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FORMULATE AND EVALUATE THE MUCOADHESIVE MICROSPHERE OF HMG CO-A REDUCTASE INHIBITOR FOR THE TREATMENT OF HYPERLIPIDEMIA

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Abstract

The purpose of this project work was to formulate and systemically evaluate in-vitro performances of simvastatin mucoadhesive microspheres for its potential use in the treatment of hypelipidemia. Simvastatin mucoadhesive microspheres, containing carbopol-934P as mucoadhesive polymer and ethyl cellulose as carrier polymer, were prepared by w/o/w double emulsion-solvent evaporation technique. Results of preliminary trials indicated that the quantity of emulsifying agent, time for stirring, mucoadhesive polymers concentration, and Drug-Polymer ratio affected various characteristics of microspheres. A 32 full factorial design was employed to study the effect of independent variables, mucoadhesive polymer concentration (carbopol-934P) (X1), and Surfactant concentration (Tween 80) (X2) on dependent variables, i.e. % mucoadhesion, particle size, and drug release profile. Microspheres were discrete, spherical, free-flowing and showed a good percentage of drug entrapment efficiency.

The morphological characteristics of the mucoadhesive microspheres were studied under a scanning electron microscope. The best batch exhibited a high drug entrapment efficiency of 76%; 95% mucoadhesion. A sustained pattern of drug release was obtained for more than 12 h. The mucoadhesive polymer-to-matrix polymer ratio had a more significant effect on the dependent variables. An *in-vitro* mucoadhesive test showed that simvastatin microspheres adhered more strongly to the intestinal mucous layer and could be retained in the intestinal tract for an extended period of time. In conclusion, the prolonged intestinal residence time and slow release of simvastatin resulting from the mucoadhesive microspheres could contribute to the provision of a sustained anti-hyperlipidemic effect.

INTRODUCTION

The oral route of drug administration constitutes the most convenient and preferred means of drug delivery to systemic circulation of body. However oral administration of most of the drugs in conventional dosage forms has short-term limitations due to their inability to restrain and localize the system at gastro-intestinal tract. Microspheres constitute an important part of these particulate drug delivery systems by virtue of their small size and efficient carrier capacity. Microspheres are the carrier linked drug delivery system in which particle size is ranges from (1-1000 µm) range in diameter having a core of drug and entirely outer layers of polymers as coating material. However, the success of these microspheres is limited due to their short residence time at 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. This can be achieved by coupling bioadhesion characteristics to microspheres and developing bioadhesive microspheres. Bioadhesive microspheres have advantages like efficient absorption and enhanced bioavailability of the drugs due to a high surface to volume ratio, a much more intimate contact with the mucus layer and specific targeting of drugs to the absorption site.

Simvastatin, a HMG-CoA reductase inhibitors used to lower cholesterol levels by inhibiting the enzyme HMG-CoA reductase, which plays a central role in the

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production of cholesterol in the liver. Increased cholesterol levels have been associated with cardiovascular diseases, so it is use for treatment for hyperlipidemia. Its short biological half-life (2 h) necessitates the need for its administration in two or three dosage forms of 20 to 80 mg per day. Thus, the development of controlled-release dosage forms would clearly be advantageous. Researchers have formulated oral controlled-release products of simvastatin by various techniques[1-5].

In context of the above principles, a strong need was felt to develop a dosage form that delivered simvastatin into the GI tract and would increase the efficiency of the drug, providing a sustained action. Thus, an attempt was made in the present investigation to use Carbopol-934P as a mucoadhesive polymer and ethyl cellulose as carrier polymer, in order to prepare mucoadhesive propranolol hydrochloride microspheres. The microspheres were characterized by %Mucoadhesion, %entrapement efficiency, in-vitro tests and factorial design was used to optimize the variables[5-7].

MATERIALS & METHODS

Materials

Simvastatin (powder) was obtained as a gift sample from INTAS Pharmaceuticals LTD. (Ahmedabad, India). Carbopol-934P (CP) was obtained as a gift sample from LOBA chemie PVT. LTD. Mumbai. Ethyl cellulose was from Oxford laboratory, Mumbai. Tween 80, sodium lauryl sulphate (SLS) and span 80 were purchased from Loba Chemie Pvt Ltd. (Mumbai, India). All other ingredients were of analytical grade.

Preparation of simvastatin mucoadhesive microspheres

Microsphere were prepared by modified w/o/w double emulsion solvent diffusion method using different polymer ratio with drug and varying concentration of surfactant in external water phase. For preparation of microsphere of simvastatin, mucoadhesive polymer, and ethyl cellulose as matrix polymer were dissolved in 30ml mixed solvent system consisting of methanol and dichloromethane in 1:2 ratio. The initial w/o emulsion was prepared by adding 2ml water containing 1.5% v/v of tween 80 to drug-polymer solution while stirring, using a magnetic stirrer at 200 rpm

for 5 min. This primary w/o emulsion was slowly added to 200ml surfactant solution containing surfactant in different ratio with syringe (20G needle). After 2 hr, 5ml of n-hexane was added to harden the microsphere and the stirring was continued for further 1 hr. The microsphere was collected by filtration and dries them at room temperature (6).

Evaluation of mucoadhesive microsphere

Micromeritic properties of microspheres

Flow properties: The flow properties of microsphere were studied by determining various parameters like the angle of repose, Carr's index, and bulk density and tapped density[13-17].

Production Yield (%)

The production yield of microsphere was calculated using the weight of final product after drying with respect to the initial total weight of the drug and polymer used for preparation of microsphere and % production yield was calculated as per the formula mentioned below[12].

$$\text{PRODUCTION YIELD (\%)} = \left[\frac{W0}{WT}\right] 100 \quad \text{..... (1)}$$

Where, W_O = Practical mass (microspheres); W_T = Theoretic lass (Polymer + Drug).

Particle size analysis

Particle size of different batches of microspheres was determined by optical microscopy. The projected diameter of microspheres from each batch was determined using ocular micrometer and stage micrometer equipped with optical microscope. Analysis was carried out by observing the slide containing microspheres under the microscope. The average particle size of the microspheres was expressed as diameter**12**.

