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Formulation Development and Optimization by 2X3 Factorial Design of Novel Prednisone Loaded Mucoadhesive Liposomal Formulation

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ABSTRACT

The aim of the present investigation was to design a mucoadhesive liposomal system of Prednisone for the treatment of arthritis, severe allergic reaction multiple sclerosis that is capable of delivering entrapped drug over an extended period of time. Mucoadhesive liposomal formulations were prepared by different concentration of lecithin and cholesterol by thin film hydration technique followed by coating of liposomes by 0.2 % w/v of chitosan and Liposomes were evaluated for entrapment efficiency, particle size, zeta potential, surface morphology and *in vitro* drug release and stability study of coated formulation. Particle size of the F4, F5 and F6 formulation was found to be 212 nm, 131 nm and 340 nm respectively and zeta potential were -164.9 mV, 165 mV and -9.6 mV, respectively. Highest entrapment efficiency was observed in the ranged of 83 % to 98% for formulation F1 -F8 and CF1-CF2 were 90.87 % to 94.68%. The percent drug release from F1-F8 was varied and affected by drug loading, soyalecithin and cholesterol concentration and followed non-Fickian diffusion mechanism. 2x3 factorial design were applied and studied the effect of parameter on entrapment efficiency and *in vitro* drug release at 2hrs, 6hrs, 12 hrs by using QI Macros^R software.

Keywords: Prednisone, mucoadhesive liposome, thin film hydration method, stability studies, *in vitro* release.

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INTRODUCTION

Mucoadhesion delivery system is designed to prolong the residence time of the dosage form at the site of application or absorption. The delivery system is facilitating intimate contact of the dosage form with the underlying absorption surface to improve and enhance the bioavailability of drugs.

The oral route remains to be the most convenient and comfortable way of drug administration, including peptide delivery. However, peptide drugs are readily degraded under the low pH of the gastric medium and by various proteolytic enzymes in the gastrointestinal (GI) tract. One of the most promising strategies in developing mucoadhesive particulate systems is surface modification, or coating, of the drug carrier particles with mucoadhesive Polymers.

The Mucoadhesion can be defined as a state in which two components, of which is used to describe the biological origin are held together for extended period of time by the help of interfacial force. The main advantage of oral mucoadhesive drug delivery system is to prolongs the residence time, rapid absorption because of enormous blood supply and good blood flow rates and increase the drug bioavailability.

Prednisone is a corticosteroid generally used alone or with other medications to treat the symptoms like arthritis, severe allergic reactions, multiple sclerosis, lupus.² Prednisone can also be used to treat pneumonia in patients with acquired immunodeficiency syndrome (AIDS). But, treatment of HIV positive patient with prednisone required as the drug is immunosuppressive and can increase the risk of opportunistic infections.³ Prednisone dose is 2.5-15 mg two to four times daily in adults and 2-14 mg/kg/day in four divided doses in children.⁴ Prednisone is classified as a class I drug of the Biopharmaceutical Classification System (BCS) and is highly soluble and permeable.

Prednisone is rapidly absorbed across the gastrointestinal tract following oral administration. The prednisone shows extensive protein binding with plasma proteins, albumin and transporting. Prednisone is metabolized by the liver to the active metabolite prednisolone, which is then further metabolized to inactive compounds. These inactive metabolites, as well as a small portion of unchanged drug, are excreted in the urine. Prednisone has plasma half-life of 1.7-4.1 hr. and biological half-life is 18–36 hr.^{5,6}

Hence, in the present investigation mucoadhesive based liposomal approach has been proposed to ensure stable Prednisone level with reduced dose throughout the treatment period which may decrease the occurrence of serious side effects.

MATERIALS AND METHOD

Prednisone was gifted from micro labs. 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. Phosphate buffer PH 7.4 were prepared as described in the Indian pharmacopoeia (1996).

METHODS

Preparation Of Prednisone Mucoadhesive Liposome.[7,8]

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. Further the optimized liposomal formulation were coated with 0.2% w/v chitosan to produce mucoadesive liposomes.

Table 1: Factorial design for the formulation of uncoated liposomes

Factorial design: 2×3 A: Drug (50mg, 100mg); B: Soya lecithin (200mg, 500mg); C: cholesterol (50mg, 100mg)

Formulation code	A	B	C
F1	+	-	-
F2	+	-	+
F3	+	+	+
F4	+	+	-
F5	-	-	-
F6	-	-	+
F7	+	+	+
F8	-	+	-

F3 and F7 formulation were taken for coating and coded as CF1, CF2 respectively and were evaluated.

EVALUATION PARAMETER OF MUCOADHESIVE LIPOSOMES. [9,10]

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

Invitro diffusion study

In-vitro release pattern of liposomal suspension was carried out in dialysis bag method. Prednisone liposomal suspension equivalent to 10 mg was taken in the dialysis bag and the bag was placed in

a beaker containing 250ml of phosphate buffer pH 6.8. The beaker was placed over magnetic stirrer having stirring speed of 100 RPM and the temperature was maintained at $37 \pm 0.5^\circ\text{C}$. 1ml sample were withdrawn periodically and were replaced by fresh buffer. The sample were assayed by UV spectrophotometer at 243.60 nm using phosphate buffer pH 6.8 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 Prednisone. 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, 0.2% and 0.4% (w /v) chitosan-coated liposomes, liposomes were spread onto each wet-rinsed tissue specimen and immediately incubated at 37°C . 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 non adhered drug, 500 μl aliquots were taken and liposomal lipids were dissolved by methanol. It was measured by a UV spectrophotometer. The concentration of prednisone eluted in the phosphate buffer pH 6.8 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 (1)

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

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 $4 \pm 2^\circ\text{C}$ and $75 \pm 5\%$ RH in stability chamber. Regular tested for % entrapment, vesicle size and drug release were fixed as physical parameters for stability testing.

RESULTS AND DISCUSSION

The λ_{max} of the Prednisone in Phosphate buffer pH 6.8 was found to be 243.60 nm and the spectra was shown in Figure 1. Standards calibration curve of Prednisone obeys the Beer's law in concentration range of 0 - 15 $\mu\text{g/ml}$ in Phosphate buffer pH 6.8 with regression of coefficient (r^2) of 0.999 and slope (n) of 0.050. This showed linear relationship between concentration and absorbance as shown in Figure 2. The melting point of the drug sample was found to be 234°C by

Thiels tube method and 239.41°C by DSC method which complied with IP standards, thus indicating the purity of drug, is shown in the DSC Figure 3. FTIR spectra of pure Prednisone showed sharp characteristic peaks 1622 cm^{-1} , 1707.66 cm^{-1} , 1666.2 cm^{-1} and 3289 cm^{-1} and Physical mixture showed the entire characteristic peaks of pure drug, confirmed no interaction between the drug and excipients. Comparative studies of FTIR graphs are shown in Figure 4-5.

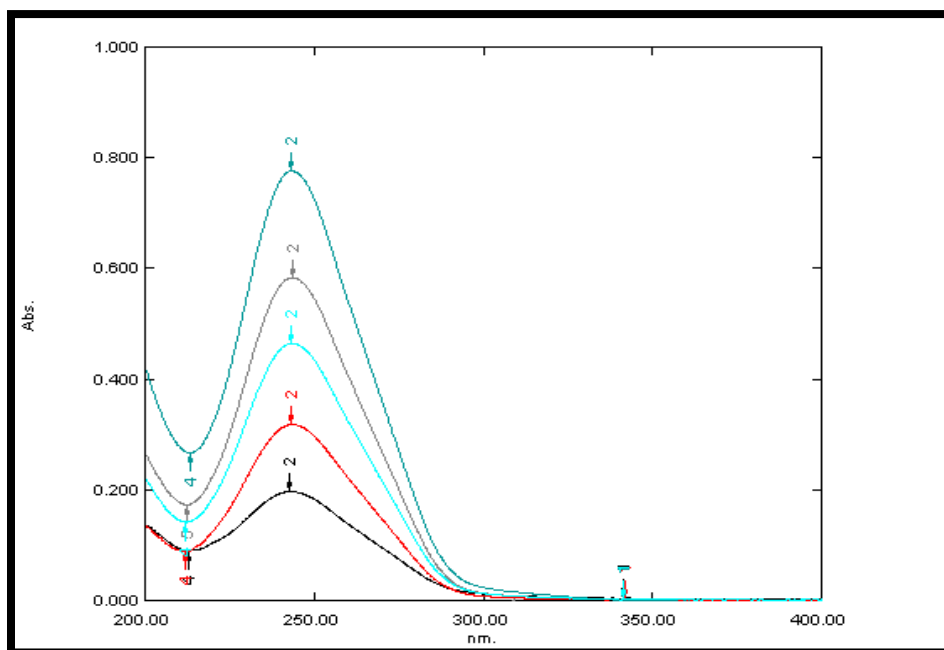


Figure 1: UV Spectra of Prednisone at 3-15 $\mu\text{g/ml}$ concentration in Phosphate buffer pH 6.8

