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Design of Experiment (DoE) Approach to Prepare, Characterize and Optimize the Gastroretentive Mucoadhesive Microspheres of Repaglinide

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Abstract

In this present investigation, gastroretentive mucoadhesive microspheres of Repaglinide was formulated, characterized and optimized by applying the design of experiment (DoE) for better release profile and sustain action of the drug. Solvent evaporation technique was used to prepare microspheres, where Methocel K15M CR (X_1), Eudragit L 100 (X_2) and rpm (X_3) were used as independent variables whereas percent cumulative drug release at 8 hours (Y_1), bond strength (Y_2) and swelling at 8 hours (Y_3) were used as dependent variables. Particle size, surface morphology, mucoadhesive bond strength, swelling study, and drug entrapment efficiency were determined to characterize the prepared microspheres. *In vitro* dissolution study was performed in 0.1N HCl (pH 1.2) media for 8 hours. Response surface of dependent variables was calculated by design expert software and it was found that most of the responses were fitted to the quadratic model. Percent cumulative drug release at 8 hours was found minimum 61.34% and maximum 87.29%, the minimum and maximum range of mucoadhesive bond strength was found 426.02 to 13335.74 N/m^2 and in case of swelling at 8 hours, it was found 157.43 and 230.22%. After analyzing the responses, proposed formula was obtained from which minimization of percent cumulative drug release at 8 hours as well as swelling and maximization of bond strength was obtained. Thermal behavior was investigated by DSC study and no interaction was found between drug and excipients from FTIR study.

Key words: Repaglinide, mucoadhesive microspheres, design of experiment.

Introduction

Product and process development problems in pharmaceutical industry usually involve a number of independent variables which are characterized by multiple objectives. Statistically valid experimental design using surface response parameters can be employed to optimize data in order to provide an economical and effective formulation (Ferreira *et al.*, 2004).

Novel drug delivery systems are always very catchy because of their superior characteristics and various advantages like safety, efficacy, sustaining action, better release profile, site specific drug delivery, economical, better patient's compliance etc. Mucoadhesive microspheres are one of the most modern means in novel drug delivery systems which are used to sustain drug delivery in a specific site for better drug release and patient compliance (Bithi *et al.*, 2017).

Currently, diabetes is considered as an incurable chronic disease which affects a large number of populations (Saha, 2018). Repaglinide, a BCS class II drug is widely used to lower the elevated blood glucose level (Culy and Jarvis, 2001). Being a BCS class II drug it has less solubility in intestinal pH and has better release profile in stomach or acidic pH (Marbury *et al.*, 1999).

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Based on these issues, an attempt was made in this investigation to prepare mucoadhesive microspheres of Repaglinide by using Central Composite Design (CCD) with the help of design expert software. By preparing mucoadhesive microspheres of Repaglinide, it is possible to retain the drug in stomach or acidic pH for a prolonged period of time and this is beneficial in the view point of pharmaceutical industry also.

Materials and Methods

Materials: Repaglinide was received as gift sample from Incepta Pharmaceuticals Ltd., Bangladesh. Methocel K15M CR was obtained from Colorcon Asia Pvt Ltd., USA and Eudragit L 100 was obtained from Evonik Industries, Germany. Other chemicals used in this experiment were of analytical grade.

Preparation of Repaglinide loaded microspheres: Emulsion solvent evaporation method was applied to prepare microsphere. The materials amount in coded form and in different formulations has been shown in table 1 and table 2, respectively. Weighted quantities of polymers were dissolved in a mixture of acetone and ethanol (1 : 2). Then the

Table 1. Coded formulation of microspheres using central composite design (CCD).

Independent variables	Low	High	Dependent Variables	Objective
	Coded -1	Coded +1		
Merhocel K15MCR (X_1)	200 mg	415 mg	% CDR at 8 hours (Y_1)	Minimize
Eudragit L 100 (X_2)	200 mg	400 mg	Bond strength (Y_2)	Maximize
RPM (X_3)	250 rpm	300 rpm	Swelling at 8 hours (Y_3)	Minimize

Table 2. Formulation of microspheres using central composite design (CCD).