Encapsulation Efficiency

To determine the amount of drug encapsulated in microspheres, a weighed amount (160 mg) of microspheres was suspended into 50 ml methanol and sonicated for 15 min in order to extract the entrapped drug completely and diluted up to 1000 ml PBS pH 6.8. The solution was filtered through whatman filter paper. 5 ml of this solution was withdrawn and diluted with 5 ml pH 6.8 PBS. This solution was assayed for drug content by UV spectrophotometer at 238.4 nm[12].

a) Encapsulation efficiency was calculated as:

$$EE = \left[\frac{\text{Actual Drug Content}}{\text{Theoriticalcal Drug Content}} \right] 100$$

…… (2)

EE= Encapsulation efficiency

Degree of Swelling

The swell ability of microspheres in physiological media was determined by swelling them in the PBS pH 6.8. Accurately weighed amount of microspheres was immersed in little excess of PBS pH 6.8 for 2 hr and washed (11). The degree of swelling was calculated using following formula:

$$\text{Degree of Swelling} = \left[\frac{Ws - Wo}{Wo} \right] 100$$

…….. (4.9)

Where, Wo is the weight of microspheres before swelling;

Ws is the weight of microspheres after swelling.

***In-vitro* Mucoadhesion Studies**

Mucoadhesion of microspheres was measured by following method: A piece of freshly cut hen intestine was obtained from a local slaughter house within one hour of killing of animal, and was cleaned by washing with isotonic saline solution. Pieces of intestinal mucosa (3cm×2cm) were mounted onto glass rod using thread. Microspheres were spread (approximately 50) onto the wet rinsed mucosal tissue specimen and the prepared glass rod was hung onto one of the groves of a USP tablet disintegration test apparatus, with continuous oxygen supply. The disintegration test apparatus was operated, giving the mucosal tissue specimen was given regular up and down movements within the beaker of the disintegration apparatus, which contained the pH 6.8 Phosphate buffer at 37°c. At the end of 30 min, and 1 hr the number of microspheres still adhering onto the mucosal tissue was counted. From this method in vitro wash off time determined by calculating total time for detach all microsphere from mucosal tissue, percent mucoadhesion was calculated by following formula[11].

$$\%\,\text{MUCOADHESION} = \left[\frac{Wa - Wl}{Wa} \right] 100$$

…….. (4)

Where, Wa = weight of microspheres applied; Wl = weight of microspheres leached out.

Micromeritic properties of microspheres

Flow properties: The flow properties of microsphere were studied by determining various parameters like the angle of repose, Carr's index, and bulk density and tapped density.

***In vitro* Drug Release Studies**

In vitro release of simvastatin from microspheres was determined by carrying out USP dissolution testing apparatus II (Basket type) at a stirring rate of 50±5 rpm at temperature 37±0.5°C. Nine hundred milliliters of HCl buffer (pH 1.2) was used as dissolution medium for first 2 hour and phosphate buffered saline (PBS, pH 6.8) was used for next 10 h. The dried microspheres were filled in basket and were placed in dissolution vessels. A 5 ml sample was withdrawn at various time intervals and the volume of the media was replenished with an equal amount of dissolution media. The samples were then analyzed spectrophotometrically[7-10].

Surface Morphology

Shape and surface morphology of microspheres was studied using scanning electron microscopy (SEM). The photographs were taken using a scanning electron microscope[22].

Compatibility Studies by FT-IR Spectroscopy

FT-IR spectroscopy was carried out to check the compatibility between drug and polymer. The FT-IR spectra of drug with polymers were compared with the standard FT-IR spectrum of the pure drug.

KINETICS OF DRUG RELEASE[23-25]

Various models are available for explaining the kinetics of drug release. They are listed below:

Zero order model

In many of the modified release dosage forms particularly controlled or sustained release dosage forms is zero order kinetics.

$W_0 - W_t = K_0 t$ …… (4.11)

Where, W_0 is the initial amount of drug in the pharmaceutical dosage form, W_t is the amount of drug in the pharmaceutical dosage form at time **t** and **K** is proportionality constant.

First order model

Most conventional dosage forms exhibit this dissolution mechanism. Some modified release preparations, particularly prolonged release formulations, adhere to this type of dissolution pattern.

$\text{Log } Q_t = \text{Log } Q_0 + K_1 t/2.303$

Where, Q_t is the amount of drug released in time **t**,

Q_0 is the initial amount of drug in the solution

K_1 is the first order release rate constant. It assumes that the drug molecules diffuse out through a gel like layer formed around the drug during the dissolution process. A plot of log % drug released versus time is linear.

Higuchi model

A large number of modified release dosage forms contain some sort of matrix system. The dissolution pattern of the drug is dictated by water penetration rate (diffusion controlled) and thus the following relationship applies:

$$Q_t = K_H t^{½}$$

Where, Q_t is the amount of drug released at time **t**

K_H is the constant for Higuchi drug release rate.

In Higuchi model, a plot of % drug unreleased (or released) versus square root of time is linear.

Hixson-Crowell Model

The simplified equations is represented as

$$Q_0 1/3 – Qt1/3 = K t$$

Where, Qt = amount of drug released in time (t),

Q_0 = initial amount of drug in solution,

K = cube root constant.

A graphic representation of cubic root of unreleased fraction of drug versus time will be linear if geometric shape of the formulation diminishes proportionally over time.

Korsemeyer and Peppas model

$$Q_t/Q_\infty = K_k t^n$$

……(4.15)

Where, K_k is the constant incorporating structural and geometric characteristic of the drug dosage form

n is the release exponent n is diffusion exponent.

if n is equal to one the release is zero-order, if n is equal to 0.5 the release is best explained by Fickian diffusion, and if 0.5 < n< 1 then the release is through anomalous diffusion or case II diffusion.

STABILITY STUDIES

Stability is defined as the ability of particular drug or dosage form in a specific container to remain with its physical,

chemical, therapeutic and toxicological specifications. Stability tests are the series of tests designed to obtain information on the stability of the pharmaceutical product in order to define its shelf life and utilization period under specified packaging and storage conditions. The purpose of stability testing is to provide information on how the quality of a drug product varies with time under the influence of variety of environmental factors such as temperature, humidity and light, and to establish a shelf life for the drug product at recommended storage conditions (20).

Factors affecting stability:

1. Storage time.
2. Storage condition.
3. Type of dosage form.
4. Container and closure system.

Stability testing of pharmaceutical product is done for the following purposes:

- ✓ To ensure the efficacy, safety and quality of active drug substance and dosage forms.
- ✓ To establish shelf life or expiration period.