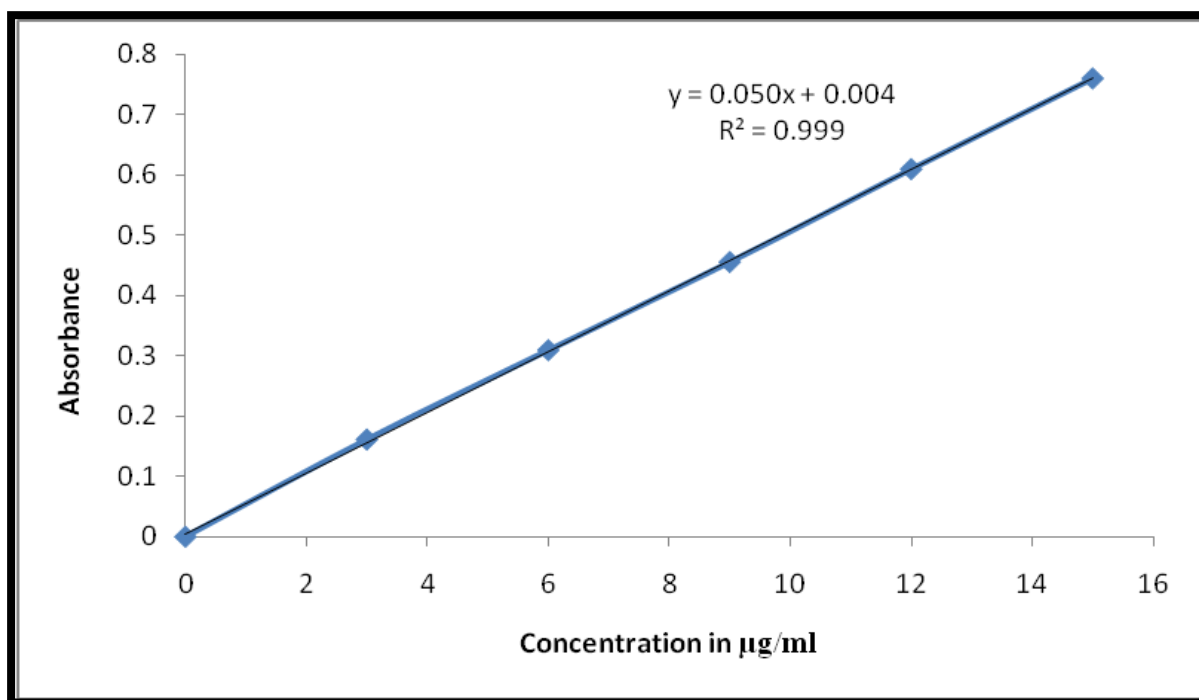


Figure 2: Plot of standard calibration curve of Prednisone

The surface morphology was studied by Scanning electron microscopy (SEM). The SEM photographs of liposomes formulation F2, F4 and F5, F8 were shown in Figure 6-9. The porous structure in the images of Figure confirmed the formation liposomes that are confirmed the incorporation of lipids and drug. The vesicles were observed by optical microscopy and sizes were measured for 100 vesicles and percentage of vesicle size distribution of different sizes were analyzed and results were depicted in Figure 10. For the formulation F1 and F5, where the concentration of Soya lecithin and cholesterol kept at low level, the maximum percentage (65%) of vesicles lies in the size range of 0.0 – 1.0 μm 20 % lies in the range of 1.0 -2.0 μm for the formulation F1 and 85 % and 10 % vesicle lies in the range of 0.0 – 1.0 μm and 1.0 -2.0 μm respectively for the formulation F2. But we have observed increase in particle size as more number of vesicle have shifted to higher range for the formulation F5 (73 % vesicle in 1.0 - 2.0 μm and 20 % vesicle in 2.0 - 3.0 μm) and F6 (65 % vesicle in 1.0 - 2.0 μm and 15 % vesicle in 2.0 - 3.0 μm). The results also showed that further increase in vesicle size when both soya lecithin and cholesterol have been kept at higher level for the formulation F3(55 % vesicle in 1.0 - 2.0 μm and 25 % vesicle in 2.0 - 3.0 μm) and F7(70 % vesicle in 1.0 - 2.0 μm and 13 % vesicle in 2.0 - 3.0 μm) shown a sharp increase in the number of vesicle to higher range. Further we have observed that for the formulation F4 (30 % vesicle in 0.0 - 1.0 μm and 63 % vesicle in 1.0 - 2.0 μm and F8 (35 % vesicle in 0.0 - 1.0 μm and 40 % vesicle in 1.0 - 2.0 μm) where soya lecithin have been kept at high level and cholesterol at low level the vesicle size have been distributed in between 0.0 to 0.2 μm but smaller than F5 and F7 but greater than F1 and F5. The vesicle size have also been studied by Microgram particle size analyzer for the formulation F4 (212 nm), F5 (131 nm) and F6 (340 nm) respectively. The results revealed that the concentrations of soya lecithin and cholesterol have significant effect on the vesicle size and increase the level increases the vesicle size.

Entrapment efficiency was observed in the ranged of 83 % to 98% for formulation F1 – F8 and 90.87 % to 94.68 for coated liposomal formulations respectively. The change in soya lecithin and cholesterol had a significant effect on entrapment of Prednisone. The % entrapment efficiency was found to decrease with increasing the cholesterol concentration. The Entrapment efficiency of all the formulation are shown in the Figure 11-12 and % Entrapment efficiency of selected formulation F3 and CF1 were found to be 82.67 % and 90.87 %, respectively. Further the effect of drug, soya lecithin and cholesterol on % EE have been studied by 2x3 factorial designs for 2 hrs, 6hrs and 12 hrs time release and observed following results as shown in the Table.5 and Figure 22. Effect of drug- increasing drug from low level to high level the % EE have decreased from 90.76

% to 88.74 % i.e. the value of %EE have been reduced by 2.01% in uncoated liposomal formulation. Effect of Soya lecithin on liposomal formulation was observed a positive effect i.e. increasing the level of Soya lecithin increases the %EE from 89.1% to 90.4 % (1.3% increased). The results also revealed that there is no interaction between the factors except that Drug vs soya lecithin showed interaction at high level.

Zeta potential of optimized formulation F4, F5 and F6 of Prednisone liposome as shown in Figure 13-15. and it was found to be -164.9 mV, 165 mV and -9.6 mV, respectively. The results revealed that the formulation F4 and F5 have higher value of zeta potential and stable but the formulation F6 showed very low level of zeta potential which indicate the formulation were poorly stable for the storage. *In vitro* release behavior of all formulations is summarized in and Figure 16-18. *In vitro* drug release of all the formulation was performed using dialysis tube diffusion technique using in phosphate buffer pH 6.8 as medium. From the results we observed that the releases of drug from coated and coated liposomes were varied according to concentration of soya lecithin and cholesterol.

The difference in the amount of drug diffused through cellophane membrane from formulations F1 to F4 and F5 to F8 attributed to variation 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 68.97 % which was lower among the formulations F1 to F5 and F7 was showed 66.98 % which was lower among the formulation F5 to F8. The percent drug release for F1 – F8 was observed at the end of 12 hrs are as follows 88.57%, 73.31%, 72.35% 76.29% for F1, F2, F3, F4 formulation and 80.61 %,69.68%,76.98%,71.58% for formulation F5,F6,F7,F8 respectively. However all the formulation release the drug in a controlled manner for 12 hrs. From these results we observed that keeping the level of both soya lecithin and cholesterol higher percentage of drug was released 88.57%(F1) and 80.61%(F5) due to less integrity of vesicle membrane the drug can diffused out easily. Further the results were also showed that keeping the level of both soya lecithin and cholesterol at high level the drug release from the formulation have been decreased 72.35%(F3) 76.98%(F7), due to high integrity membrane of vesicle and increased vesicle size. The *in vitro* release profile of coated formulation were also resemble to the respective liposomal formulation (F3 is coated and coded as CF1 and F7 is coated and coded as CF2) in which we observed 81.86% and 72.37% drug release for CF1 and CF2 formulation respectively. Further the effect of drug, soya lecithin and cholesterol on % CDR have been studied by 2x3 factorial design for 2 hrs, 6hrs and 12 hrs time release and observed following results as shown in the Figure 23-25. Effect of drug- increasing level of drug

from low level to high level the % CDR in 2hrs, 6hrs and 12hrs have increased from 17.95 % to 20.65%, 52.17 % to 53.18% and 74.71% to 82.29% respectively. In liposomal formulation % increases in the % CDR when the drug is level is changed from low to high were observed as follows 2.70% (2hrs), 1.01 % (6hrs) and 7.58 % (12hrs). The results suggested that increasing the drug from low to high increases the drug release from the formulation. Effect of soya lecithin on liposomal formulation showed the increasing the level of soya lecithin from low to high level, increasing the %CDR from 18.8 % to 19.8%, 47.9% to 57.5%, 78.0% to 79.0% for 2 hrs, 6 hrs and 12 hrs respectively. The differences in positive effect on the drug release were observed 1.0 %, 9.6% and 1.0 % for 2 hrs, 6 hrs and 12 hrs respectively. The results showed that the effect of soya lecithin in altering the release of drug was very low as compared to the effect of drug. Effect of cholesterol on liposomal formulation was observed that as the level of cholesterol changed from low to high level resulted negative effect on the drug release i.e decreasing the the %CDR from 22.0 % to 16.6%, 53.3% to 52.0%, 79.3% to 77.7% for 2 hrs, 6 hrs and 12 hrs respectively. These results suggested that as we increasing level of cholesterol the membrane of the liposome vesicles become more rigid and retard the release of drug. Further we observed from the results drug Vs cholesterol and soyalecithin Vs cholesterol didn't show any in interaction effect. But, drug vs soya lecithin produced interaction effect when both drug and soyalecithin have been kept at low level at 2 hrs drug release. Similar effect have been observed at 12 hrs except that soyalecithin vs cholesterol produced strong interaction effect. We got also a mixed response at 6 hrs. This difference in the effect may be due to the changes in vesicle size and rigidity in the surface level on the liposomal formulation.