Formulation code	Repaglinide (mg)	PEG 4000 (mg)	Original Values		
			X_1 (mg)	X_2 (mg)	X_3 (rpm)
F1	6	2	126.71	300	275
F2	6	2	307.50	300	233
F3	6	2	200	400	250
F4	6	2	307.50	131.82	275
F5	6	2	200	400	300
F6	6	2	200	200	250
F7	6	2	415	200	300
F8	6	2	307.50	300	317
F9	6	2	200	200	300
F10	6	2	307.50	300	275
F11	6	2	307.50	300	275
F12	6	2	415	400	300
F13	6	2	415	200	250
F14	6	2	307.50	468.18	275
F15	6	2	307.50	300	275
F16	6	2	415	400	250
F17	6	2	488.29	300	275

required amount of Repaglinide was added and dissolved into the mixture by using sonicator and vortex mixture in order to prepare internal phase solution. For the preparation of external phase, 60 ml light liquid paraffin was emulsified with 1% tween 80 and taken in a 500 ml beaker and stirred using overhead stirrer. After that, the internal phase was slowly introduced by drop-wise into the external phase, while stirring at required rpm held by the mechanical stirrer equipped with a three-blade propeller, at room temperature. The whole system was run for three hours. After 3 hours, the microspheres were separated by filter paper and the excess of paraffin oil was removed by continual washing (5 to 6 times) with n- Hexane (50 ml) and then the obtained microspheres were lastly dried overnight in desiccators at room temperature to get free-flowing sphere-shaped yields (Srivastava *et al.*, 2005; Rahman *et al.,* 2016; Ayon *et al.*, 2014).

Percentage yield: The percentage yield of the dried microspheres was calculated as per the following formula (Chaturvedi *et al.*, 2012):

$$\text{Percentage of yield} = \frac{\text{Weight of microspheres obtained}}{\text{Weight of drug + polymer}} \times 100\%$$

Particle size analysis: The particle size analysis and size distribution of the microspheres were determined by using sieving method (Behera *et al.*, 2008). Microspheres that were retained on each sieve were collected and weighted, and the average particle size was calculated based on the following formula:

$$\text{Average particle size} = \frac{\Sigma(\%\ \text{Retained} \times \text{di})}{\Sigma\ \%\ \text{Retained}}$$

Here, di is the arithmetic mean of the upper and lower openings of the sieve on which the portion of microspheres was recollected (Abdallah *et al.*, 2012).

Drug entrapment efficiency: Drug entrapment efficiency of Repaglinide loaded microspheres was measured by using UV Spectrophotometer having absorbance at 243 nm. A small amount of microspheres was taken in a mortar and were triturated properly with pestle until fine powder was formed. Required amount of fine powders were taken in a volumetric flask containing 40 ml of acidic media (0.1N HCl) which was then subjected to mixing using vortex mixture and sonicator. The volume of the solution was later adjusted to 100 ml with the acidic media and absorbance was taken at 243 nm by doing proper dilution. This method was repeated in order to obtain entrapment efficiency for rest of the formulations (Farheen *et al.*, 2017).

$$\text{Entrapment Efficiency} = \frac{\text{Amount of drug actually present}}{\text{Theoretical drug load expected}} \times 100\%$$

Swelling measurement: Around 100 mg of drug loaded microspheres from each batch were placed separately in a vessel which contains 100 ml of 0.1N HCl of pH 1.2 and temperature was kept at 37 ± 0.5°C. The microspheres were periodically removed at pre-determined intervals of 1st hour, 2nd hour, 3rd hour, 4th hour, 5th hour, 6th hour, 7th hour and 8th hour then re-weighed the microspheres after removing excess water with the help of filter paper (Chaturvedi *et al.*, 2012).

$$\%\ \text{swelling} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100$$

In vitro dissolution study: In vitro dissolution studies of the microspheres were conducted in acidic media using USP dissolution apparatus type I (Basket type). For performing the dissolution study, weighed quantities of microspheres were placed in basket in 900 ml of acidic dissolution medium having pH 1.2 and stirred at 50 rpm at 37 ± 0.5ºC. At pre-determined time intervals of 1, 2, 3, 4, 5, 6, 7 and 8 hrs, 5 ml aliquot was withdrawn from the dissolution media. At each interval, the withdrawn medium was replaced with an equivalent amount (5 ml) of fresh 0.1 N HCl of dissolution medium. Collected samples

were analyzed by determining the absorbance through UV spectrophotometer at 243 nm after suitable dilution to determine the amount of Repaglinide released from the microspheres.

Surface morphology study: Scanning Electron Microscope (SEM) was utilized to contemplate the morphology and surface topology of the microspheres. The microsphere from the advanced clump were mounted on the SEM test wound which were covered with a twofold sided staying tape, fixed lastly covered with gold (200 Aº) under lessened weight (0.001 tor) for 15 minutes utilizing particle sputtering gadget. The gold covered samples were filtered utilizing SEM (s-3400N, Hitachi) under various amplification like 20 kV X 50, 20 kV X 200 and photograph of micrographs of appropriate amplification were dried totally before examination.