Procedure:

From the nine batches of simvastatin loaded microspheres, formulation F8 was tested for stability studies. sample stored at:

- ✓ 25± 2^{o}C and 60 ± 5% RH.
- ✓ 40 ± 2^{0}C and 75 ± 5 % RH

After 30 days, the drug release of selected formulation was determined by the method discussed previously *in vitro* drug release studies and mucoadhesion behaviour was also carried out for the same formulation.

RESULTS AND DISCUSSION

Compatibility Studies by FT-IR Spectroscopy

The FT-IR spectra of drug with polymers were compared with the standard FT-IR spectrum of the pure drug. Drug is compatible with polymer showm in Figure No 8.

- All the microsphere were prepared by W/O/W double emulsion solvent diffusion method. Carbopol 934P was selected as mucoadhesive polymer and ethyl cellulose was selected as matrix polymer. The composition of all prepared formulkation is depicted in Table 1.

Micromeritic properties of microspheres

All formulations were evaluated for angle of repose, bulk density, tapped density, % compressibility and Hausner's ratio. Results are shown in Table 2.

Angle of repose of all formulation svaried from 20.09 to 29.37. Angle of repose less than 30 indicates good flow property. Compressibility index vary from 19.78 % to 26.55 %. Compressibility index 12 to 16% indicates good compressibility and 16 to 22 indicate fair passable.

Hausner's ratio varies from 1.25 to 1.36. Hausner's ratio less than 1.25 indicates good compressibility. Here all these results showed good flow property and compressibility

Surface morphology of Mucoadhesive microspheres of optimized batch F8 by scanning electron microscopy

The morphological characteristics of the mucoadhesive microspheres were studied under a scanning electron microscope. The microspheres of formulation batch good spherical in shape shown in Figure No 1.

Production yield

The % yield of all the 9 formulations was found to be ranging between 59.00 to 88.05% shown in Table No 3. It was found that concentration of carbopol decrease than increase the % Production Yield. Formulation Batch F8 shows maximum yield 88.05%.

Particle size

The Mean Particle Sizes of all the 9 formulations were found to be ranging between 225 ± 2.3 to 987.5 ± 5.7 µm shown in Table No 3. It was found that as the polymer quantity increases relative to drug the mean particle size also increases due to higher proportion of the Polymer which forms relatively bigger particle.

Entrapement efficiency

The percentage Entrapment efficiencies of all 9 formulations were found to be ranging between 37.44 to 66.80% shown in Table No 3. It was found that as the Mucoadhesive polymer quantity increases relative to drug the percentage entrapment decreases. This is because as total polymer quantity increases relative to drug the amount of Polymer increases while drug quantity remains constant. Thus high amount of the Polymer results in formation of some microspheres without drug since the entire drug have been entrapped with

optimum quantity of polymer, which decreases the overall percentage entrapment.

% Mucoadhesion

The % Mucoadhesion of F1-F9 formulation was found between 62% to 92% shown in Table No 3. The concentration of carbopol increase than % mucoadhesion also increase, but it is possible only in case of spherical microsphere, the microsphere irregular in shape than % mucoadhesion also low.

Sphericity of microsphere

Shape of microsohere observe in optical microscope was shown in Table No. 4. Microsphere of F8 formulation was good in sphericity and flowing properties.

***In Vitro* Drug release:**

From the results, it was observed that as the polymer quantity increases relative to drug the dissolution rate decreases. This is because as the polymer quantity increases relative to drug the size of the microspheres increases and as the size increases the overall surface area for the erosion decreases and thus the dissolution decreases.

The formulation F8 gives drug release upto 96.31% in 12hr which is higher than other formulation shown in Table No 5 and Figure No 2,3, and 4.

Kinetic modeling and mechanism of Drug release

Dissolution profiles were fitted to various model and release data were analyzed on the basis of Korsmeyer Peppas equation, Zero order, First order, Hixon Crowell and Higuchi kinetics.

From the Korsmeyer Peppas equation, the diffusion exponent ranges from 0.580 to 1.650. From the results, all formulations showed non-Fickian release. Coefficients of correlation (R^2) were used to evaluate the accuracy of the fit. The R^2 values are given in Table 6 and 7.

Results of Analysis of variance (ANOVA)

ANOVA was done using Microsoft Excel. Results of ANOVA for Y_1, Y_2 and Y_3 are shown in Table 8.

Y_1 and Y_3 variables show significant F value, less than 0.05. So, this two variables showed significant change in the responses.

Contour plot and surface plot of the design

Here, contour plots and surface plots were drawn using the Statgraphic 16.1.17. These types of plots are useful in study of the effects of two factors on the response at one time shown in figure 5,6, and 7.

Stability studies

Stability studies of the prepared Simvastatin microspheres were carried out by storing the best formulation at 25±2^{o}C & 60±5% RH and at 40 ± 2^{0}C/ 75 ± 5 % RH for 1 month. For optimized formulation batch F8 show negligible change in drug release, % entrapement efficiency, % mucoadhesion, *in vitro* wash off time shown in 9 and 10.

The percentage of drug release before and after storage was found to be similar. Dissolution profiles before and after storage are nearly overlapable. The change in the drug release pattern i.e. dissolution profile was not significantly different from the one month's previous microsphere dissolution profile.

CONCLUSION

The present study has been satisfactorily attempted to formulate a mucoadhesive microsphere of an antihyperlipidemic drug like simvastatin with a view of enhancing absorption of the drug. From the experimental results it can be concluded that,

- The IR spectra revealed that there was no interaction between polymers and drug, hence they are compatible.
- % entrapment efficiency was higher for carbopol based microspheres with EC ratio 1:6 than microsphere with other ratio. While practical yield obtained was higher for Microspheres containing higher amount of EC.
- The particle size analysis revealed that all formulations gave particles in the range of 225-1000 µm which is suitable for mcroparticulate system.
- SEM analysis of the microspheres revealed that F8 formulation was smooth and spherical with ideal surface morphology.
- Increase in the mucoadhesive polymer led to increase in mucoadhesion and degree of swelling. However, higher amount of carbopol showed higher mucoadhesion and swelling degree..
- Stability studies for one month revealed that the formulation F8 was stable up to 25 ^{o}C(60% RH) and 40^{o}C (75% RH) . It should be stored in a cool and dry place.

ACKNOWLEDGMENTS

The authors wish to thank Intas pharmaceutical, Ahmedabad, for sparing gift sample of Simvastatin. The authors are thankful to the Matushree V.B. Manvar College of Pharmacy, Dumiyani, Rajkot for extending the laboratory facilities to carry out the Research work.

