AMERICAN JOURNAL OF PHARMTECH RESEARCH
Journal home page: http://www.ajptr.com/

Effect of Chitosan on Mucoadhesive Liposomal Delivery System For Repaglinide

Shravya Lakshmi S*[1], Parthiban S[1], Senthil kumar G P[2], Tamizh Mani T[3]
1.Department of Pharmaceutics, Bharathi College of Pharmacy, Bharathinagar, Maddur Taluk, Mandya District, Karnataka, India.

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 and 0.3 % 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 C2F2 formulation was found to be 413.5 nm, 830.9 nm and -40.9 mV, -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, CF1 and C2F2 90%, 95% and 94%. The percent drug release from F1-F3, CF1-CF3 and C2F1-C2F3 was observed as follows F1- 79.04%, F2- 76.77%, F3- 64.32%, CF1-66.65%, CF2- 62.12%, CF3-56.54% and C2F1- 59.1%, C2F2-56.56%, C2F3- 53.45% which follows first order drug release and non-Fickian diffusion mechanism. And mucoadhesive strength from CF2- 60%, C2F2- 74%.

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

*Corresponding Author Email: shravyals1993@gmail.com
Received 12 April 2017, Accepted 19 April 2017

Please cite this article as: Lakshmi SS *et al*., Effect of Chitosan on Mucoadhesive Liposomal Delivery System For Repaglinide. American Journal of PharmTech Research 2017.

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]

Liposomes are colloidal structures formed by the self-assembly of amphiphilic lipid molecules in solution. Liposomes are self-enclosed and often spherical, with the lipid bilayer encapsulating an inner solution phase. They have long been used as models for biological membranes because they have a structure and functions similar to those of biological membranes. [2]

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.[3]

Coating of the liposomal surface with chitosan may improve the stability of the liposome in the gastrointestinal tract. Furthermore, being a biocompatible, mucoadhesive, and nontoxic polymer, chitosan can have additional benefits, such as a prolonged residence time in the gastrointestinal tract and enhanced membrane permeability.[4]

Chitosan is natural cationic polysaccharide derived from deacetylation of chitin, which is, after cellulose, the most abundant polymer found in nature. Due to its biodegradable, biocompatible, mucoadhesive and non-toxic nature, it has been widely used in numerous drug delivery systems. Compared to other delivery systems, chitosan nanoparticles have a special feature. They can adhere to the mucosal surface and transiently open the tight junction between epithelial cells. Some reports have indicated that chitosan can increase membrane permeability, both *in vitro* and *in vivo*. [5]

Diabetes mellitus is a chronic metabolic disorders characterized by a high blood glucose concentration (hyperglycemia), glycosuria, hyperlipidemia, negative nitrogen balance, and sometimes ketonemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. Diabetes is now a days that affects 371 million people worldwide, and 187 million of them do not even know they have the diabetes, according to

the International Diabetes Federation (IDF). Researchers estimate that the diabetes dilemma will only increase. By 2030, they expect 552 million people will have the disease.[6, 7]

Repaglinide, a fast and short-acting Meglitinide analog was chosen as the drug candidate since it is indicated for the development of a dosage form with increased gastric retention time. 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 has melting point of 130-131 ℃ and Mol. wt. 452.58. It belongs to class BCS class-II compound with poor solubility and high permeability. These properties make it suitable for transdermal delivery. [8, 9]

Therefore, in this study, The thin film hydration method was used to prepare the Soya lecithin and cholesterol liposome which was coated with chitosan. Mucoadhesive liposomes were prepared to improve the bioavailability of orally administration peptide and possibly phytochemicals by the optimization of coating mucoadhesive material. The vesicle size before and after coating, entrapment efficiencies for model drug repaglinide, and the mucoadhesive strength of the delivery systems based on different concentration of chitosan coating were used as parameters in evaluating the optimal formulation.

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:

Preparation of liposomes[10]

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.

Coating of liposomes [11]

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
C2F1	50	500	100	0.3
C2F2	50	500	200	0.3
C2F3	50	500	300	0.3

EVALUATION PARAMETER OF MUCOADHESIVE LIPOSOMES [12,13]

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** [14,15]

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

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

Stability studies as per ICH guidelines [16]

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.