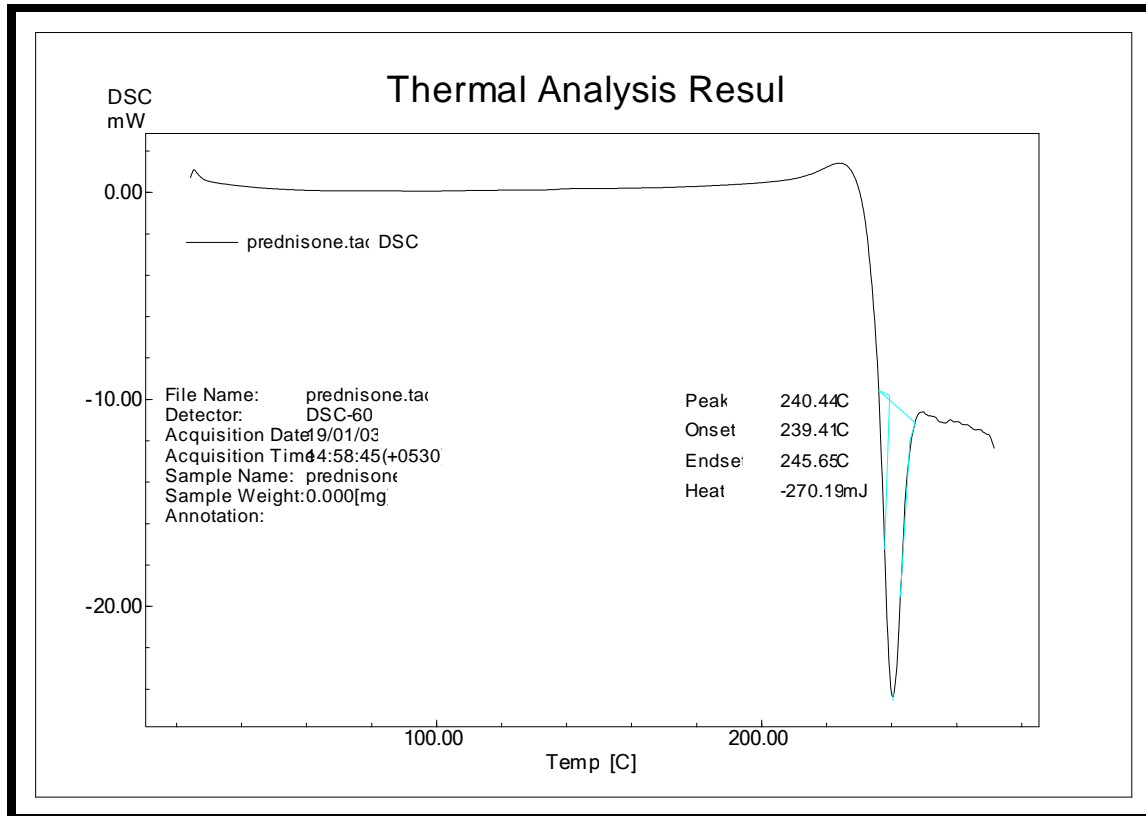


Figure 3: DSC Thermograph of Prednisone

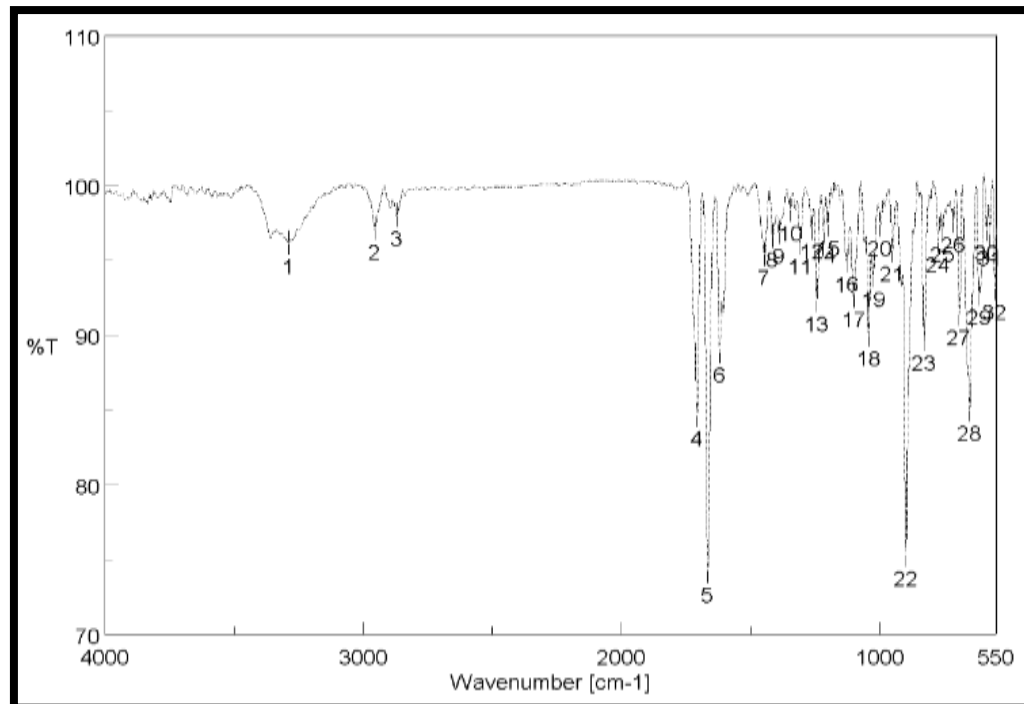


Figure 4: FT-IR Spectroscopy of Prednisone

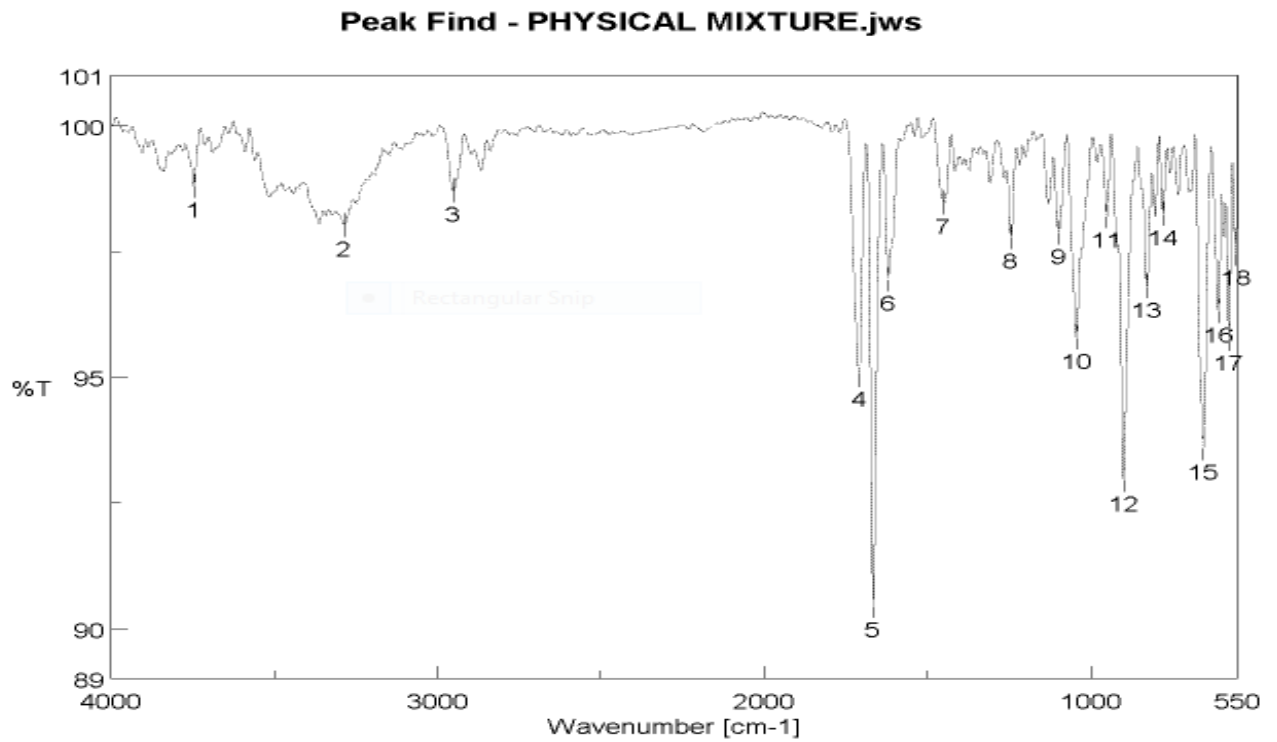


Figure 5: FT-IR Spectroscopy physical mixture of Prednisone+SoyaLecithin+Cholesterol