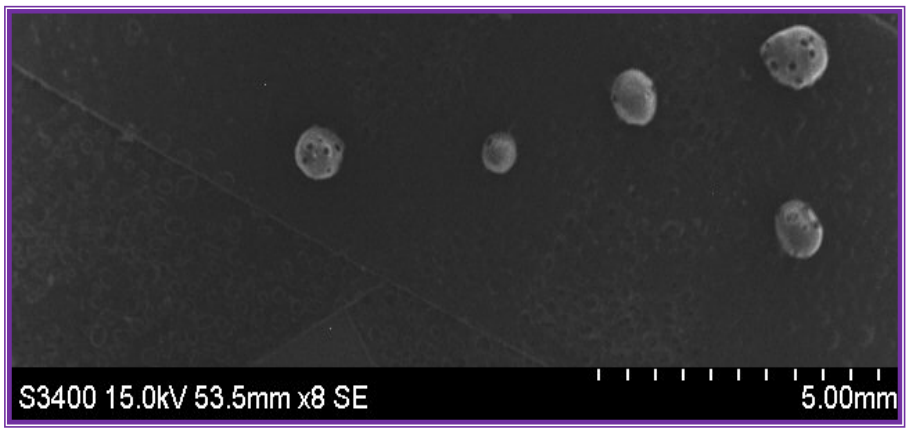


Figure 1 SEM Photograph of optimized F8 formulation

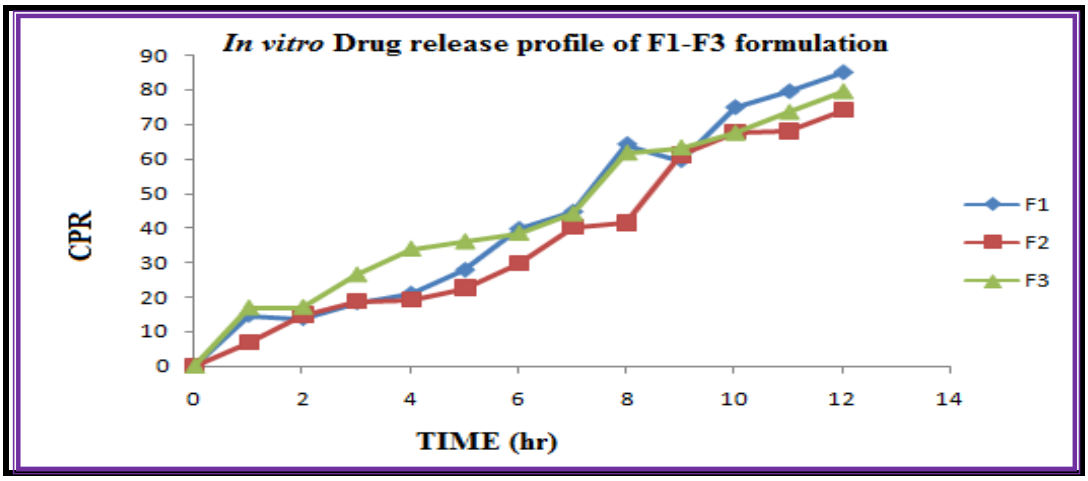


Figure 2 *in vitro* drug release profile of F1 – F3 formulation

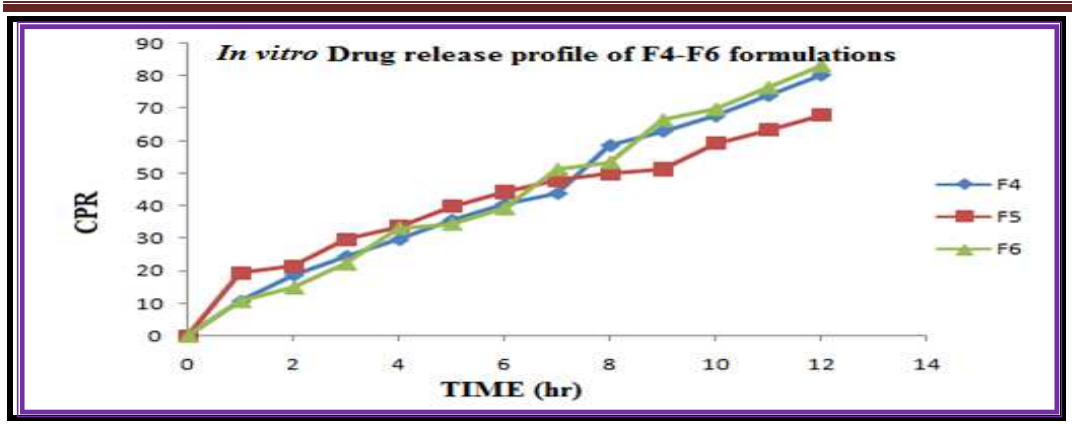