RESULTS AND DISCUSSION

FTIR spectra of pure Repaglinide showed sharp characteristic peaks at 3309.96, 3086.21, 2931.91, 2800.73, 1689.70, 1627.97 and 1219.05 Physical mixture showed the entire characteristic peaks of pure drug, confirmed no interaction between the drug and excipients. Comparative studies of FTIR graphs are showed in Figure 1-2. The surface morphology was studied by Scanning electron microscopy (SEM). The SEM photographs of optimized liposomes formulation F2 and C2F2 as shown in Fig. 3-4. The porous structure in the images of Fig confirmed 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 liposome of 413.5 nm and coating of liposomes in concentration of 0.3% C2F2 is 830.9 nm. Shown in the fig.5-6. The % entrapment efficiency was found to decrease with increasing the cholesterol concentration. % Entrapment efficiency of selected formulation F2 , CF2, C2 F2 were found to be 82 %, 89 % and 90 %. It is shown in the Table 2. Zeta potential of optimized formulation F2 and C2F2 of Repaglinide liposomes as shown

in Figure 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 .3-5 and Figure 9-11. *In vitro* drug release of all the formulation was performed using dialysis tube diffusion technique using in 0.1N HCl pH 1.2 as medium. From the results we observed that the release of drug from uncoated and coated liposomes were 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 and C2F1 to C2F3 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 and C2F3 was showed 53.45% which was lower among the formulation C2F1 to C2F3. The percentage of mucoadhesive strengths was calculated by Equation (1) and the results demonstrated that the higher polymer-coated liposomes have higher strength. After 3 hrs of incubation, more than 60% of the originally entrapped Repaglinide was retained on the intestinal mucosa in the case formulation CF2 and 74 % was retained on the intestinal mucosa for chitosan coated liposomes C2F2 and the results are shown in table 6. Percent mucoadhesion was calculated and found the mucoadhesive strength was 60% and 74% which showed sufficient mucoadhesive property.