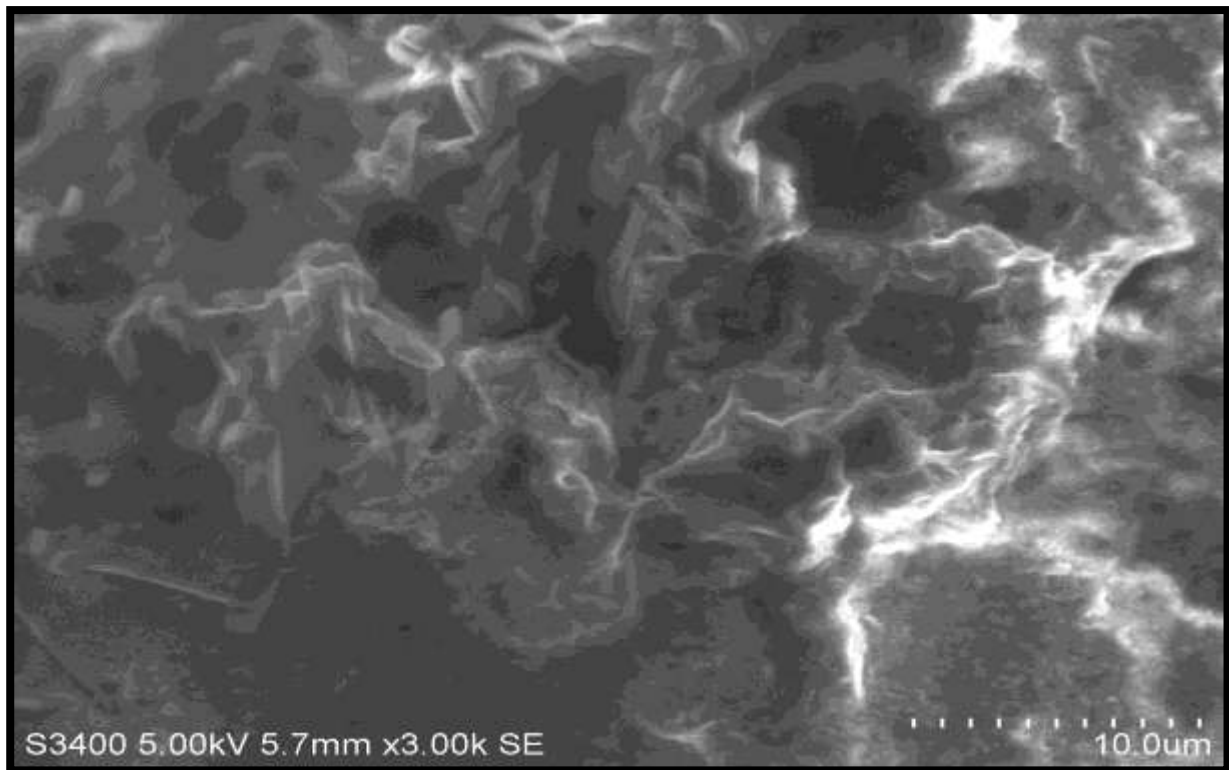


Figure 6: SEM of liposomes formulation F2

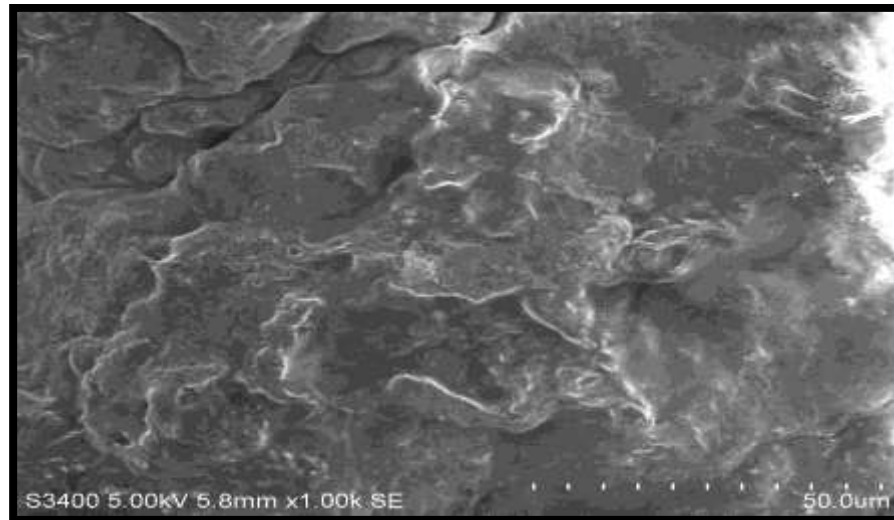


Figure 7: SEM of liposomes formulation F4

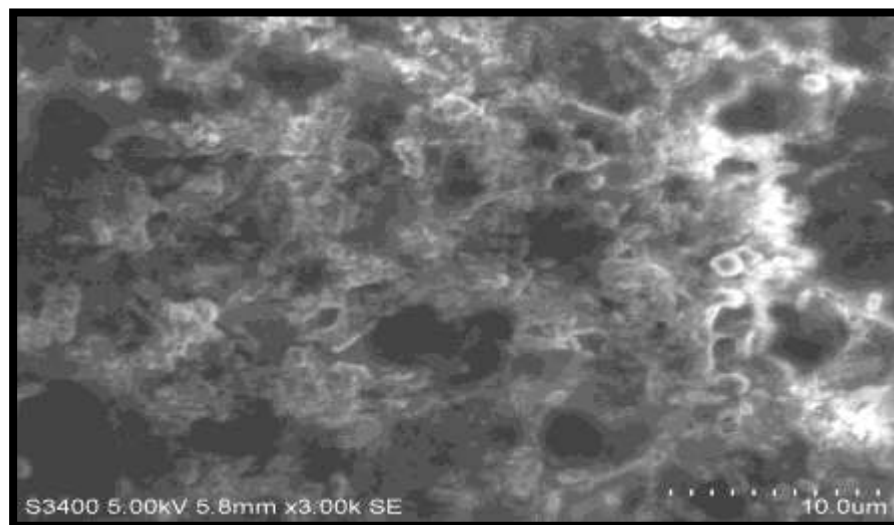


Figure 8: SEM of liposomes formulation F5

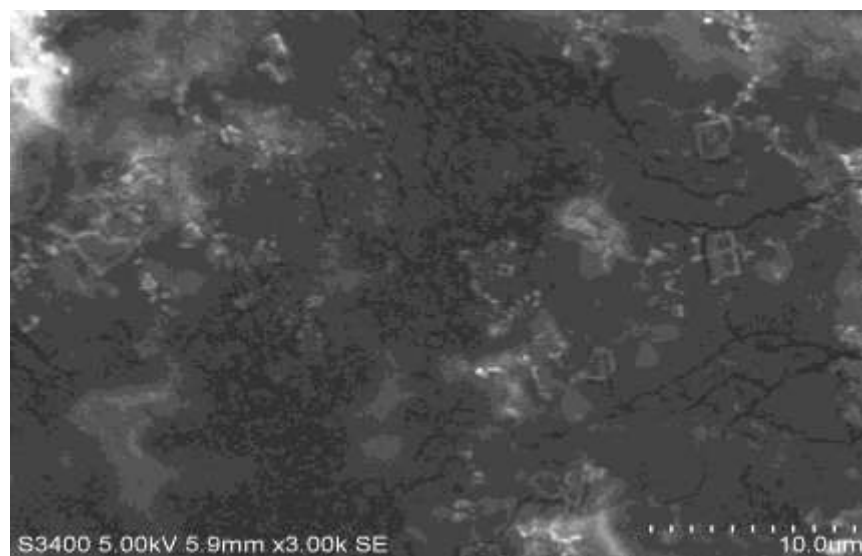


Figure 9: SEM of liposomes formulation F8

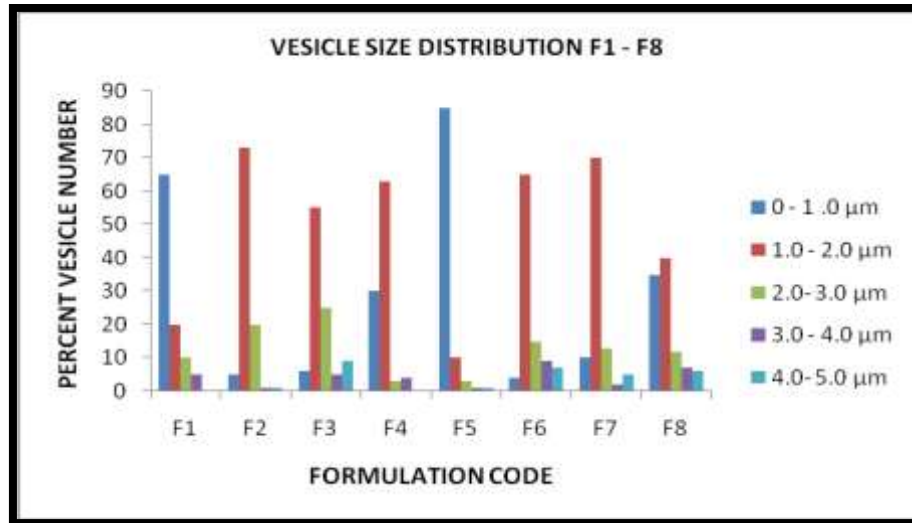


Figure 10: Vesicle size distribution of F1-F8

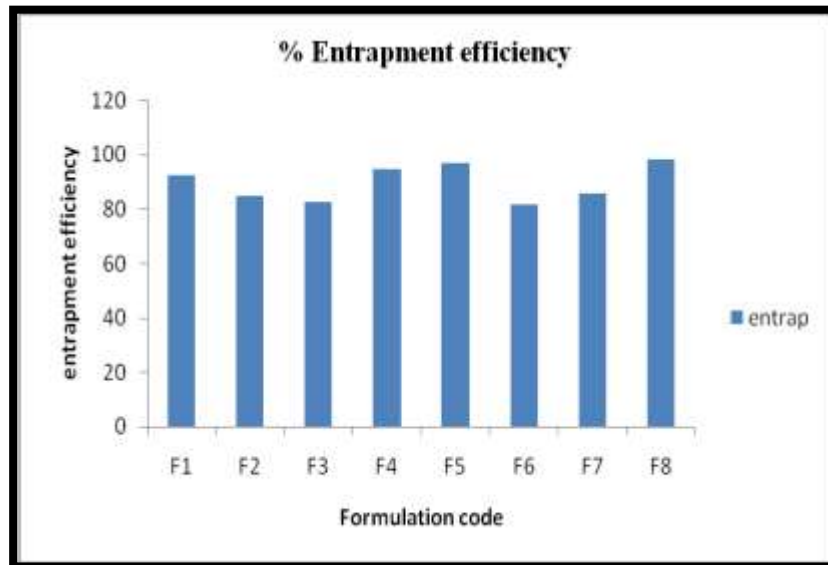


Figure 11: % entrapment efficiency of formulation F1- F8

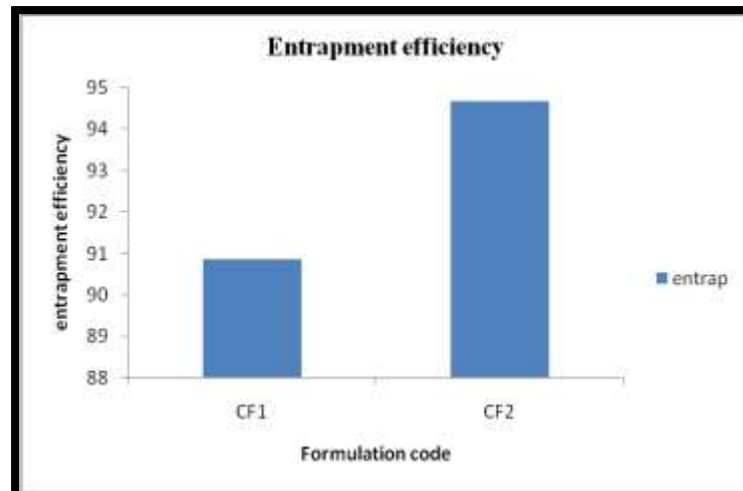