Ex vivo mucoadhesion test: A modified balance method was set up for determining the mucoadhesive strength of the prepared microspheres (Karmoker *et al*., 2019; Kyada *et al*., 2014). For preparing such balance fresh goat intestinal mucosa was collected from a local slaughterhouse and used within 2 hours of slaughter. The mucosal membrane was detached by removing the underlying fat and loose tissues. After that the membrane was washed with distilled water and then cut into pieces. Two pieces of goat stomach mucosa were attached to the bottom of two glass vials separately. From that one glass vial was fixed on the surface of the floor and other piece was tangled with the balance on left hand side. A glass beaker was kept on right hand side which was placed on the balance. The right and left sides were balanced by adding extra weight on both the left and right hand side of the balance. Approximately 100 mg of microsphere from each batch was placed between the two glass vials covered with goat stomach mucosa and 2-3 drops of acidic solution (0.1N HCl of pH 1.2) were added on it for wetting. Then the two vials were gently pressed together to remove the presence of air. The balance was kept in this position for 5 minutes. As the amount of microspheres was not more so microspheres formed the single layer along the surface of disk. Water was added slowly at 1ml/min to the beaker placed at right hand pan of the balance until the microsphere separated from the goat stomach mucosal membrane. The water in ml (1 ml equivalent to 1 gram) required to isolate the microsphere from the mucosal surface gave the measure of mucoadhesive strength. The strength of the mucoadhesive microspheres were calculated using following equations:

$$\text{Force of adhesion (N)} = \frac{\text{Mucoadhesion strength} \times 9.81}{1000}$$

$$\text{Bond strength (N/m}^2\text{)} = \frac{\text{Force of adhesion}}{\text{Surface area of vial}}$$

Fourier transform infrared spectrophotometry (FTIR): The FTIR spectrum of the pure drug, mixture of drug and specific polymers and optimized microsphere formulation were obtained to prove the chemical integrity and compatibility of the drug in the formulation. It was performed by KBr pellet method having the range of 4000 - 500 cm^{-1} for 30 times.

Differential scanning calorimetery (DSC) for thermal analysis: Differential scanning calorimeter was used to measure the specific heat and enthalpies of transition. Thermograms were obtained by using a differential scanning calorimeter (Shimadzu DSC 60) at a flow rate of 20ml/min and heating rate of 10ºC/ min over a temperature range of 30°C to 300°C.

ANOVA and response surface analysis: Analysis of variance and response surface analysis was conducted for cumulative percent of drug release using Design Expert software (State-Ease Inc.), where $p < 0.05$ was considered as a statistically significant difference.

Results and Discussion

Percentage yield, mean particle size and drug entrapment efficiency: Percentage yield, mean particle size and drug entrapment efficiency of the formulated batches are presented in table 3.

From table 3 it is found that the yield result was suitable and the range of the result was from 78.60 %

to 98.76 %. F1 shows lowest percentage yield whereas F10 has highest percentage yield of microspheres. This outcome reveals that during preparation of Repaglinide microspheres, fewer amounts of polymers were lost as the washing procedure was done carefully.

Table 3. Percentage yield, mean particle size and drug entrapment efficiency of mucoadhesive Repaglinide microspheres.

Formulation code	Percentage yield (%)	Mean particle size (μm)	Drug entrapment efficiency (%)
F1	78.60	388.76	66.34
F2	97.50	316.45	78.84
F3	96.20	318.43	77.17
F4	89.14	360.71	88.51
F5	97.50	339.19	76.61
F6	95.53	390.94	73.53
F7	97.34	389.91	81.83
F8	97.36	319.16	78.45
F9	98.07	315.72	74.38
F10	98.76	342.12	79.46
F11	98.61	340.62	78.89
F12	95.97	530.27	88.51
F13	98.10	371.62	82.51
F14	92.48	344.17	84.38
F15	98.36	358.92	78.58
F16	95.26	535.74	68.92
F17	94.41	402.16	85.53