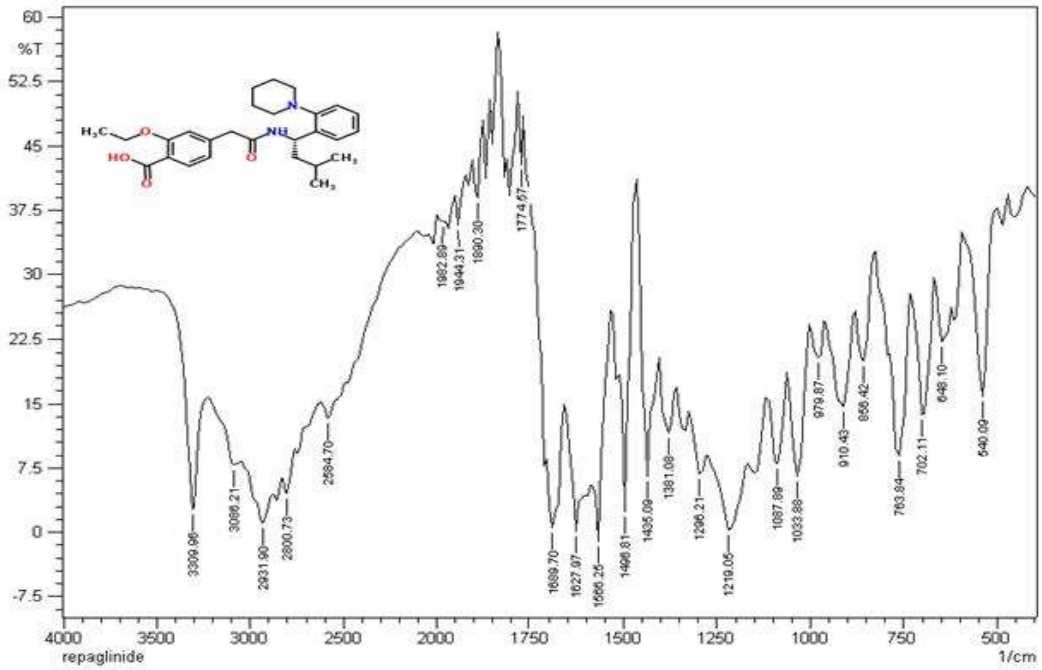

Figure.1: FT-IR Spectroscopy of Repaglinide

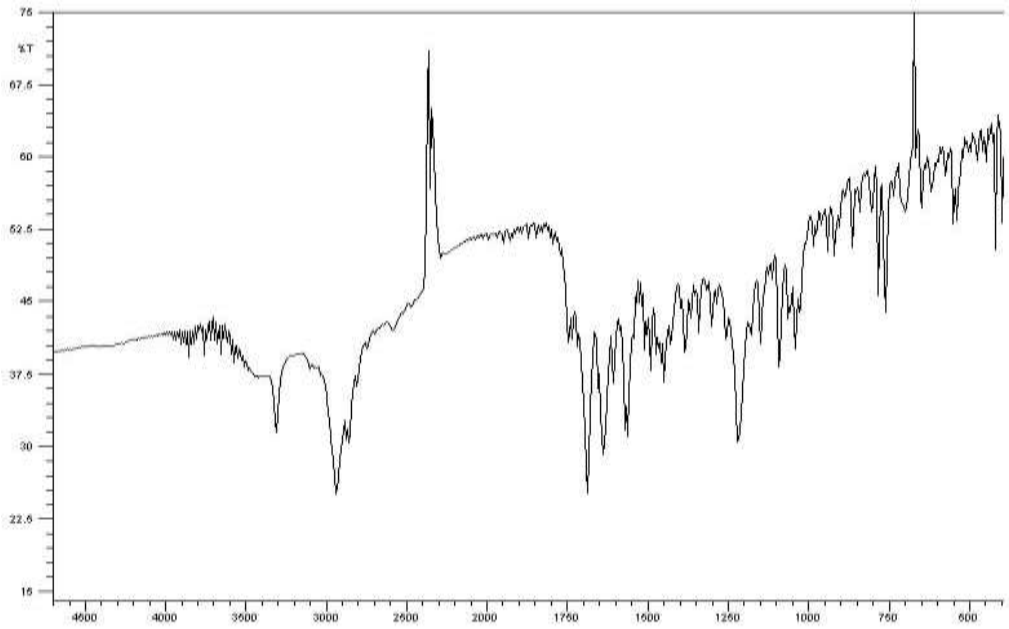

Figure.2: FT-IR Spectroscopy physical mixture of Repaglinide+Soya Lecithin+Cholesterol