Figure 3 *in vitro* drug release profile of F4 – F6 formulation

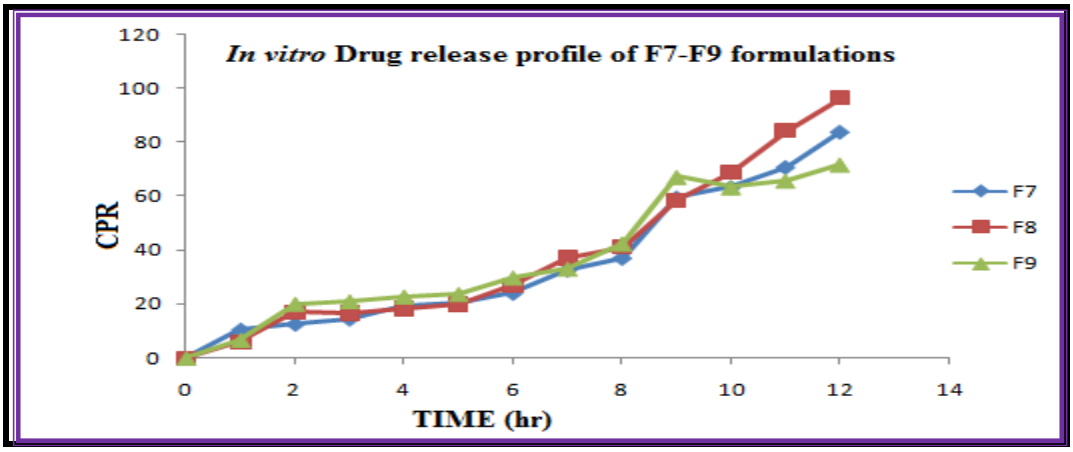
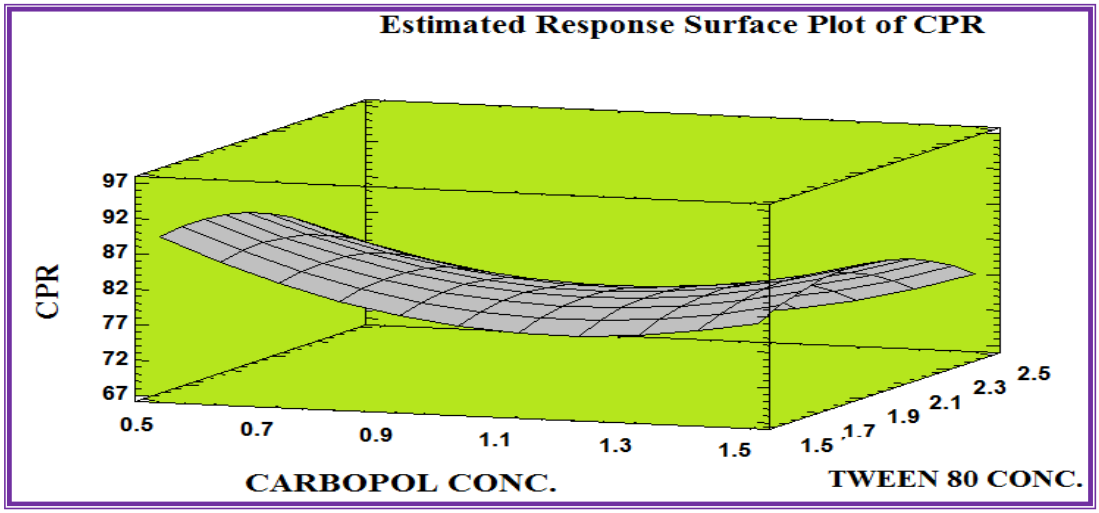
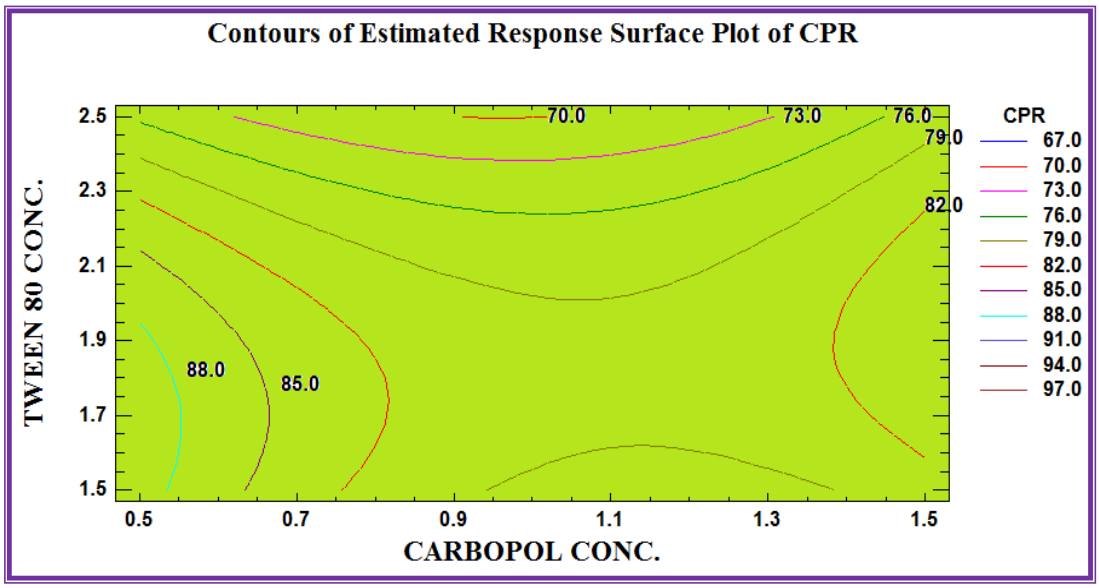


Figure 4: *in vitro* drug release profile of F7 – F9 formulation

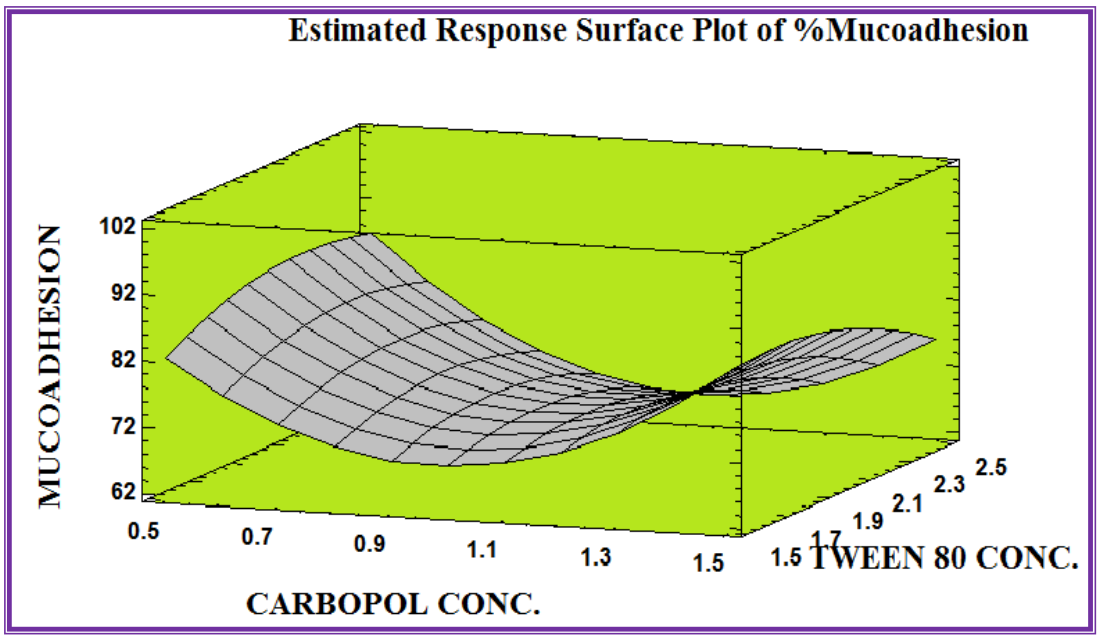


(a)