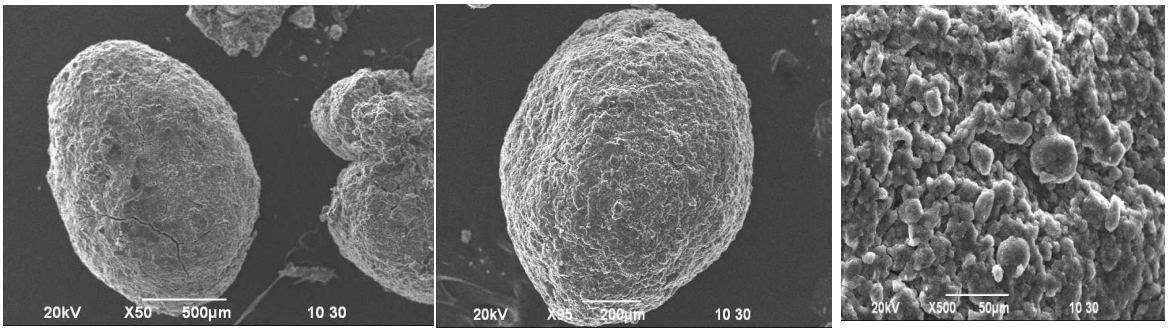
Particle size of microsphere increases as the concentration or polymeric ratio increases. F12 and F16 have a bigger particle size whereas F2 and F9 have smaller particle size. This could be due to the accumulation of more polymers in the formulation leading to larger size particles. Besides, rpm may play a vital role in increasing or decreasing particle size of the microspheres. Like the size of the particle of F6 > F9 and similarly the size of the particle of F16>F12, in both cases rpm were less where larger particles were formed. More rpm causes the size of the particles to be small. According to the definition of microsphere, size of microspheres should be between 1-1000 μm. So, it can be said that the prepared formulations were microsphere in terms of their particle size.

Drug entrapment efficiency (DEE) of the prepared microspheres of different formulations ranges from 66.34% - 88.51%. %DEE was increased with increased polymeric concentration. F4 and F12 exhibited the highest %DEE of 88.51% and F1 showed minimum %DEE of 66.34%. Considering the amount of polymers used in F1, F4 and F12 current investigation suggests that drug entrapment efficiency increases with increasing polymeric concentration.

Surface morphology study: Surface morphology of prepared microspheres is presented in figure 1. Shape of the microspheres is a vital consideration which shows the uniformity of ingredients of the particle. The shape of microspheres provides the uniformity of ingredients along with the property and dissolution configuration of the prepared microspheres. The nature of the surface of microspheres was another parameter for analysis. If surface is rough, then there is more chance of wetting

and contact of water with it than the smoother one. The rough texture might be the root of holding the moisture at peaks, cracks or ridges as variably seen from formulation to formulation. This persistence of moisture for more time might cause weakening of the matrix system.

Percent swelling and mucoadhesive bond strength: Figures 2 and 3 represents the percent of swelling and mucoadhesive bond strength, respectively. Percent swelling is more in case of F4 as compared to other formulationsprobably because F4 contains less amount of Eudragit L100. The % swelling was found in the range of 157.43% to 230.22%.



A) 50 times magnification B) 95 times magnification C) 500 times magnification

Figure 1. Surface morphology (A, B, C) of prepared Repaglinide mucoadhesive microspheres.

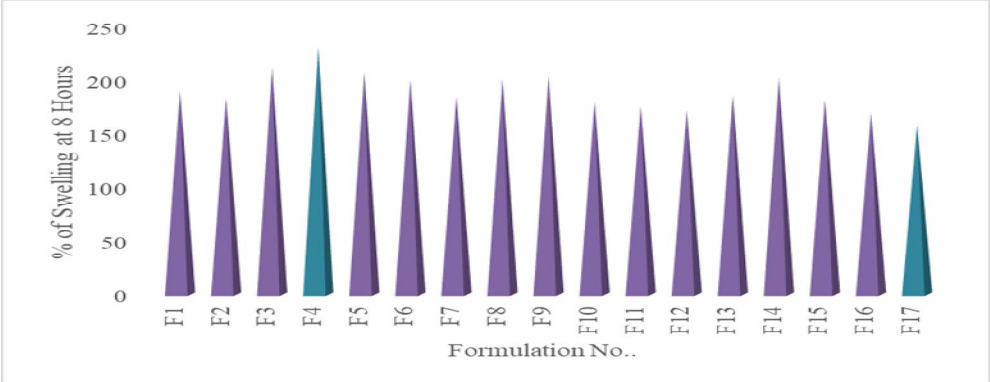
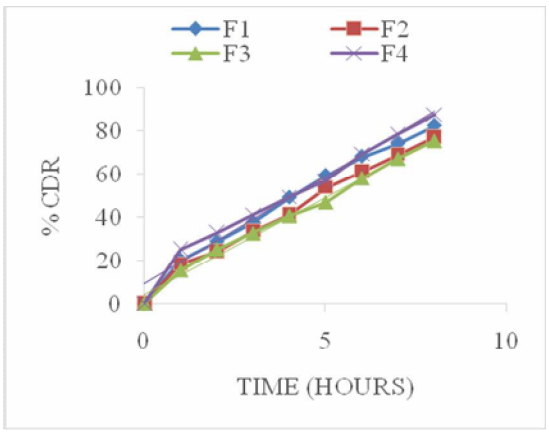


Figure 2. Percent of swelling of Repaglinide mucoadhesive microsphers at 8 hours.