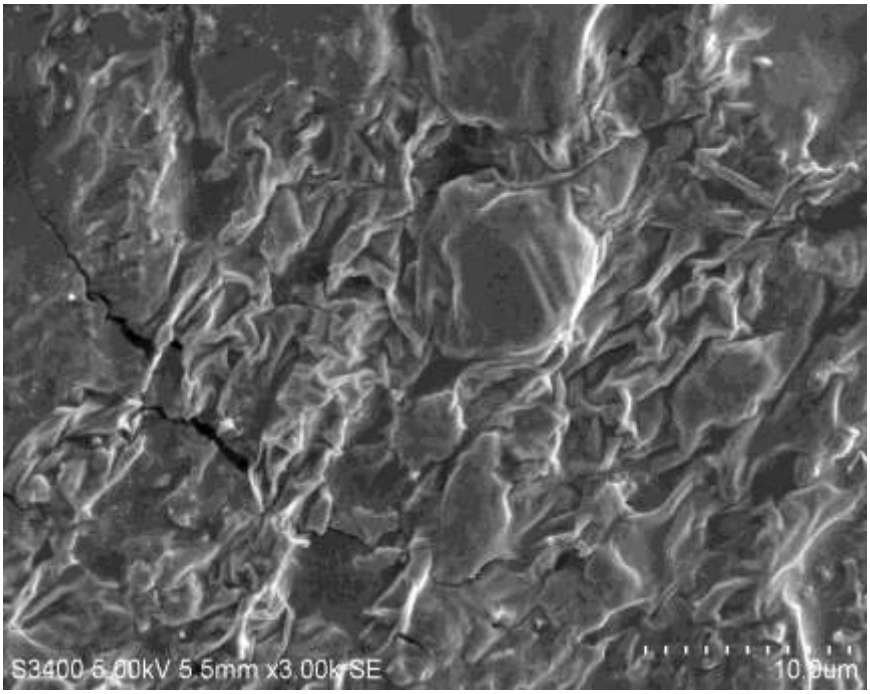

Figure.3: Scanning Electron micrograph of liposomes formulation F2

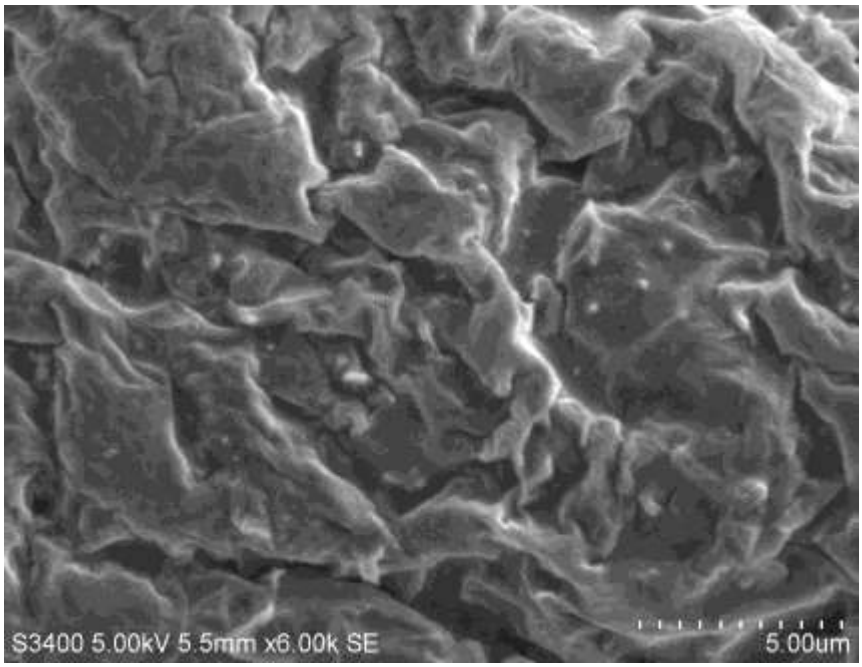

Figure.4: Scanning Electron micrograph of liposomes formulation C2F2

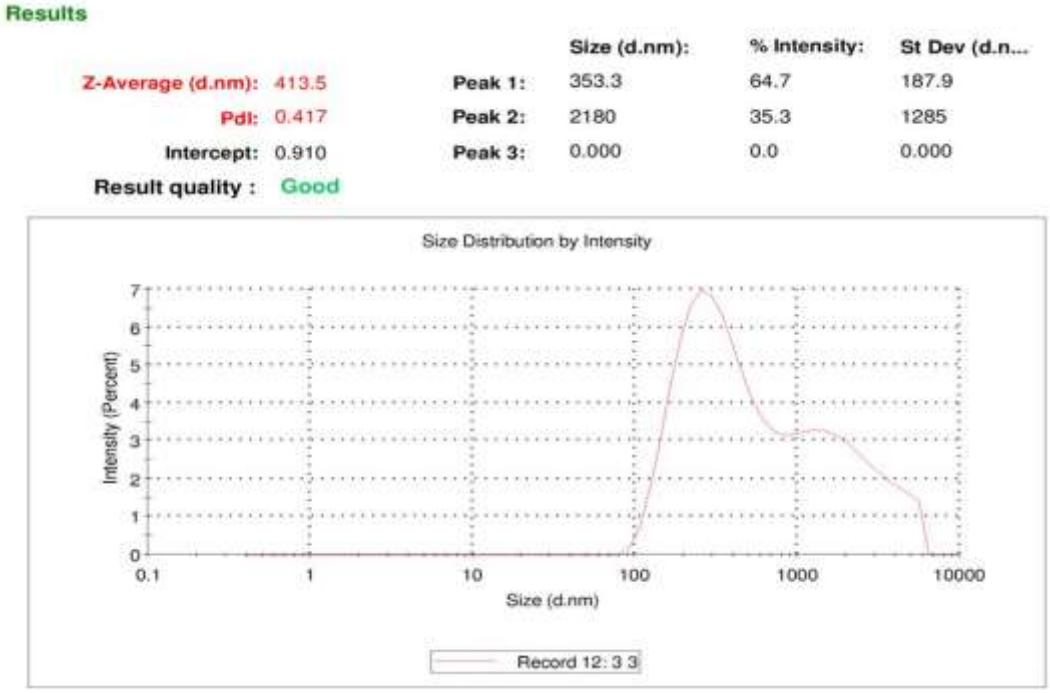

Figure.5: Particle size data for liposome formulation F2