Figure 12: Entrapment efficiency of Coated liposomal formulation CF1-CF2

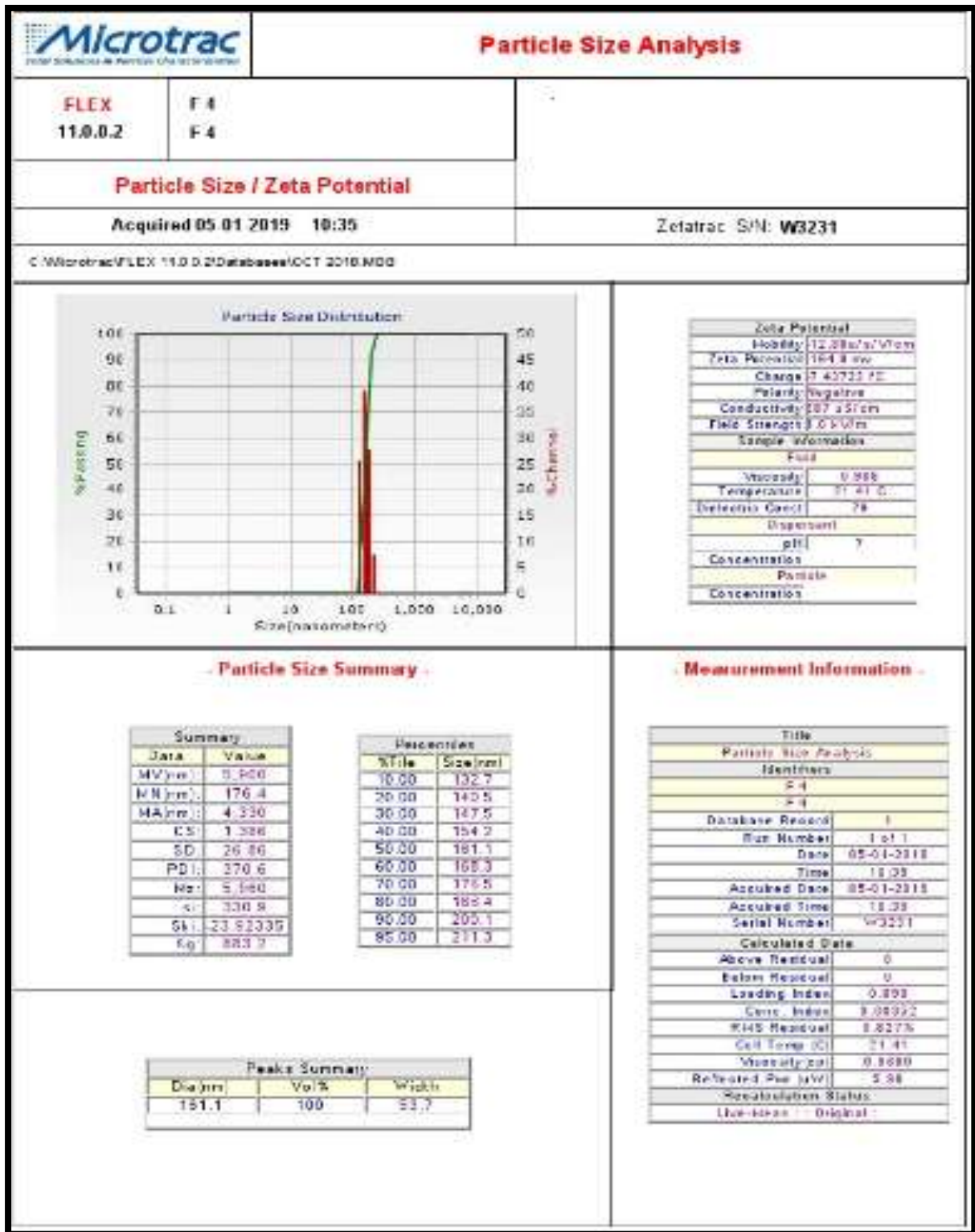


Figure 13: Particle size analysis of formulation F4

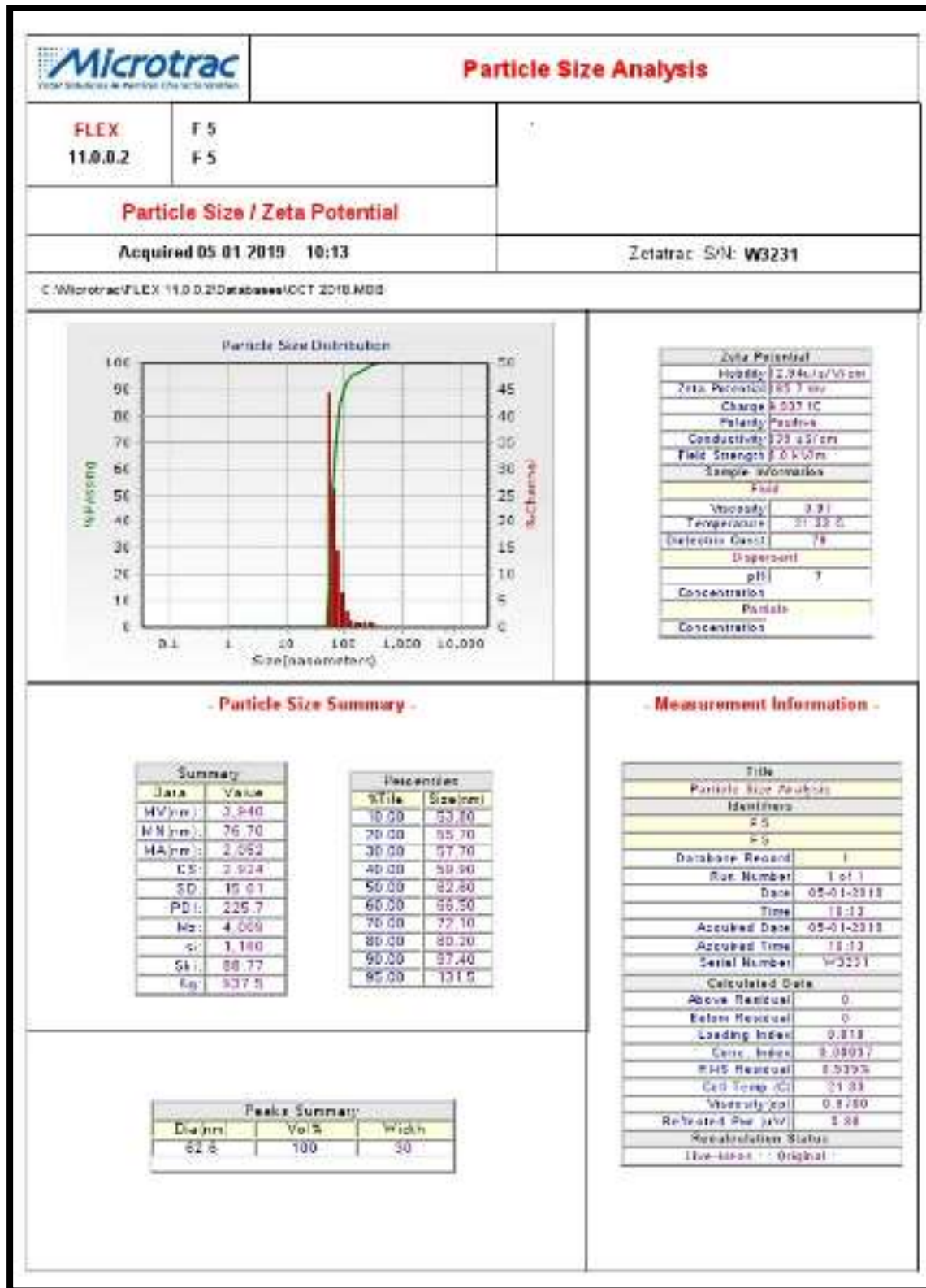


Figure 14: particle size analysis of formulation F5

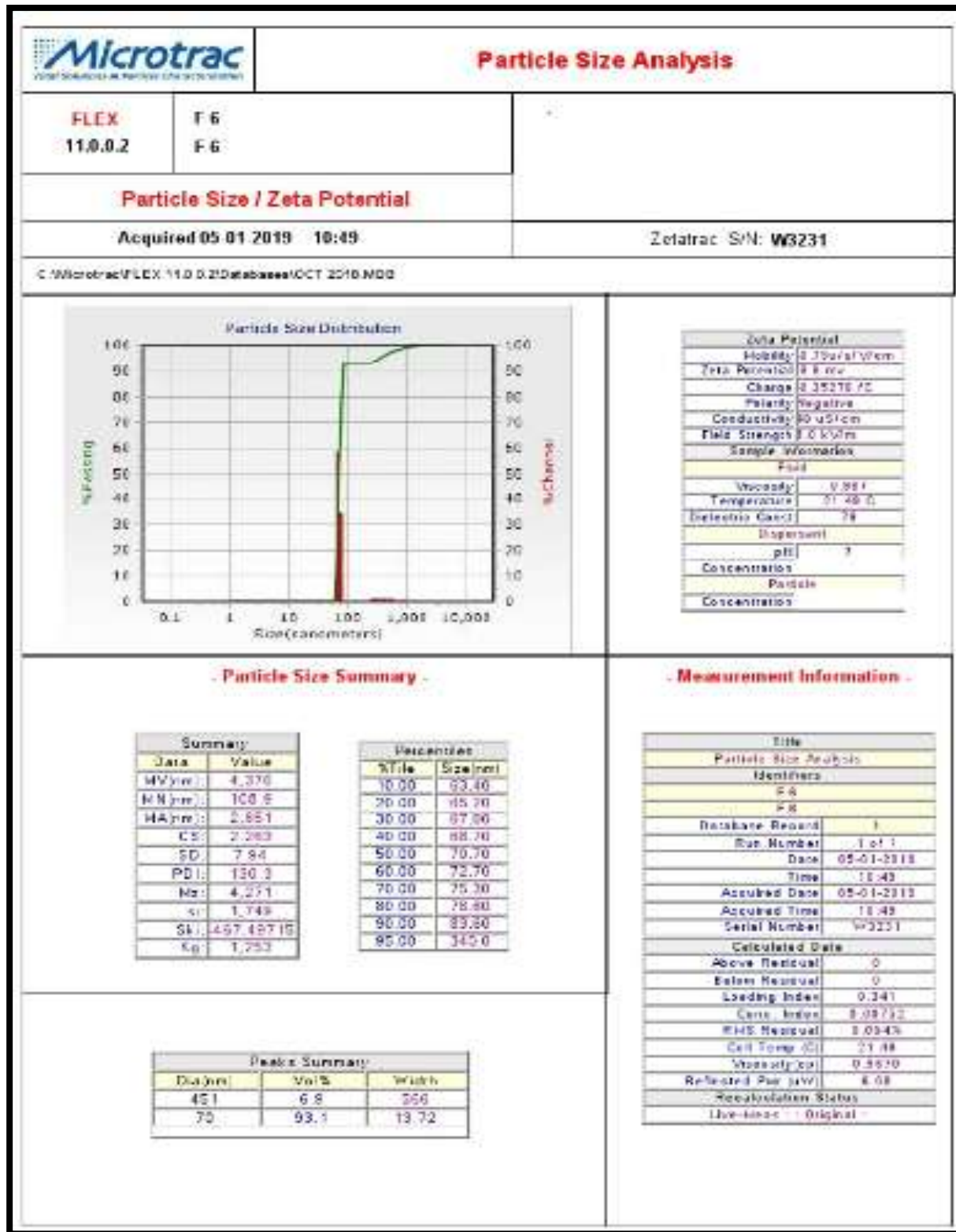


Figure 15: Particle size analysis of formulation of F6

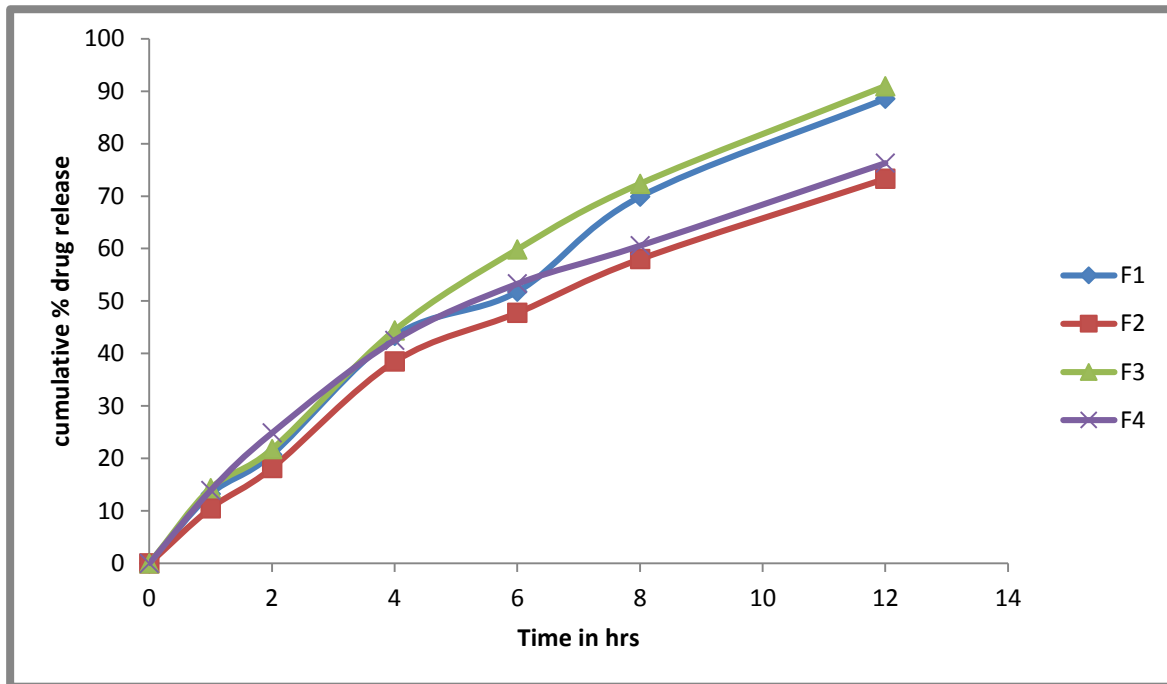


Figure 16: Cumulative % drug release formulation F1- F4

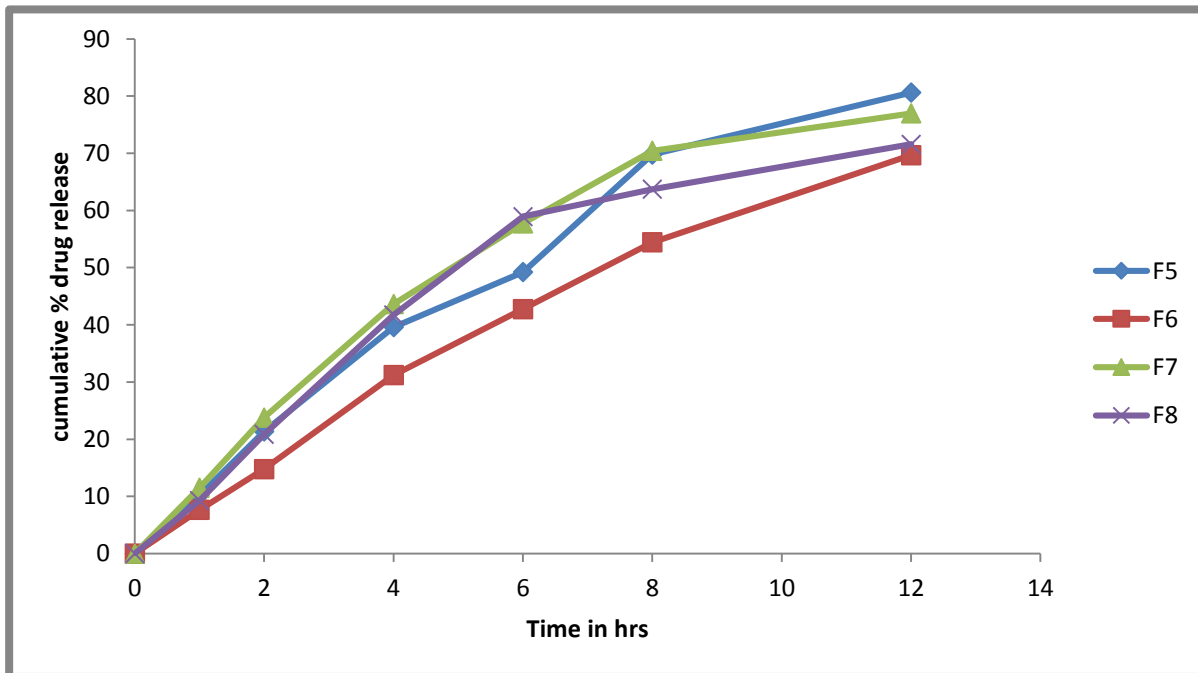


Figure 17: Cumulative % drug release formulation F5- F8