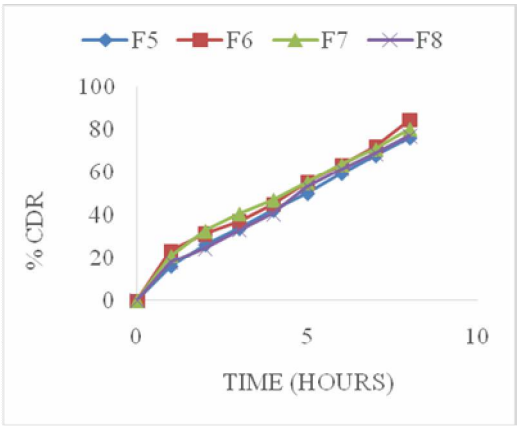
Figure 3. Mucoadhesive bond strength of Repaglinide mucoadhesive microsphers at 8 hours.

From the figure 3, it is clear that F1 has the weakest bond and F17 has the strongest bond with mucous layer of goat stomach. So F17 has good mucoadhesive properties compared with others.

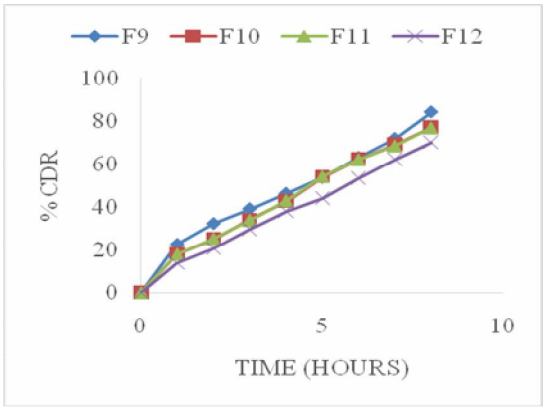
In vitro dissolution study: Data of cumulative drug release (CDR) presented in figure 4 (A-D). It has been established that the rate of drug release from the prepared microspheres were determined by the ratio of polymeric concentration that have been used and the rate of drug release usually declined with increasing the amount of polymers. The rate of drug release decreases with increasing content of the polymers: Eudragit L 100 and Methocel K15M CR. The presence of these polymers lessened amount of drug present near to the surface of the preparation and the second fact might be that due to increasing the polymer amount, the amount of uncoated drug decreases.



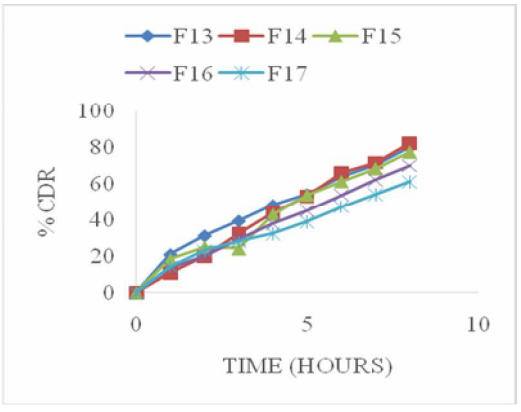
A) % CDR of F1, F2, F3 and F4



B) % CDR of F5, F6, F7 and F8



C) % CDR of F9, F10, F11 and F12



D) % CDR of F13, F14, F15, F16 and F17

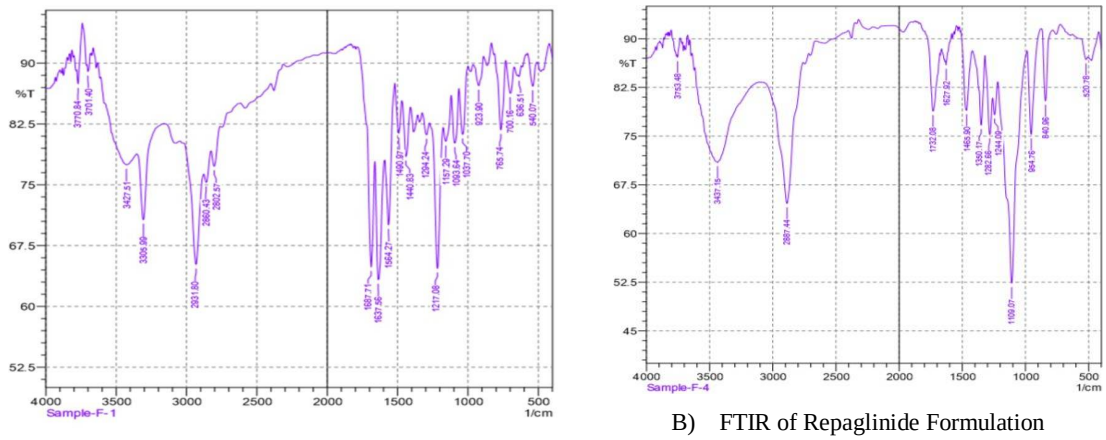
Figure 4. Percent cumulative drug release (CDR) of F1-F17 (A-D).