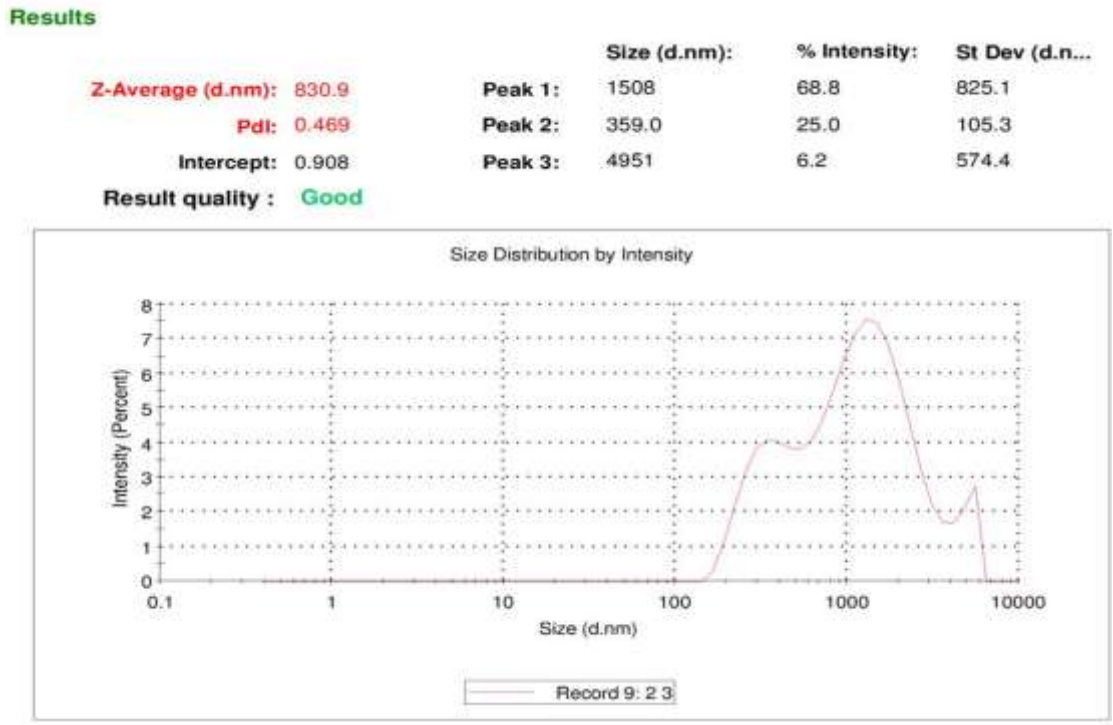

Figure.6: Particle size data for coating of liposome formulation C2F2

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
C2F1	-	94
C2F2	-	90
C2F3	-	80

The vesicle size of F2 and C2F2 formulation from particle size analyzer was found to be 413.5 and 830.9 nm as shown in Figure 3