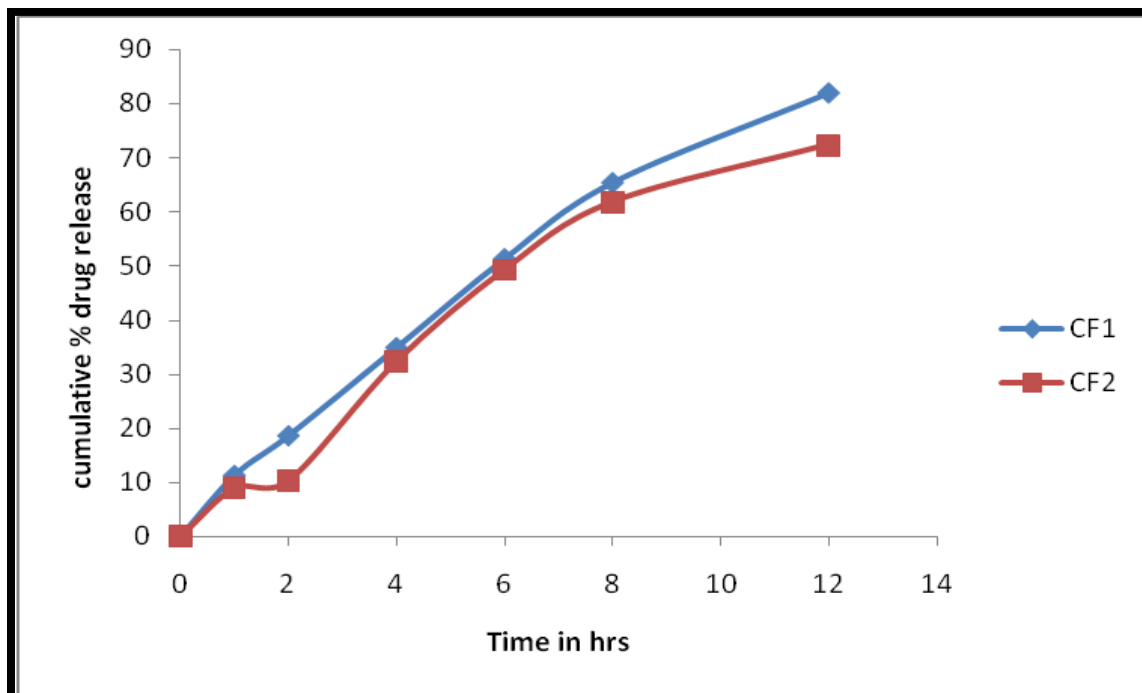


Figure 18: % cumulative drug release of coated formulations CF1-CF2

Result of modelling fitting

Table 3: Data for different kinetic model

Formulation code	Zero order	First order	Higuchi plot	Peppas plot r^2	'n'
F1	0.963	0.622	0.973	0.714	1.306
F2	0.959	0.478	0.970	0.578	1.166
F3	0.966	0.611	0.969	0.705	1.315
F4	0.938	0.567	0.987	0.668	1.234
F5	0.958	0.632	0.959	0.74	1.325
F6	0.959	0.689	0.974	0.800	1.336
F7	0.914	0.594	0.967	0.712	1.307
F8	0.894	0.611	0.953	0.745	1.334
CF1	0.956	0.651	0.976	0.744	1.317
CF2	0.923	0.704	0.959	0.803	1.373