(b)

Figure 5: (a) Contour plots and (b) 3 D surface plot for Y_1



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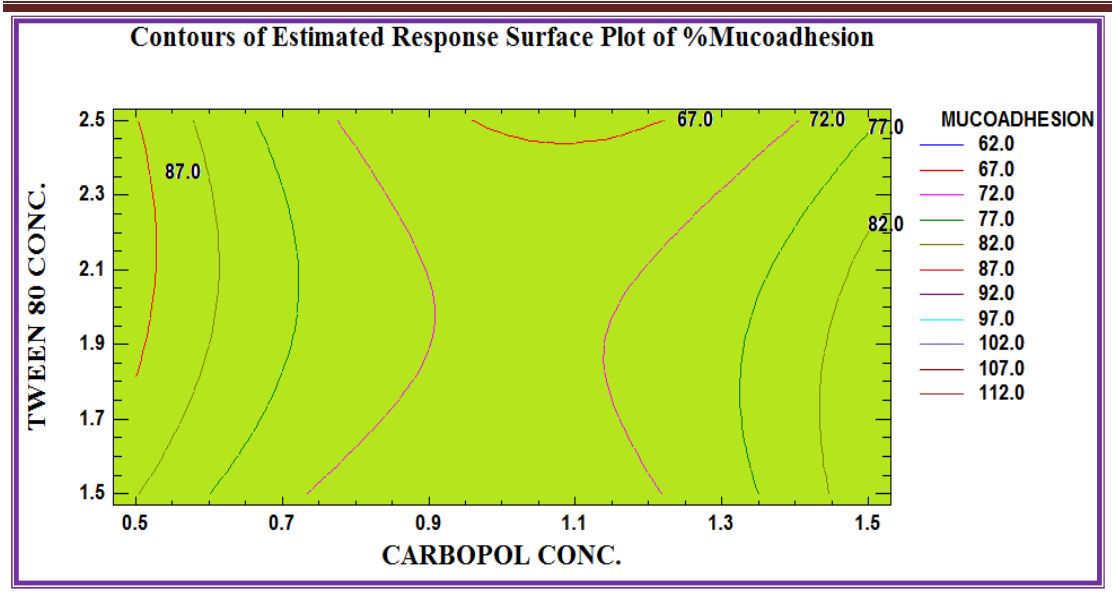
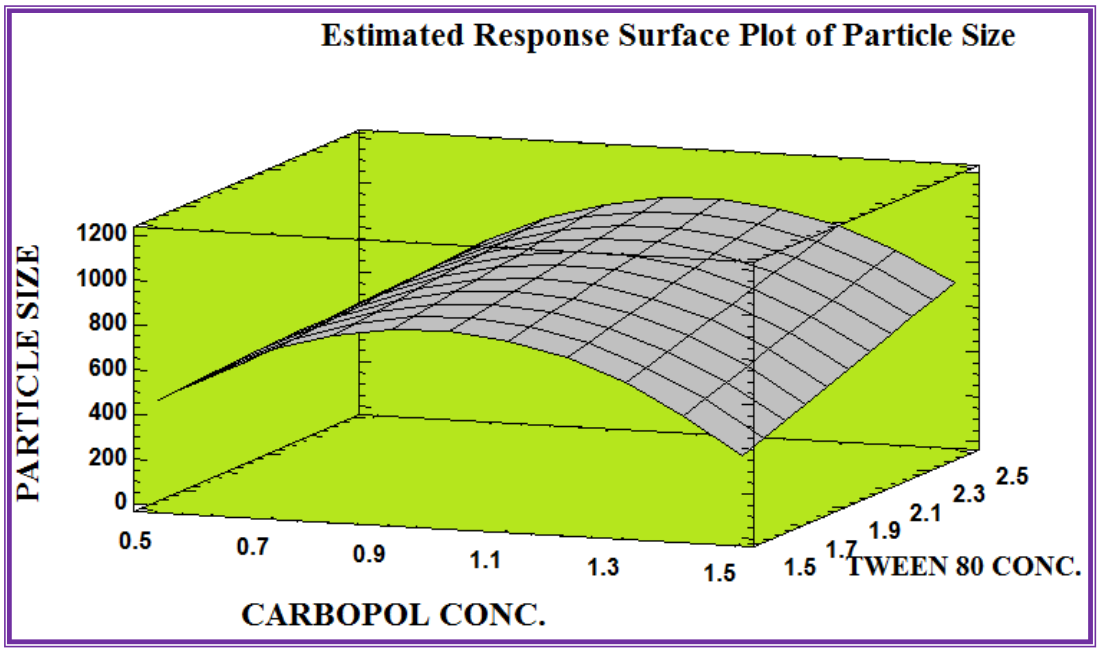
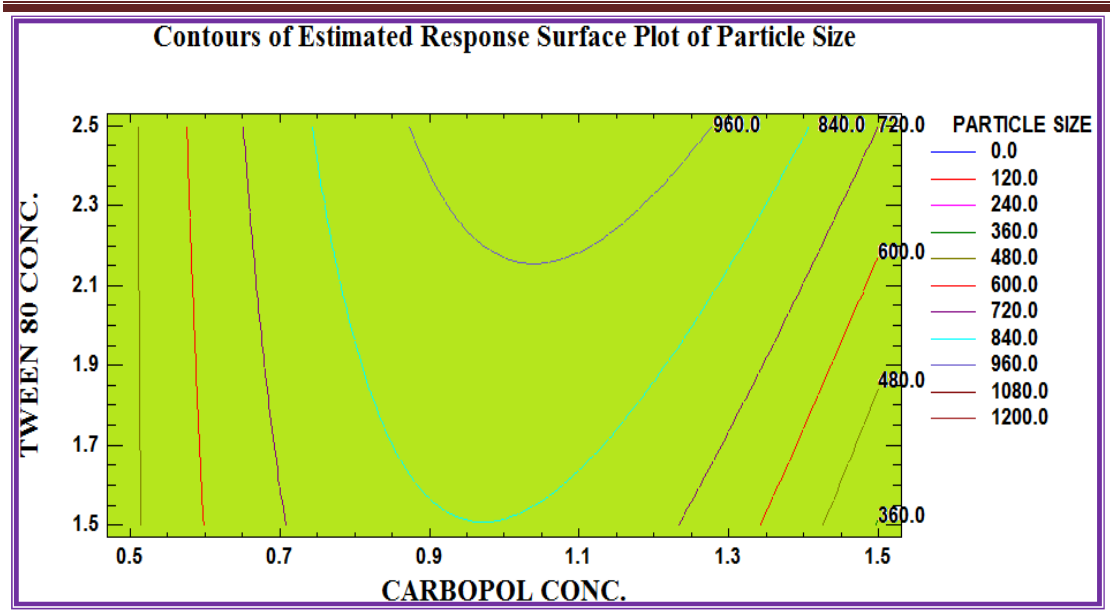