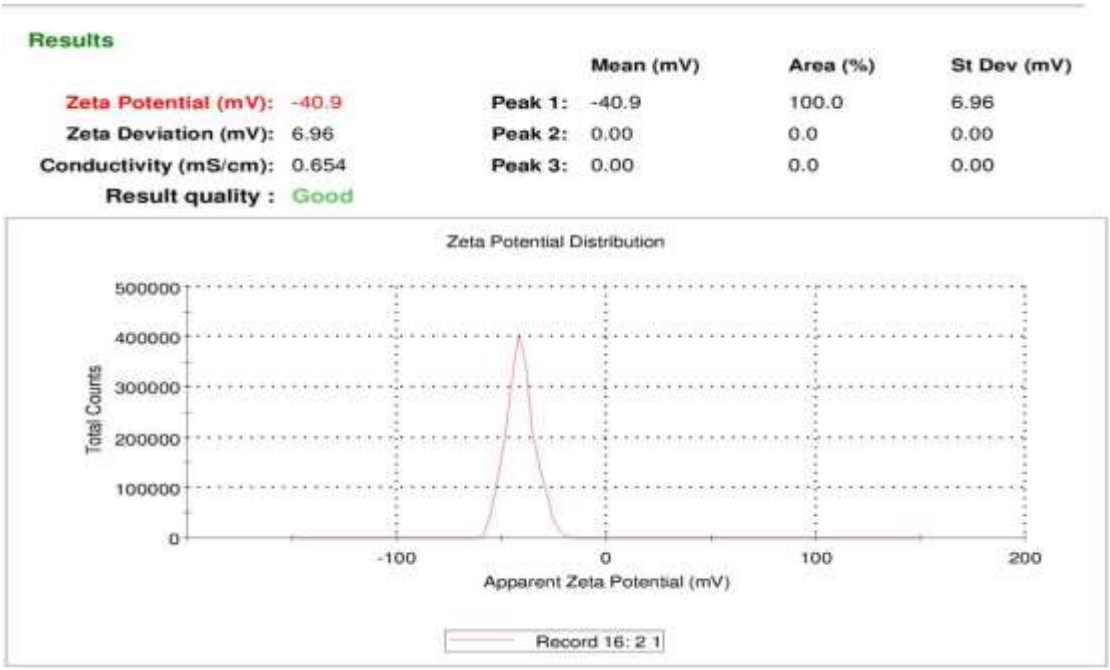

Figure 7: Zeta potential of optimized liposomes formulation F2

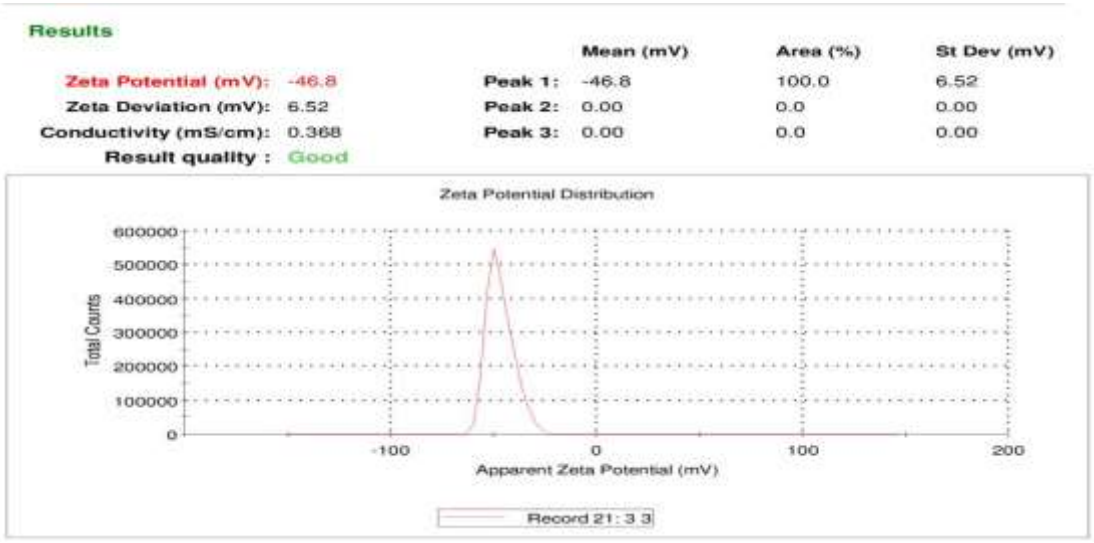

Figure.8: Zeta potential of optimized liposomes formulation C2F2

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

Table 5: *In-vitro* study of coating liposomes formulation C2F1 to C2F3

Time (hrs)	% Cumulative drug release		
	C2F1	C2F2	C2F3
0	0	0	0
1	8.43	6.84	3.9
2	18.67	13.56	8.8
4	28.56	27.88	20.9
6	42.65	36.66	34.56
8	51.97	47.86	47.12
12	59.1	56.56	53.45

