):	413.5	Peak 1:	353.3	64.7	187.9
PdI:	0.417	Peak 2:	2180	35.3	1285
Intercept:	0.910	Peak 3:	0.000	0.0	0.000
Result quality :	Good				

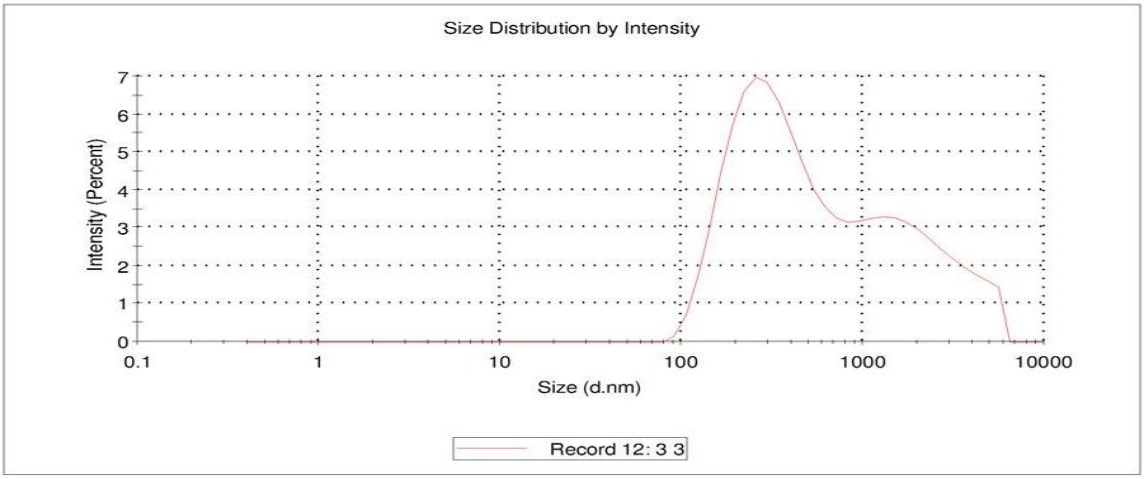

Fig.5: Particle size data for liposome formulation F2

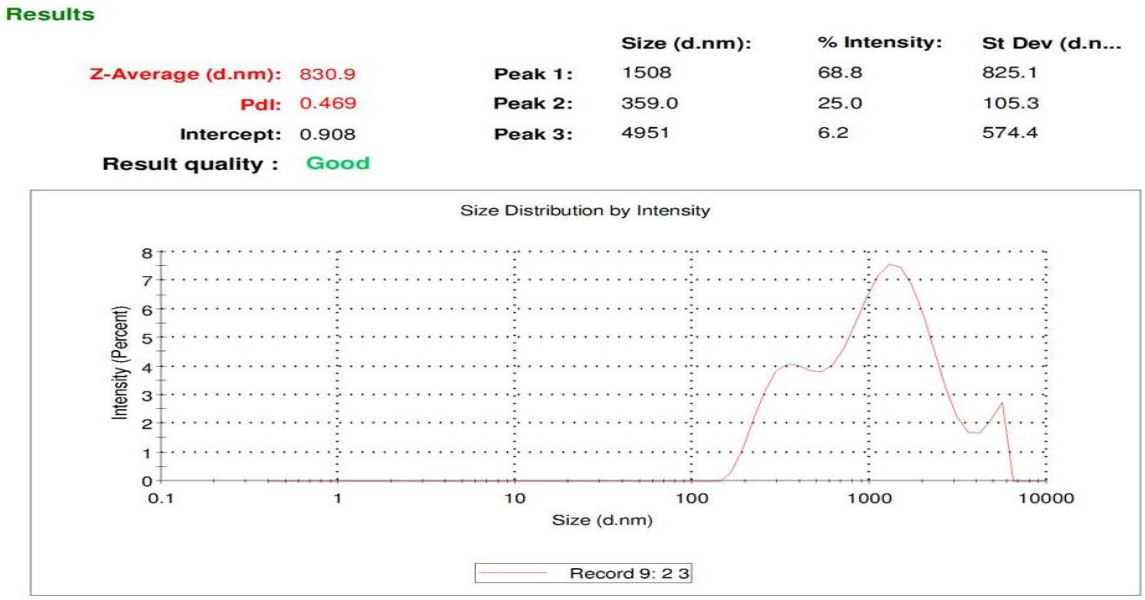

Fig.6: Particle size data for coating of liposome formulation CF2

Table 2. Vesicle size and % Entrapment efficiency of mucoadhesive liposomes formulations

Formulation code	Average vesicle size in µm	% Entrapment efficiency
F1	7.94	90
F2	10.90	82
F3	15.67	75
CF1	-	95
CF2	-	89
CF3	-	81

The vesicle size of F2 and CF2 formulation from particle size analyzer was found to be 413.5 and 830.9 nm as shown in Fig. 3.