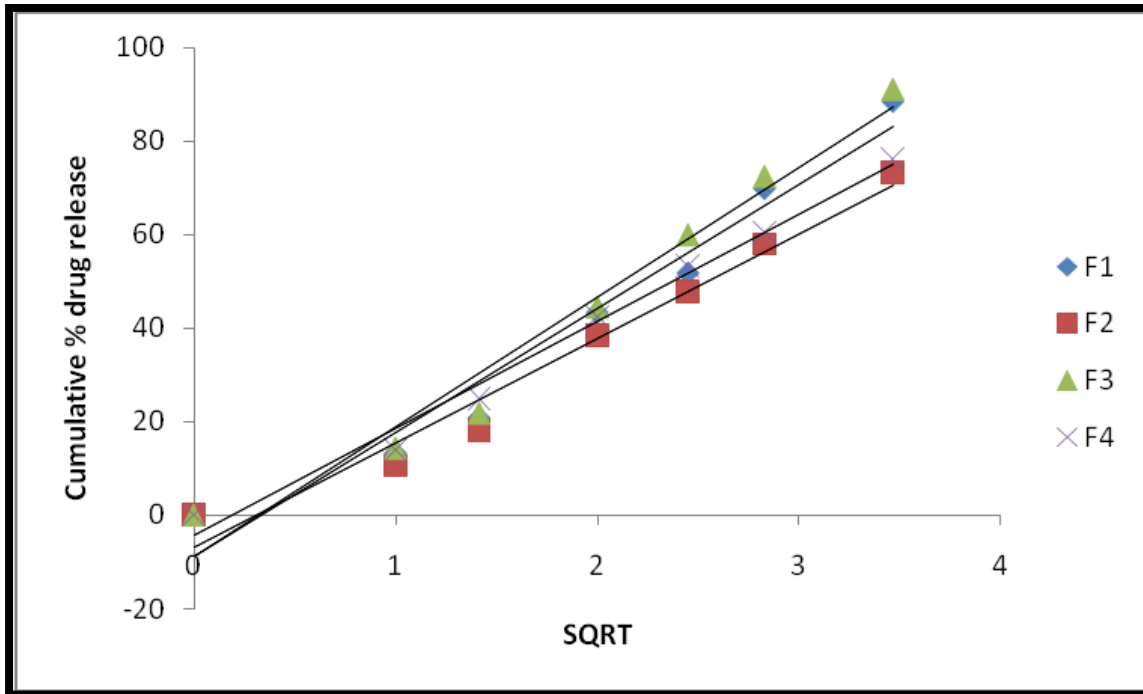


Figure 19: Higuchi release kinetic profile of formulation F1-F4

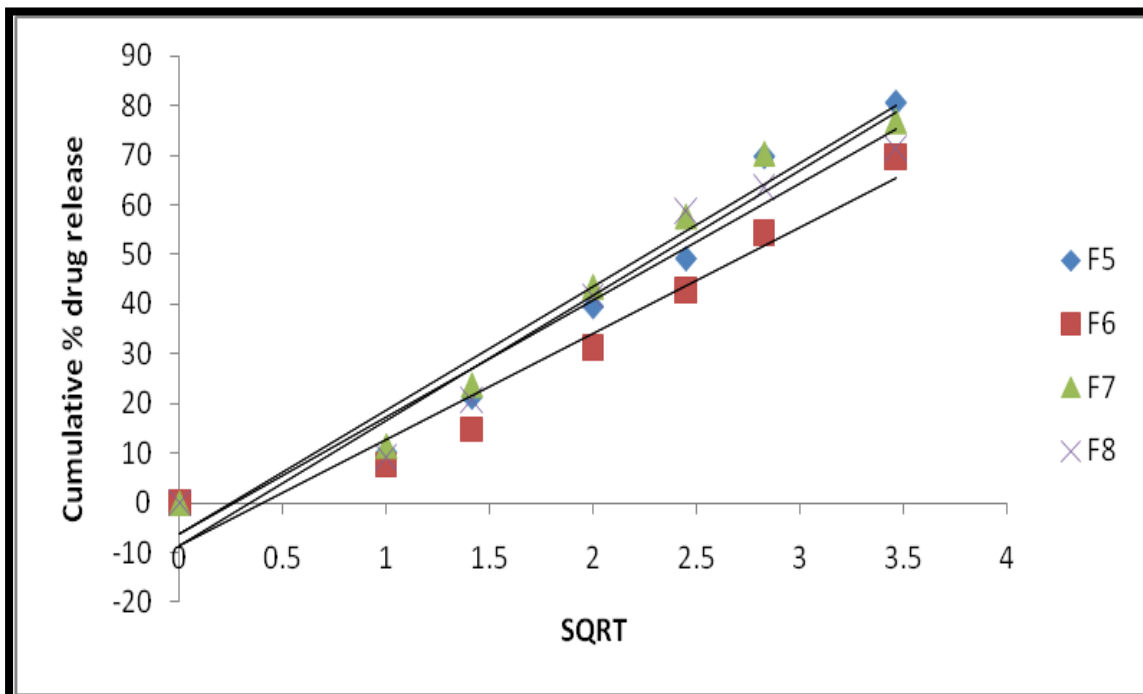


Figure 20: Higuchi release kinetic profile of formulation F5-F8

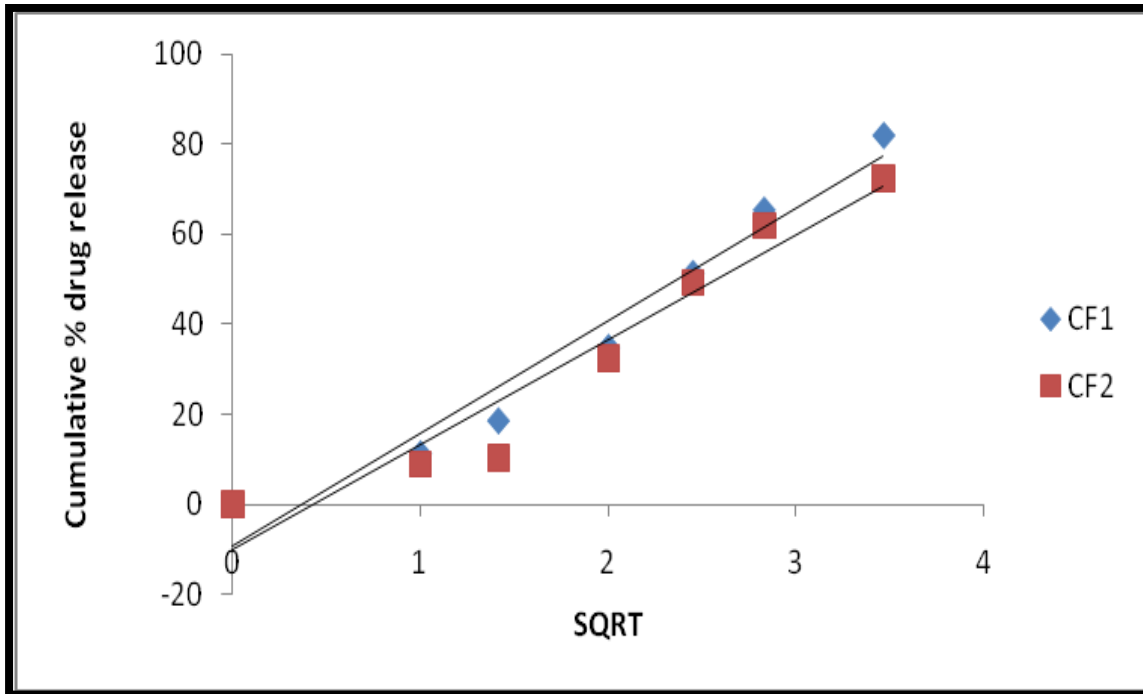
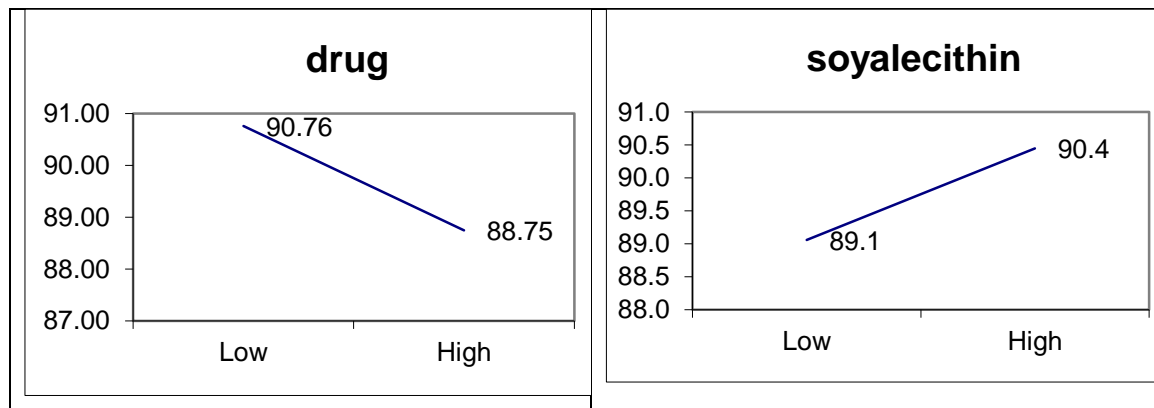


Figure 21: Higuchi release kinetic profile of coated formulations CF1-CF2

Table 4: Accelerated stability studies for optimized coated at 40°C ±2°C and 75±5%

Parameter	Duration in months					
	0	3		6		
	CF1	CF2	CF1	CF2	CF1	CF2
Entrapment	90.87	94.68	90.32	94.13	90	89.80
% CDR	81.86	72.37	81.39	72	79.84	71.76



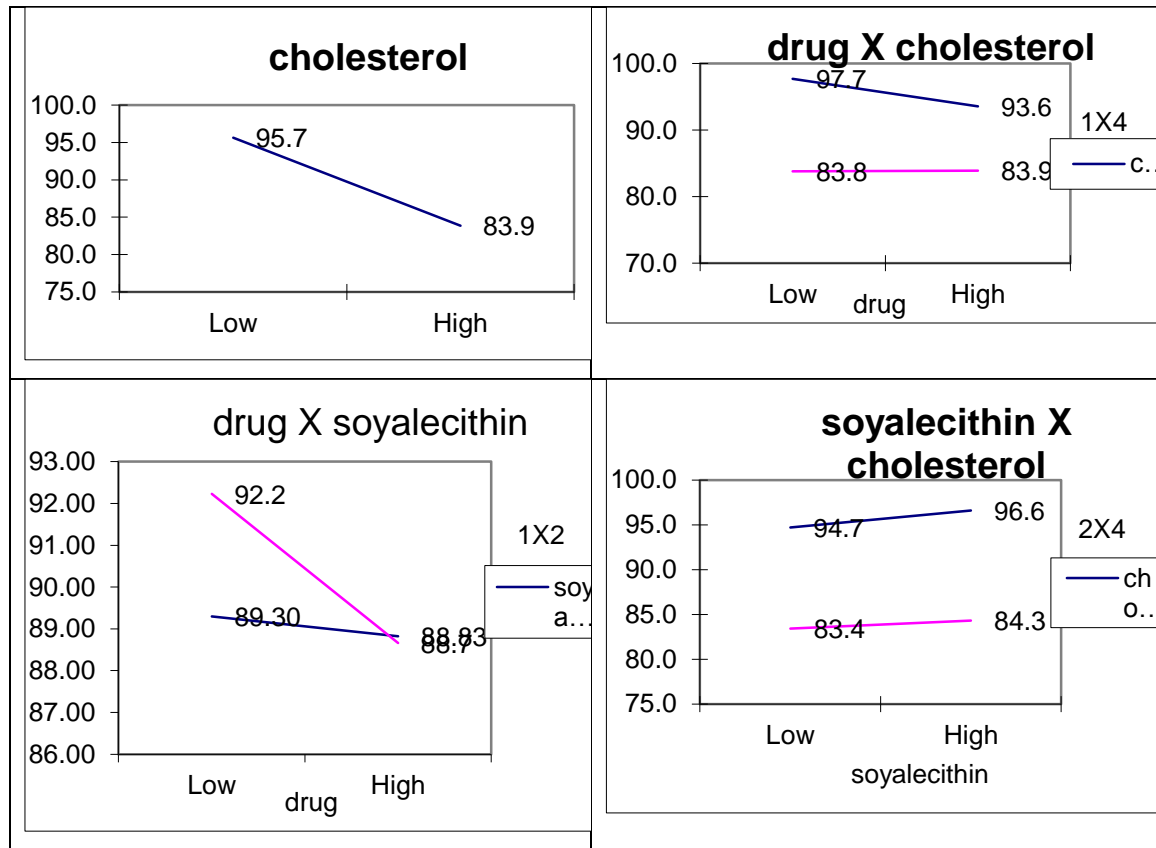
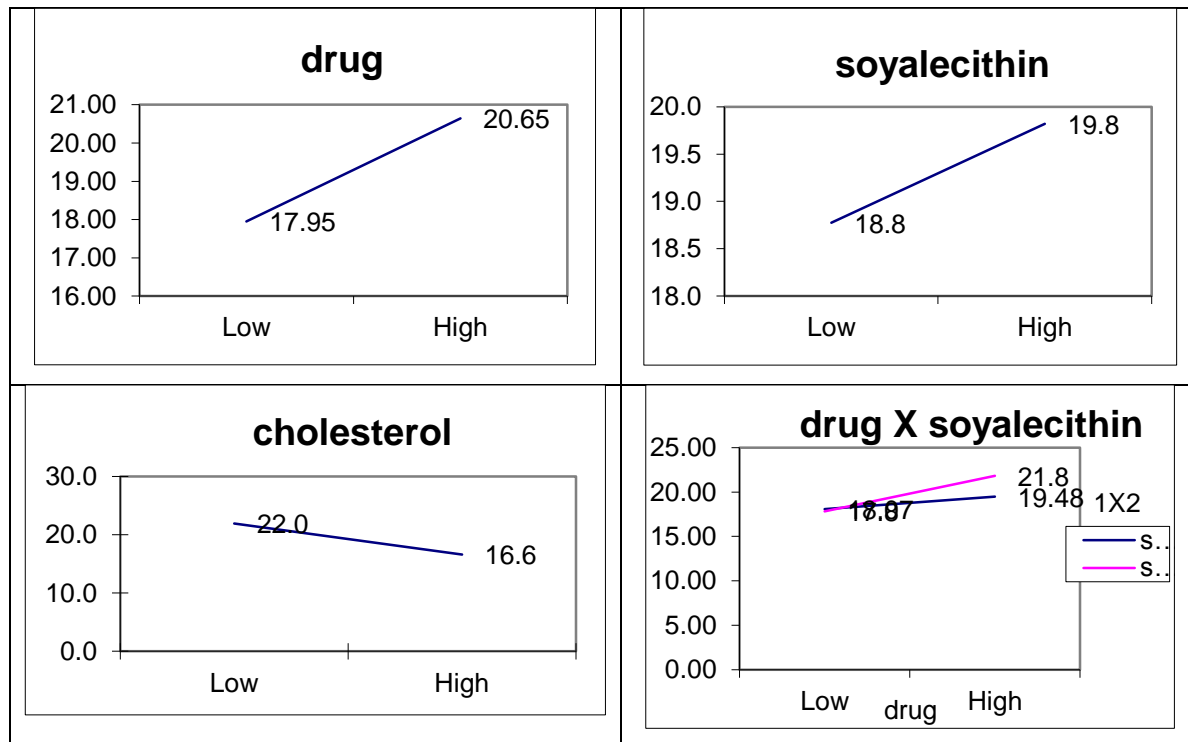