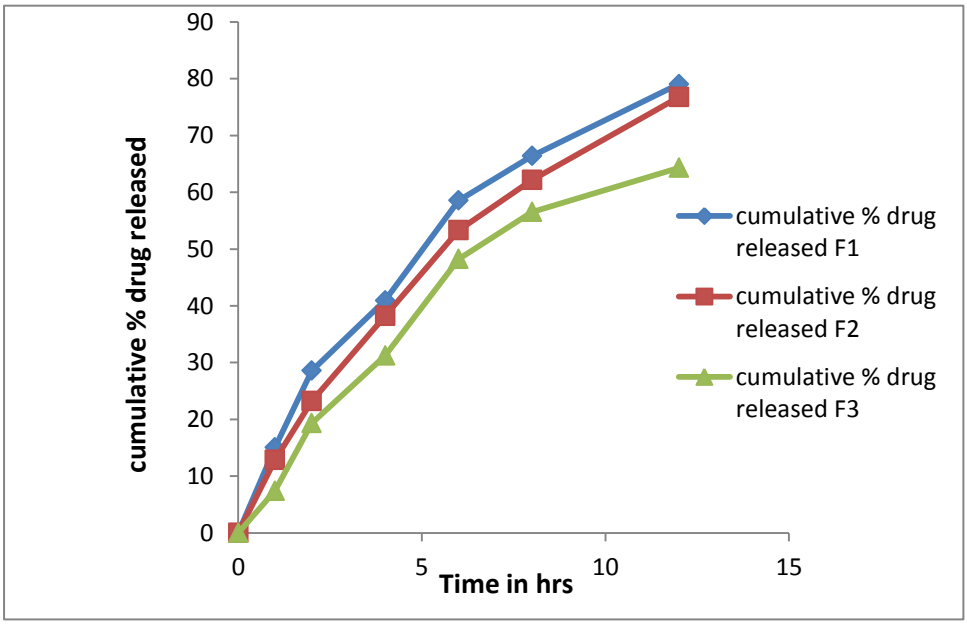

Figure.9: % Cumulative drug release of liposomes formulation from F1-F3

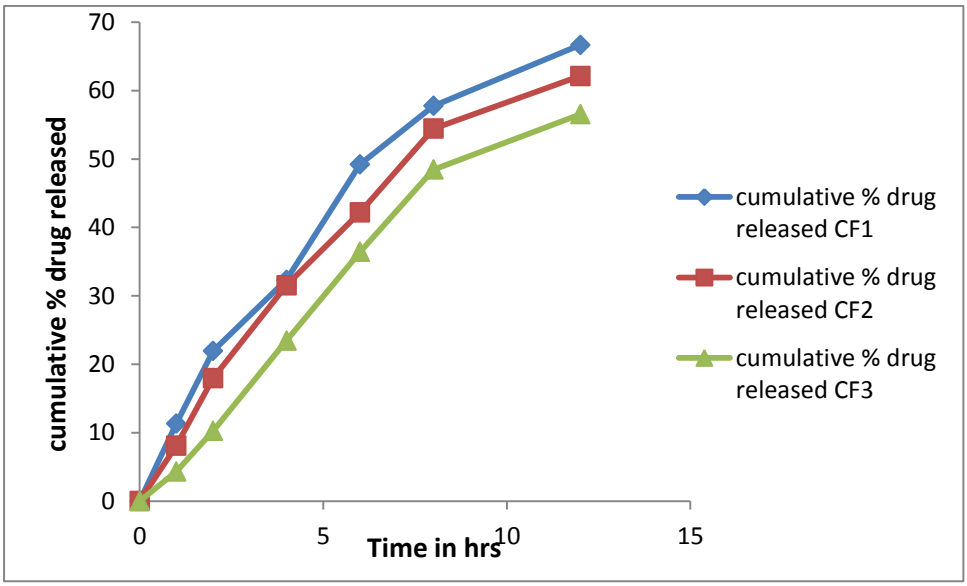

Figure.10: % Cumulative drug release of liposomes formulation from CF1-CF3

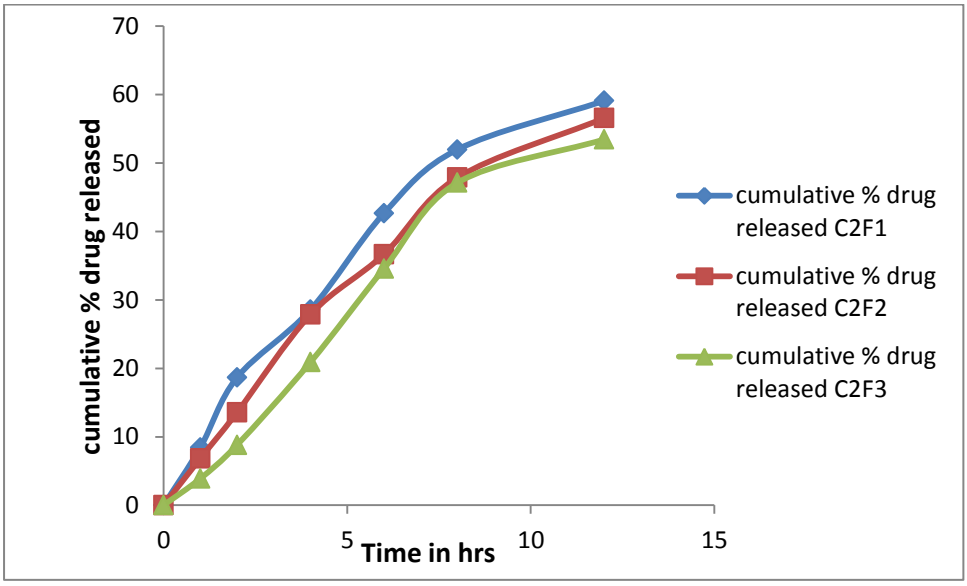

Figure.11: % Cumulative drug release of liposomes formulation from C2F1-C2F3

Table 6: 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 liposomes exhibited good mucoadhesive property *in vitro* test.