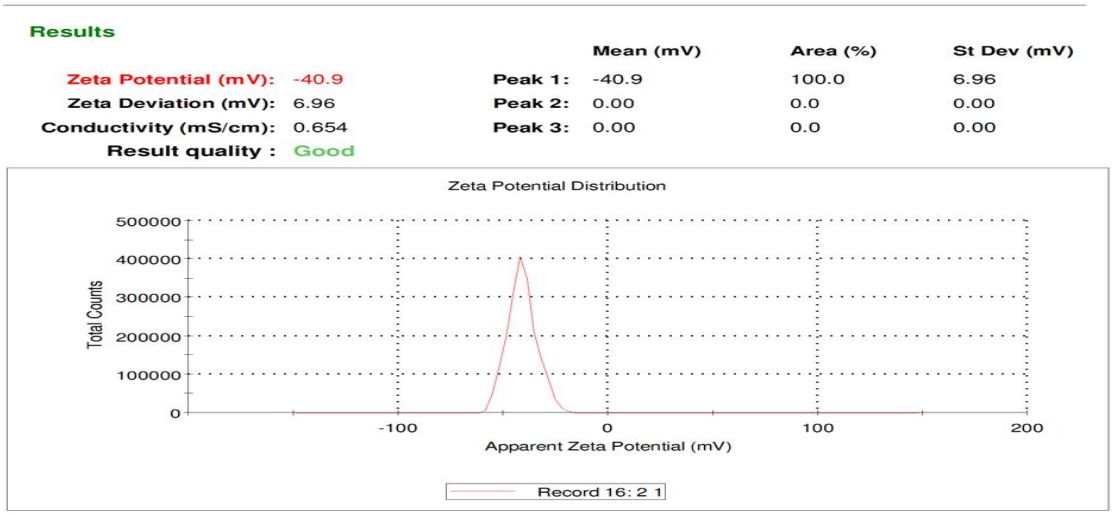

Fig7: Zeta potential of optimized liposomes formulation F2

Results

		Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV): -46.8	Peak 1:	-46.8	100.0	6.52
Zeta Deviation (mV): 6.52	Peak 2:	0.00	0.0	0.00
Conductivity (mS/cm): 0.368	Peak 3:	0.00	0.0	0.00
Result quality : Good				

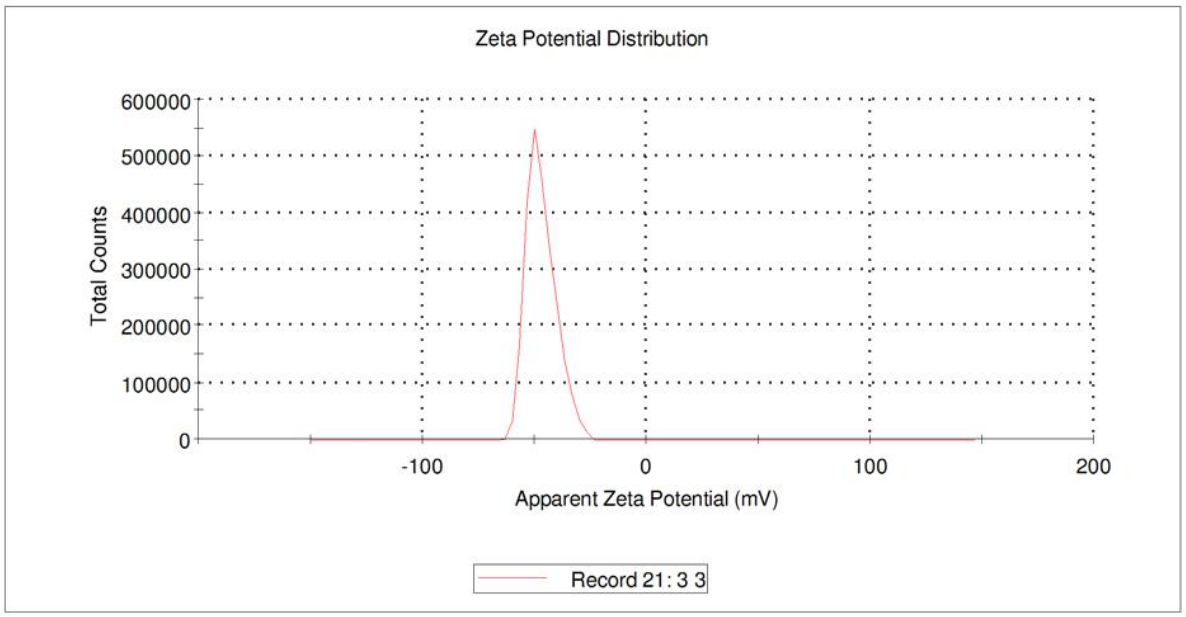

Fig.8: Zeta potential of optimized liposomes formulation CF2

Table 3: *In-vitro* study of liposomes formulation F1 to F3

Time (hrs)	% Cumulative drug release		
	F1	F2	F3
0	0	0	0
1	15.06	12.84	7.41
2	28.58	23.21	19.33
4	40.94	38.22	31.23
6	58.55	53.33	48.24
8	66.39	62.16	56.54
12	79.04	76.77	64.32