PEG 4000 which was used as surfactant help in forming porosity on the surface of the microsphere to enhance the release of drug but the amount of the surfactant was kept fixed and less amount was used compared to other polymers that were used. So, for this reason percentage of drug release was decreased mainly when polymers concentration or amount was rose.

Drug-excipients compatibility study by FTIR: No interaction was found between drug and excipients by FTIR study. FTIR result is presented in Figure 5. FTIR spectrum indicates that there are no chemical

interactions takes place between the drug and the polymers. Again, no identified variation is observed with the help of peak values of the overlapping spectrum of drug, polymer and microspheres.

DSC study: DSC study reveals (Figure 6) that the thermal behavior of the drug and the physical mixture did not overlap with each other, that means due to thermal heat their physical state changes and for this the graph shift towards right. This means that at high temperature, the drug and the polymers were not stable.



A) FTIR of Repaglinide (Pure Drug)

B) FTIR of Repaglinide Formulation

Figure 5. FTIR spectrum of Repaglinide (A) and Repaglinide formulation (B).

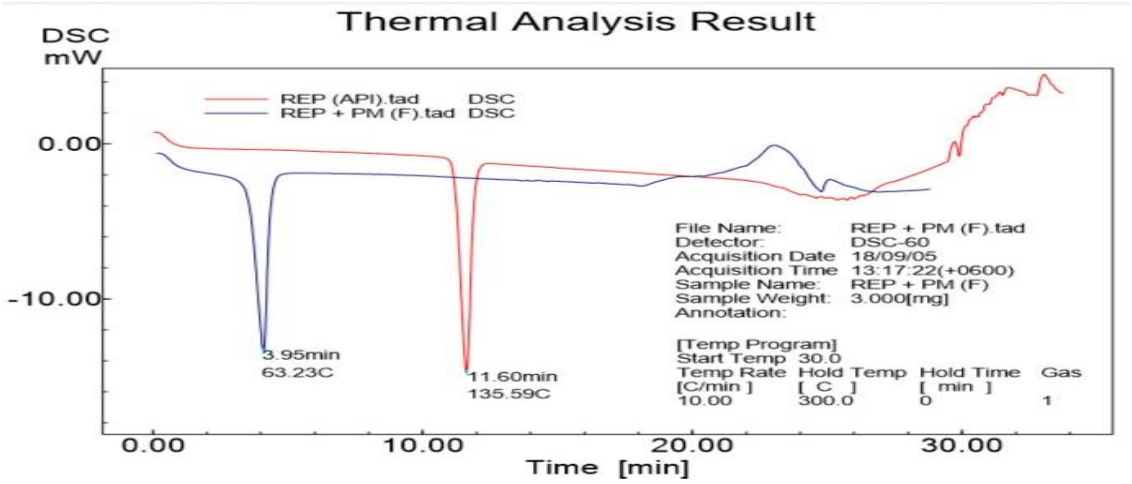


Figure 6. DSC graph of Repaglinide (red) and physical mixture of Repaglinide formulation (blue).

Analysis of % CDR at 8 hours (Y_1), mucoadhesive bond strength (Y_2) and swelling at 8 hours (Y_3): Final equation in terms of coded factors for Y_1, Y_2 and Y_3 are tabulated in table 4. On the other hand, ANOVA analysis is presented in table 5. Response surface graphical presentations for all the effects are shown in figures 7 - 9.