Figure 22: Factorial design for entrapment efficiency



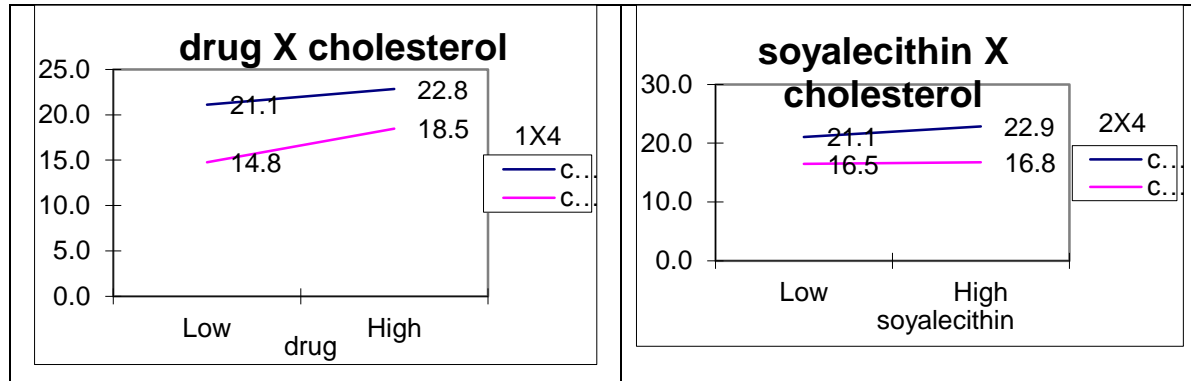


Figure .23: Factorial design of %CDR for 2 hrs

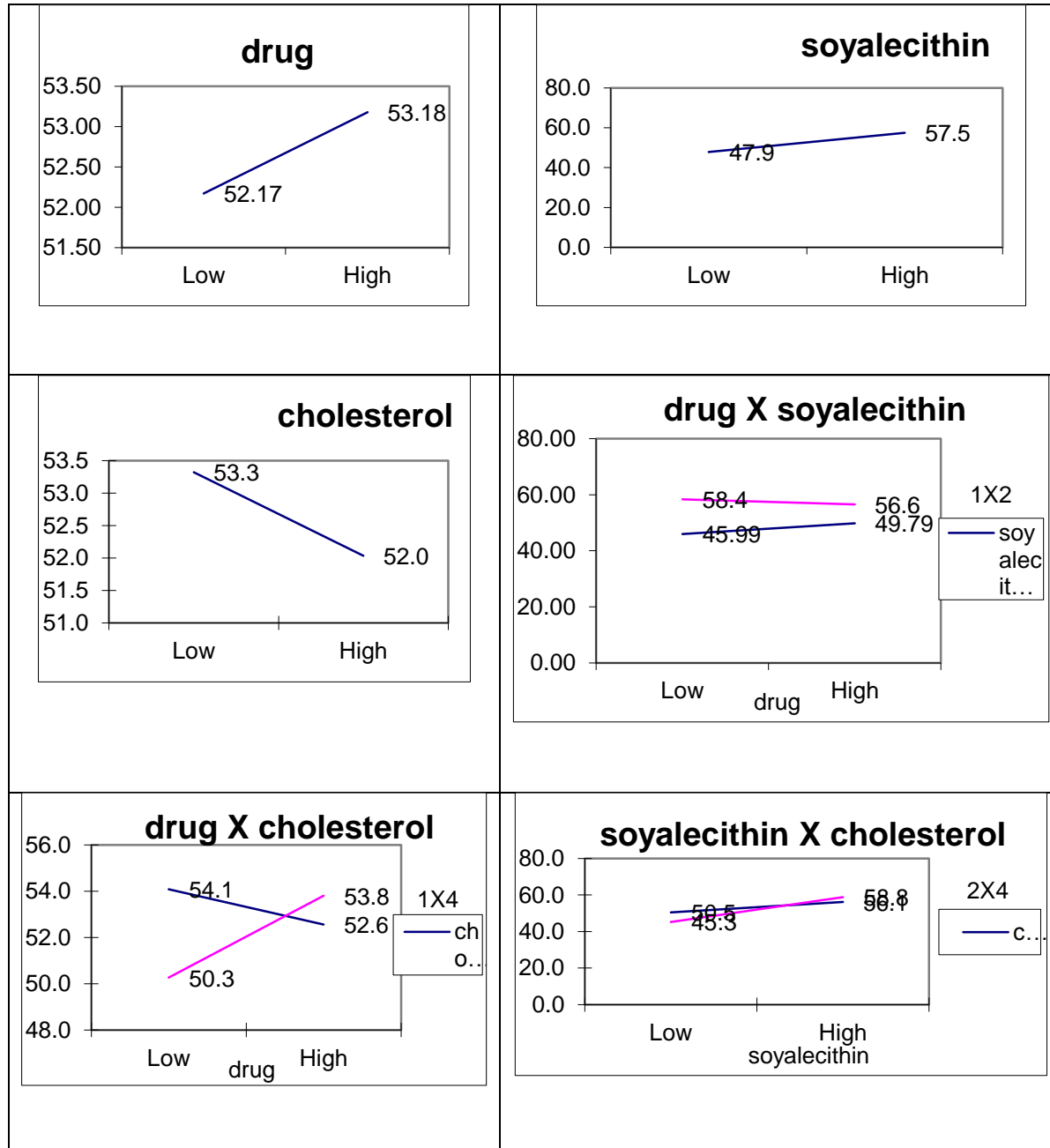


Figure 24: Factorial design of %CDR for 6 hrs

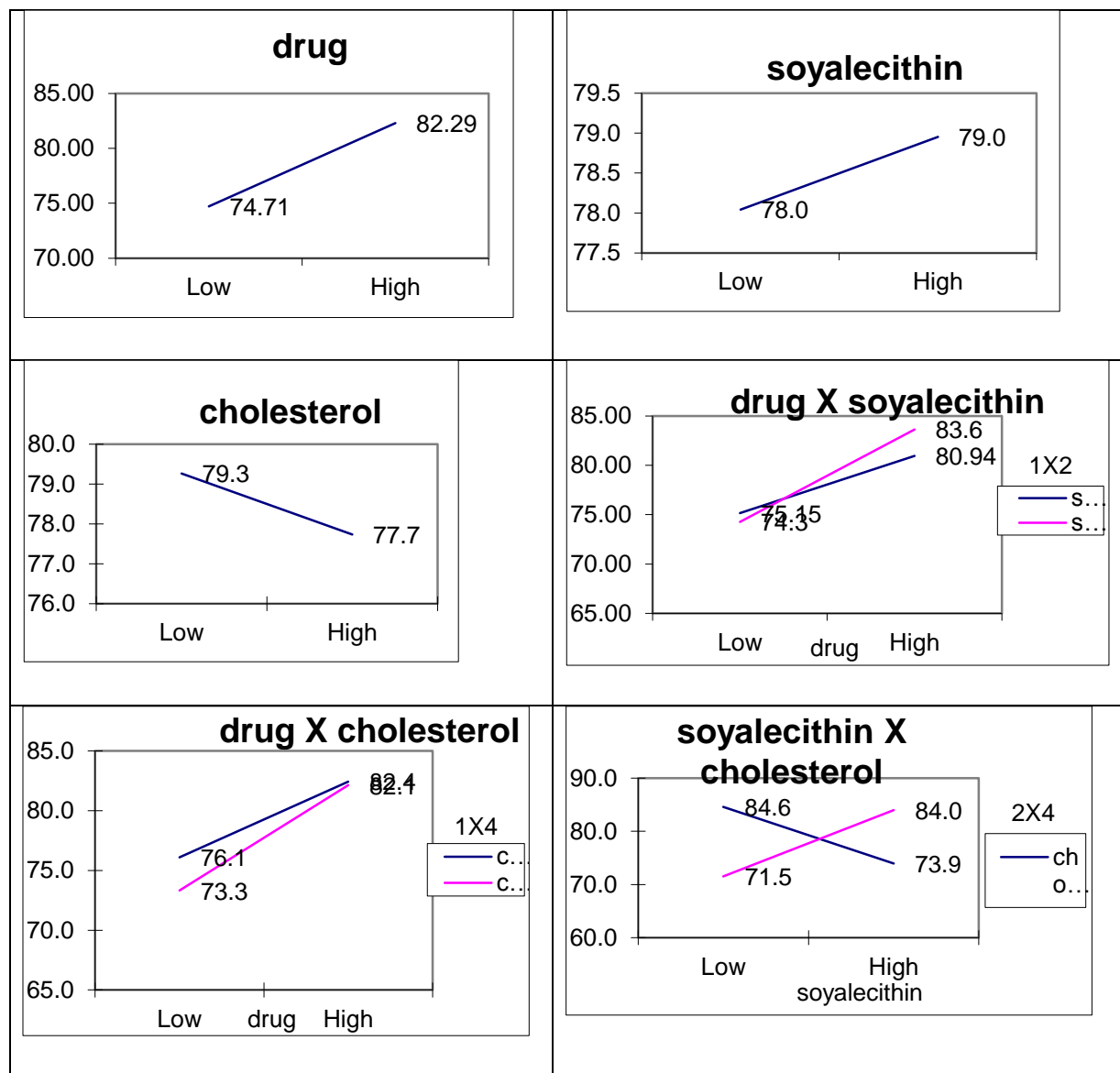


Figure 25: Factorial design of % CDR for 12 hrs

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 Prednisone was retained on the intestinal mucosa in the case formulation CF1 and 78 % was retained on the intestinal mucosa for chitosan coated liposomes CF2 . Percent mucoadhesion was calculated and found the mucoadhesive strength was 63 % and 78 % which showed sufficient mucoadhesive property. The application of different drug release model kinetics is given in Table. 5.9-5.18. Release profile represented graphically in Figure 19-21. It was found that all the formulation follows Higuchi plot. The ‘n’ values for all the formulation were found to be more than 0.5. This indicates that the release approximates non-Fickian diffusion mechanism. As shown in Table. Stability studies of

mucoadhesive liposome formulations of CF1 and CF2 as shown in Table.4, respectively, that negligible change in % Entrapment efficiency and % CDR revealed that the formulations are stable on storage.

CONCLUSION

In this study, a mucoadhesive liposomal formulation of Prednisone was developed and study the effect of parameters by 2x3 factorial design to develop desired 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 Prednisone.

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