(b)

Figure 6: (a) Contour plots and (b) 3 D surface plot for Y_2



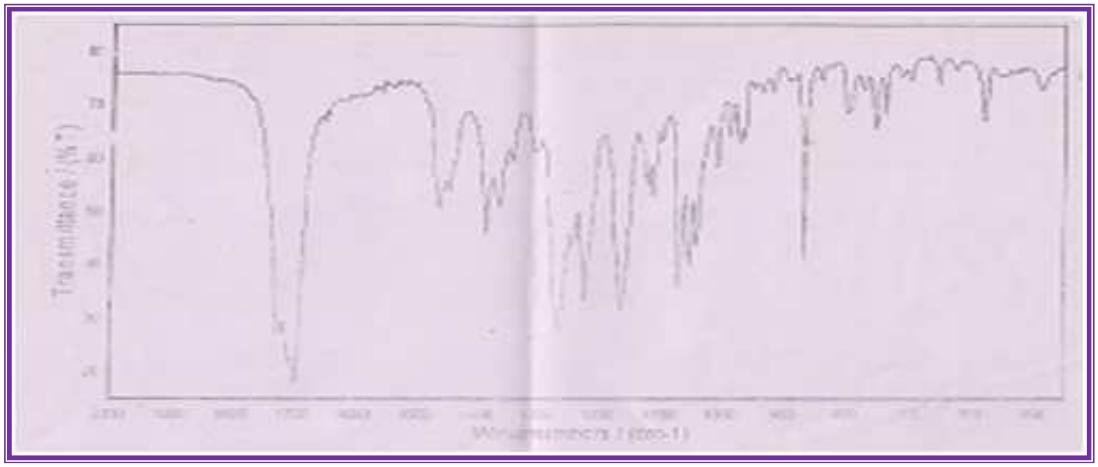
(a)



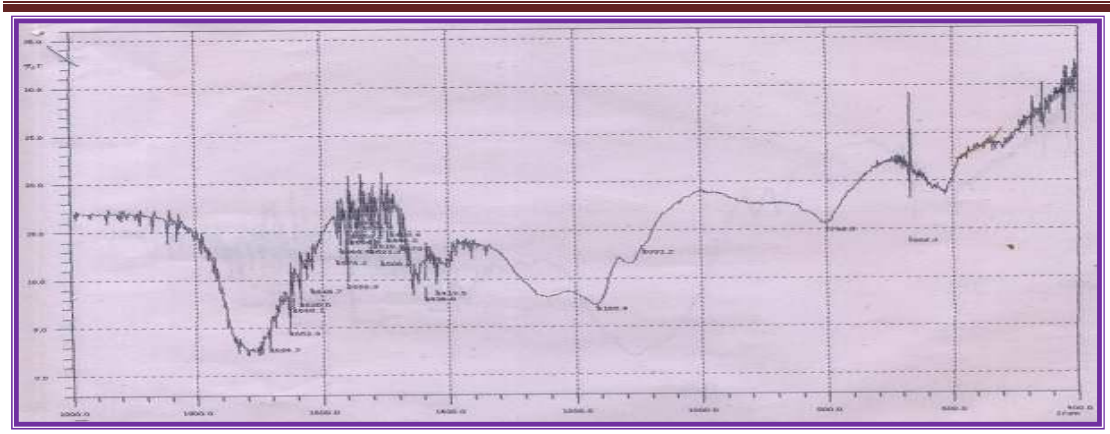
(b)

Figure 7:(a) 3-D surface plots and (b) Contour plot of responses for Y3

FT-IR spectra of Drug alone and Drug with other excipient



(A) Simvastatin alone



(B) Simvastatin + Excipients (Carbopol 934p, Ethyl cellulose, DCM, Methanol)

Figure 8: FTIR Spectrum of Simvastatin and with Excipients

Table 1

Various batches of Simvastatin mucoadhesive microspheres, prepared using the 3^2 full factorial design layout.

Batch	Independent variables		Dependent Variables		
	X_1	X_2	Y_1(%)	Y_2(%)	Y_3(µm)
F1	1	-1	84.97	92	225
F2	1	0	74.01	76	650
F3	0	-1	79.38	62	987.5
F4	0	0	80.26	74	925
F5	0	1	67.92	70	875
F6	1	1	83.18	78	737.5
F7	-1	-1	83.95	82	437.5
F8	-1	0	**96.31**	**94**	**350**
F9	-1	1	71.81	82	587.5

Translation of Coded Level in Actual Unit			
Independent variables	**Low (-1)**	**Medium (0)**	**High (+1)**
Carbopol concentration	0.5	1	1.5
Tween 80 concentration	1.5	2	2.5

Dependent variables	
Y_1	Particle Size
Y_2	% Mucoadhesion
Y_3	% cumulative drug release

Table 2

Powder blend properties of microsphere of formulation F1 – F9

Code	Angle of Repose (o)	Bulk Density (g/ml)	Tapped Density (g/ml)	Carr's Index (%)	Hausner's ratio (%)
F1	22˚79'	0.356	0.543	18.75	1.38
F2	28˚95'	0.322	0.523	24.5	1.62
F3	30˚78'	0.453	0.642	38.5	1.41
F4	31˚89'	0.432	0.521	27.1	1.21
F5	29˚56'	0.331	0.431	27.5	1.32
F6	27˚91'	0.412	0.541	22.9	1.31
F7	30˚69'	0.421	0.532	20.2	1.26
F8	24˚26'	0.425	0.521	18.5	1.22
F9	26˚39'	0.432	0.544	20.6	1.25