F values of Y_1, Y_2 and Y_3 indicates that, models are significant since in all the cases $p < 0.05$. Incase of Y_1, A, B, B^2 are significant model terms. The "Lack of Fit F-value" of 149.43 implies the Lack of

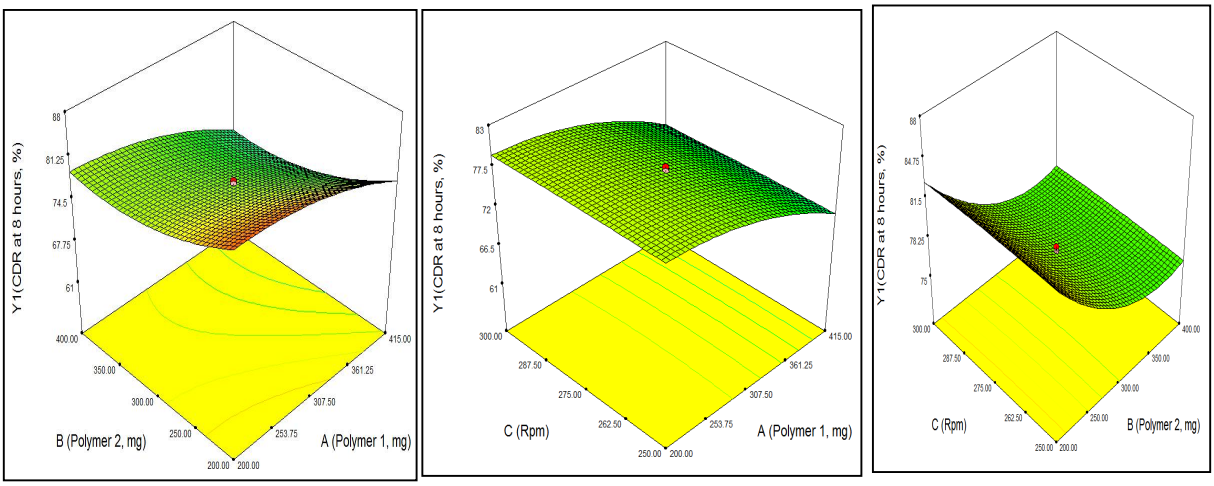
Fit is significant. A negative "Predicted R-Squared" indicates that the overall mean is a better predictor of response than the current model. "Adequate Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. The ratio for Y1 is found to be 9.615 which indicates an adequate signal. This model can be used to navigate the design space.

Table 4. Final equation in terms of coded factors.

Final equation in terms of coded factors
Y_1 = 77.17-3.99A-3.30B-0.018C-0.43AB-3.750C-003AC+0.094 BC-1.97A^2+2.64B^2-0.11C^2
Y_2 = 914.56+238.56A-67.97B-2.95C
Y_3= 178.94-12.00A-4.53B+2.09C-5.81AB+0.35AC-0.52BC-2.57A^2+12.49B^2+3.91C^2
Here, A= Amount of Methocel K15MCR, B= Amount of Eudragit L100, C= RPM

Table 5. ANOVA analysis for different responses (Y_1, Y_2 and Y_3).

Criteria	Y_1	Y_2	Y_3
F value	5.11	43.902	10.46
p value	0.0215	< 0.0001	0.0027
Lack of fit	149.43	5.950	5.92
R^2	0.8678	0.9102	0.9308
Predicted R^2	–0.0032	0.8395	0.4928
Precision	9.615	20.708	12.780



i. ii. iii.

Figure 7. Response surface shows the effects of i. A and B ii. A and C iii. B and C on Y_1.

For Y2, The "Lack of Fit F-value" of 5.95 implies the Lack of Fit is not significant relative to the pure error. There is a 15.26% chance that a "Lack of Fit F-value" this large could occur due to noise. Non-significant lack of fit is considered good.The "Predicted R-Squared" of 0.8395 is in reasonable agreement with the "Adjusted R-Squared" of 0.8894. "Adequate Precision" measures the signal to noise ratio. Ratio of 20.708 indicates an adequate signal since it is above 4.

For Y3, The "Lack of Fit F-value" of 5.92 implies the Lack of Fit is not significant relative to

the pure error which is a good indication. There is a 15.08% chance that a "Lack of Fit F-value" this large could occur due to noise. The "Predicted R-Squared" of 0.4928 is not as close to the "Adjusted R-Squared" of 0.8419 as one might normally expect. This may indicate a large block effect or a possible problem with the model and/or data. Things to consider are model reduction, response tranformation, outliers to measures the signal to noise ratio. Any value greater than 4 is desirable for "Adequate Precision". The ratio of 12.780 indicates an adequate signal. Therefore, this model can also be used to navigate the design space.