Table 4: *In-vitro* study of coating liposomes formulation CF1 to CF3

Time (hrs)	% Cumulative drug release		
	CF1	CF2	CF3
0	0	0	0
1	11.34	8.12	4.32
2	21.95	17.98	10.26
4	32.33	31.54	23.45
6	49.22	42.21	36.43
8	57.76	54.43	48.44
12	66.65	62.12	56.54

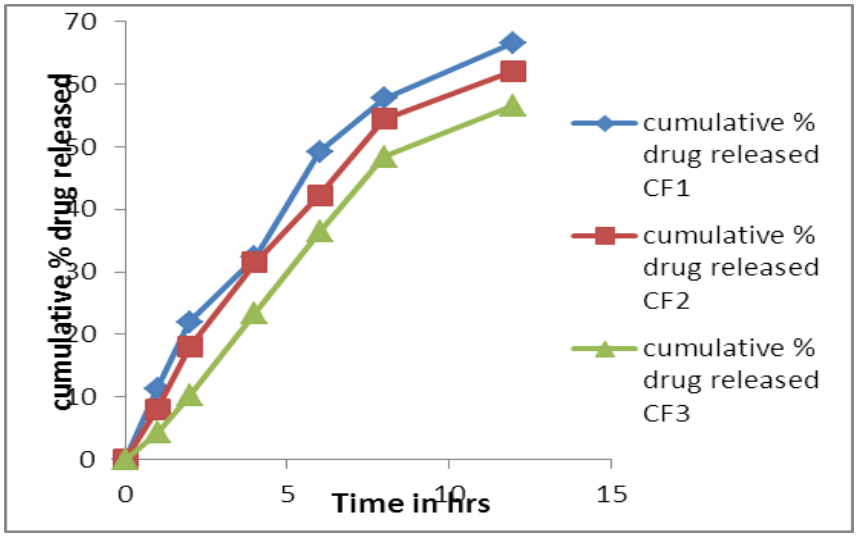

Fig.9: % Cumulative drug release of liposomes formulation from F1-F3

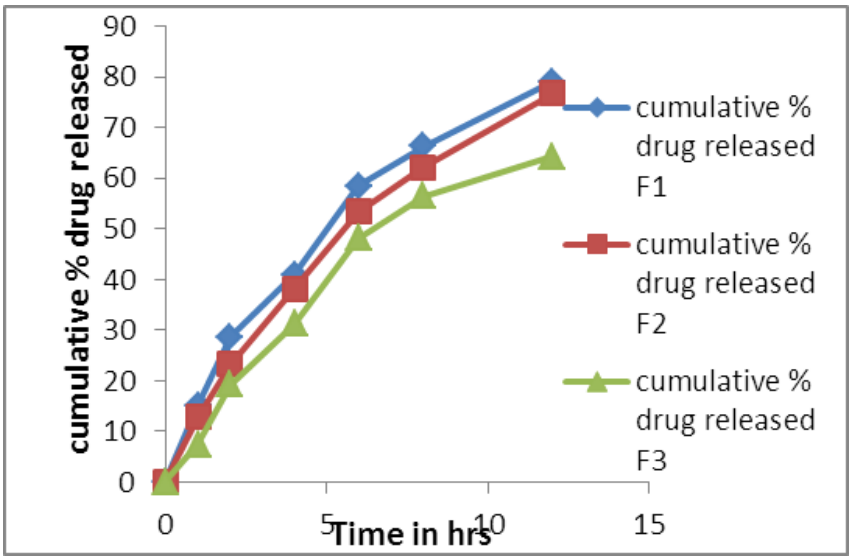

Fig.10: % Cumulative drug release of liposomes formulation from CF1-CF3

Table 5: Effect of storage condition on the stability of the optimized formulation CF2 at 4 ± 2 ℃ and 75 ± 5 % RH

Parameters	Duration in months			
	0	1	3	6
% Entrapment efficiency	90	89.95	89.91	89.86
% CDR	56.56	56	55.45	54.98

CONCLUSION

In this study, a mucoadhesive liposomal formulation of repaglinide was developed with desirable drug delivery properties. The chitosan-coated liposome had good *in vitro* stability, strong mucoadhesiveness, and enhanced cellular uptake. Therefore, the chitosan-coated

liposomal formulation appears to have the potential to improve the bioavailability of repaglinide.

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