Table 3

Result of Evaluation of formulation F1-F9

Batch Code	Average particle size (µm)	% Yield	% Entrapment efficiency	% mucoadhesion	Degree of Swelling
F1	214.25±4.42	69.67	71.5	77.49	110
F2	262.59±3.15	81.46	67.45	82.22	90
F3	297.43±2.58	72.58	70.67	75.45	60
F4	347.842±3.95	79.32	64.87	79.99	60
F5	326.48±4.38	79.97	53.95	50.14	110
F6	310.31±7.17	80.76	51.25	45.67	100
F7	355.12±4.12	77.86	79.75	**90.55**	20
F8	366.129±1.11	79.21	64.55	**90.85**	90
F9	362.45±5.69	71.34	75.63	85.23	100

Table 4

Sphericity of microsphere of formulation F1-F9

Batch Code	Sphericity of Microsphere
F1	Spherical free flowing
F2	Slightly irregular
F3	Slightly irregular
F4	Slightly irregular
F5	Sphere not formed
F6	Spherical free flowing
F7	Good spherical and free flowing
F8	Good spherical and free flowing
F9	Slightly irregular

Table 5

***In vitro* Drug release profile of formulation F1-F9**

Time	Batch Code								
Hr	F1	F2	F3	F4	F5	F6	F7	F8	F9
1	14.74	6.89	16.85	10.72	19.34	10.72	10.53	5.93	6.7
2	13.78	14.74	17.04	18.76	21.44	14.93	12.63	17.04	20.1
3	18.38	18.57	26.42	24.5	29.67	22.21	14.55	16.65	21.06
4	20.87	19.14	33.89	29.67	33.5	33.31	19.11	18.40	22.78
5	27.76	22.59	35.99	35.61	39.82	34.46	20.67	20.11	23.74
6	39.63	29.68	38.48	40.59	44.23	39.25	24.12	26.99	29.87
7	44.61	40.1	44.04	43.84	48.06	51.31	32.74	37.14	33.12
8	64.14	41.35	61.65	58.59	49.97	53.42	37.15	41.1	42.5
9	59.55	61.08	63.18	62.99	51.31	66.44	59.55	58.4	67.2
10	74.86	67.78	67.41	67.65	59.23	69.76	63.37	68.74	63.37
11	79.46	67.97	73.40	73.95	63.58	76.47	70.84	84.25	65.84
12	84.97	74.01	79.38	80.26	67.92	83.18	83.95	**96.31**	71.81

Table 6

R^2, k values of release profile of each formulation made of formulation stage corresponding to Zero-order, First-order, Higuchi and Hixon Crowell kinetics

Batch	Zero Order		First Order		Higuchi		Hixon Crowell	
	R^2	K	R^2	K	R^2	K_H	R^2	K_{HC}
F1	0.9609	7.017	0.8670	0.104	0.7529	19.739	0.9039	0.031
F2	0.9528	6.053	0.8754	0.084	0.7345	16.972	0.9046	0.025
F3	0.9478	6.894	0.9381	0.106	0.8650	19.760	0.9548	0.031
F4	0.9820	6.858	0.9515	0.104	0.8571	19.564	0.9729	0.030
F5	0.9788	6.238	0.9239	0.094	0.9699	18.222	0.8874	0.027
F6	0.9882	7.031	0.9397	0.107	0.9813	19.984	0.9661	0.031
F7	0.9022	5.979	0.7947	0.081	0.9092	16.585	0.8299	0.024
F8	0.8861	6.631	0.7556	0.092	0.9082	18.293	0.9242	0.028
F9	0.9190	5.993	0.8597	0.084	0.9313	16.928	0.9446	0.025

NOTE: R^2= coefficient of determination, k_0=Zero-order release constant, k_1= First-order release constant, kH= Higuchi release constant, kHC= Hixon Crowell release constant

Table 7: R^2, n, kKP values of release profile of each formulation made of formulation stage corresponding to Korsmeyer Peppas

Batch	Korsmeyer Peppas			Mechanism of drug release
	R^2	N	Kkp	
F1	0.9670	1.124	5.344	Non-Fickian
F2	0.9647	1.184	4.039	Non-Fickian
F3	0.9688	0.824	10.111	Non-Fickian
F4	0.9894	0.890	8.729	Non-Fickian
F5	0.9827	0.580	15.464	Non-Fickian
F6	0.9902	0.941	8.003	Non-Fickian
F7	0.9587	1.500	1.973	Non-Fickian
F8	0.9681	1.650	1.559	Non-Fickian
F9	0.9207	1.065	5.196	Non-Fickian

NOTE: R^2= coefficient of determination, n= diffusional exponent, kKP= Korsmeyer Peppas release constant

Table 8

ANOVA for dependent variables

Source	Degree Of Freedom	Sum Of Squares	Mean Square	F Value	Significance
For Y_1 = % CPR					
Regression	5	253.8651	50.77302	10.79906	0.039078
Residual	3	14.10484	4.701615		
Total	8	267.97			
For Y_2 = % Mucoadhesion					
Regression	5	576.1111	115.2222	1.435625	0.4071
Residual	3	240.7778	80.25926		
Total	8	816.8889			
For Y_3= Particle Size					
Regression	5	458.1889	91.63778	2.742607	0.0217868
Residual	3	100.238	33.41266		
Total	8	558.4269			

Table 9

Evaluation of formulation F8 for Stability

Tested after time (days)	Average particle size (µm)	% Entrapment efficiency	% Mucoadhesion	*In vitro* wash off time
At 30 ± 2 °C / 65 ± 5 % RH				
0 Days	350.05±2.9	63.61	94	6.05
30 Days	349±2.9	62.11	92	5.55
At 40 ± 2 °C / 75 ± 5 % RH				
0 Days	350.05±2.9	63.61	94	6.05
30 Days	347.05±1.5	63.45	94	6.12

Table 10

% *in vitro* drug release profile of formulation F8 for Stability

Time (hr.)	Tested after time (1 month)		
	BATCH F4 Initial	At 25±2°C & 60±5% RH	At 40 ± 2 °C / 75 ± 5 % RH
		60 days	60 days
1	5.93	7.34	5.45
2	17.04	17.38	17.99
3	16.65	18.86	19.31
4	18.38	19.36	20.85
5	20.11	21.42	21.94
6	26.99	25.90	24.67
7	37.14	37.33	37.21
8	41.10	42.23	40.90
9	58.40	58.97	55.50
10	68.74	69.29	69.89.
11	84.25	86.10	84.05
12	94.48	95.44	94.15

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