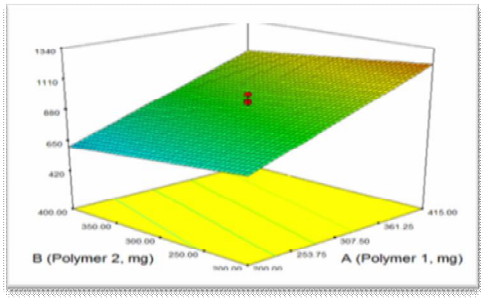
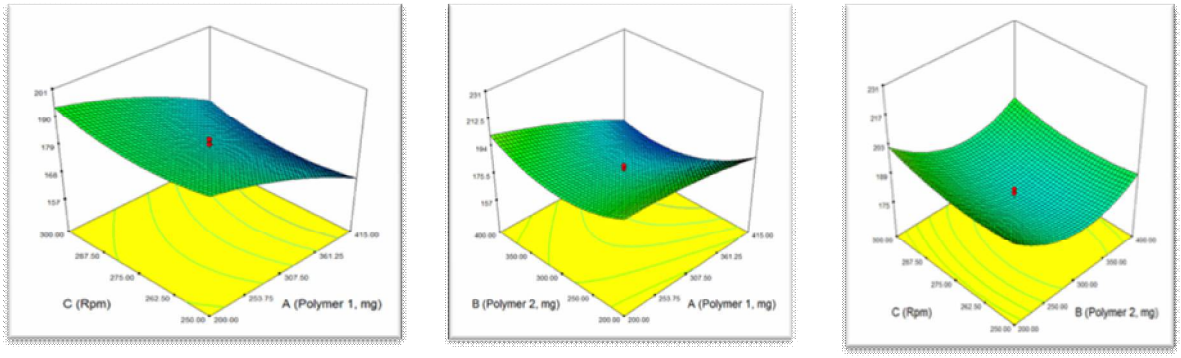


Figure 8. Response surface shows the effects of A and B on Y_2.



i. ii. iii.

Figure 9. Response surface shows the effects of i. A and C ii. A and B iii.B and C on Y_3.

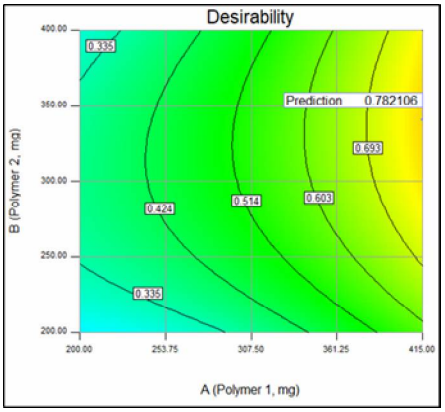


Figure 10. Contour plot shows the effects of A and B on desirability.

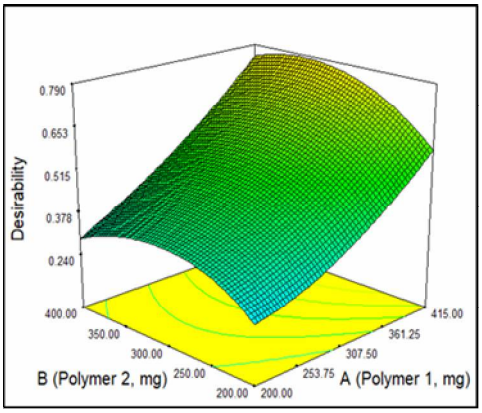


Figure 11. Response surface shows the effects of A and B on desirability.

Table 6. Optimization of Y_1, Y_2, and Y_3.

Methocel K15M CR (mg)	Eudragit L100 (mg)	rpm	Y_1	Y_2	Y_3	Desirability
415	340.41	265	70.11	1126.74	161.95	0.782

Obtaining responses are optimized by desirability functions described by Derringer and Suich (1980). Possible solutionis stated in table 6.

Minimum Y_1 (CDR at 8 hours), minimum Y_3 (Swelling at 8 hours) and maximum Y_2 (Mucoadhesive bond strength) were obtained for Methocel K15MCR at 415 mg, Eudragit L100 at 340.41 mg and rpm at 265 with desirability of 0.782. The 2D contour plot and 3D surface plot of overall desirability functions are presented in figure 10 and figure 11, respectively.

Conclusion

Repaglinide loaded mucoadhesive microspheres were prepared by solvent evaporation technique. The method was easy, economical and modest. When the amount of polymer increases then the release rate decreases and the ratio of Eudragit L 100 and Methocel K15M CR varied while the amount of PEG 4000 was kept constant in all the formulations. F4 was the best formulation as because after 8 hours this formulation gave the highest % of drug release along with highest mucoadhesive bond strength. This study presented that polymeric blend of Eudragit L 100, Methocel K15M CR and PEG 4000 contained in microspheres could be useful carrier for Repaglinide drug.

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