Repaglinide release from these mucoadhesive liposomes was slow and extended over longer periods of time and depended on composition of the coat. Drug release was diffusion controlled and followed first order kinetics with non-Fickian diffusion. These mucoadhesive Liposomes are, thus, suitable for oral controlled release of repaglinide.

REFERENCE

1. Takeuchi H, Yamamoto H, Kawashima Y. Mucoadhesive nanoparticulate systems for peptide drug delivery. Adv Drug Deliv Rev. 2001 Mar 23;47(1):39-54.
2. Imura T, Otake K, Hashimoto S, Gotoh T, Yuasa M, Yokoyama S, Sakai H, Rathman JF, Abe M. Preparation and physicochemical properties of various soybean lecithin liposomes using supercritical reverse phase evaporation method. Colloids and Surfaces B: Biointerfaces. 2003 Feb 1;27(2):133-40.
3. Takeuchi H, Yamamoto H, Niwa T, Hino T, Kawashima Y. Enteral absorption of insulin in rats from mucoadhesive chitosan-coated liposomes. Pharm Res. 1996 Jun 1;13(6):896-01.
4. Jung IW, Han HK. Effective mucoadhesive liposomal delivery system for risedronate: Preparation and *in vitro/in vivo* characterization. Int J Nanomedicine. 2014;9:2299-2306.
5. Wu Y, Yang W, Wang C, Hu J, Fu S. Chitosan nanoparticles as a novel delivery system for ammonium glycyrrhizinate. Int J Pharm. 2005 May 13;295(1):235-45.
6. Tripathi KD. "Essentials of medical pharmacology." 6th *ed*. New Delhi: Jaypee brothers medical publishers; 2003.
7. Michelle Castillo. CBS News. [Internet] 2012 Nov 14 [cited 2016 Mar 10]. Available from: http://www.cbsnews.com/news/371-million-people-have-diabetes-globally-about-half-undiagnosed/
8. Yadav AP. Satheesh Madhav NV. "Development and evaluation of novel Repaglinide biostrip for translabial delivery". Int Res J Pharm. 2013;4(5):198-202.
9. Vijayan V, Jayachandran E, Anburaj J, Srinivasa Rao D, Jayaraj Kumar K. Transdermal delivery of Repaglinide from solid lipid nanoparticles in diabetic rats: *In vitro* and *in vivo* studies. J Pharm Sci Res. 2011;3(3):1077-81.
10. Patel DB, Patel JK. Liposomal drug delivery of metronidazole for the local treatment of vaginitis. Int J Pharm Sci Nanotechnol. 2009;2(1):248-57.
11. Bang SH, Hwang IC, Yu YM, Kwon HR, Kim DH, and Park HJ. Influence of chitosan coating on the liposomal surface on physicochemical properties and the release profile of nanocarrier systems. J Microencapsul. 2011 Nov 1;28(7):595-04.

12. Mady MM, Darwish MM, Khalil S, Khalil WM. Biophysical studies on chitosan-coated liposomes. Eur Biophys J. 2009 Oct 1;38(8):1127-33.
13. Mady MM, Darwish MM. Effect of chitosan coating on the characteristics of DPPC liposomes. JAR. 2010 Jul 31;1(3):187-91.
14. Chowdary KP, Rao YS. Design and *in vitro* and *in vivo* evaluation of mucoadhesive microcapsules of glipizide for oral controlled release: a technical note. AAPS Pharm Sci Tech. 2003 Sep 1;4(3):1-6.
15. Rao S, Varalakshmi ST, Chandana RV. Design and *in vitro* evaluation of mucoadhesive microcapsules of aceclofenac for oral controlled release. Int J Pharm Pharm Sci. 4(5):305-308.
16. Liu N, Park HJ. Chitosan-coated nanoliposome as vitamin E carrier. J Microencapsul. 2009 May 1;26(3):235-42.