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Formulation and evaluation of mucoadhesive microspheres of simvastatin using a 2^3 full factorial approach

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Abstract: The objective of the current investigation was to optimize and formulate mucoadhesive microspheres loaded with simvastatin utilizing guar gum as the mucoadhesive polymer and stabilizing the particles with magnesium stearate so that the formulation could control the release of the drug thereby reducing its dosing frequency and improving the bioavailability. The microspheres were prepared using emulsion coacervation method and the micromeritic features were evaluated. The yield of all the batches of microspheres was found to be reasonably good ranging from 52.96 % to 75.48 %. The drug loading was found to be 70.46 ± 0.130 to 74.63 ± 0.535 % while the size of microspheres ranged from 13.34 ± 0.092 µm to 17.73 ± 0.217 µm. Swelling study was performed on all the formulation for 24 h and swelling index ranged from 2.95 to 4.27. The particles size of the formulation was found to be 14.04 µm with polydispersity index of 0.701. The mucoadhesion time of the formulation was obtained to be 6 h 19 min. The *in vitro* release of simvastatin was studied for 12 h and it was found that the microsphere formulation was able to sustain the release of the drug for more than 12 h with 73.2 % drug released at the end of the 12th hour.
Keywords: Microsphere, patch, optimization, simvastatin, emulsion coacervation

1. Introduction

The relative ease of administration as well as the traditional belief makes oral drug delivery as the most widely recommended route of drug administration. The last two decades of research in drug delivery have witnessed numerous scientific advancements leading to novel approaches to modify the release of drugs by modifying the delivery system.[1] Mucoadhesion is one such advancement of the modified release systems that allows for prolonged release of drug and enhanced bioavailability. Mucoadhesives are synthetic or natural polymers which interact with the mucus layer covering the mucosal epithelial surface and mucin molecules constituting a major part of the mucus. They localize the formulation at a particular region of the body thereby improving bioavailability of drugs with low bioavailability.[2-8] The increased contact time and localization of the drug due to the strong interaction between the polymer and mucus is essential for the modification of tissue permeability. Mucoadhesive drug delivery systems are delivery systems, which utilize the property of certain polymers to adhere to mucus membranes upon hydration[9] and hence can be used for targeting a drug to a particular region of the body for extended period of times[10]. Simvastatin is a hyperlipimic drug that lowers the level of lipoproteins in blood.[11,12] It has attaracted considerable attention due to its potential to prevent cardiovascular diseases by retarding the accelerated atherosclerosis in hyperlipoproteinemic individuals.

The bioavailability of simvastatin is reported to be 5-30% on oral administration and its protein binding is very high. This makes it a good candidate for mucoadhesive delivery. The objective of this work was the development and investigation of mucoadhesive microspheres of simvastatin in order to improve its bioavailability and reduce the dosing regimen. Treatment of disease requires maintenance of uniform concentration of drug in blood for a long period of time.

2. Material and Methods

Calibration curve of simvastatin in methanol

A stock solution of simvastatin (100 mg/100 ml) was prepared in methanol. Diluted simvastatin solution (10 mg / 100 ml) in methanol was prepared from the stock solution. Then, serial dilutions were prepared from that diluted into simvastatin solution in ethanol to obtain different concentrations ranging from 2.5 to 45 µg/ml. The

absorbance of these serial dilutions was determined spectrophotometrically at λ_{max} 238 nm, using methanol as a reference. Each sample was analyzed in triplicate and the results are presented as mean ± SD. The measured absorbance was plotted against the corresponding concentrations to obtain the standard calibration curve.

Formulation of mucoadhesive microspheres[13]

Simvastatin microspheres were prepared according to the oil-in-oil emulsification-coacervation method using guar gum as the polymer. Briefly, Guar gum was dissolved in 15.0 mL of acetone in a 250 mL beaker with stirring at room temperature. Guar gum (110 mg) and magnesium stearate (0.1 g) were dispersed in the polymer solution. The resulting milky white dispersion was added drop-wise into a beaker containing a mixture of liquid paraffin (50 mL) and span 60 (0.5 g) and homogenized using a paddle stirrer at 500 rpm for 2 h. The formed microspheres were separated by filtration and washed many times with n-hexane to make them completely free from oil. The microspheres were dried at room temperature and stored at 4°C until used. Eight batches of the microspheres were prepared for optimization of the process variables by varying the ratio of drug to polymer, stirring speed and amount of droplet stabilizer utilizing a 2^3 full factorial approach, as shown below in Table 1. The particles size (minimum size) and mucoadhesive ability (maximum) were taken as the desired responses for optimization.

Table 1: Batch Formula for formulation of mucoadhesive microspheres

Formulation Code	Simvastatin (mg)	Guar gum (mg)	Magnesium stearate (g)	Stirring Speed (rpm)
F1	100	200	0.1	500
F2	100	200	0.2	500
F3	100	100	0.2	500
F4	100	100	0.1	500
F5	100	100	0.2	250
F6	100	200	0.1	250
F7	100	100	0.1	250
F8	100	200	0.2	250

Evaluation of micromeritic characteristics

Angle of repose, Carr's Index, Bulk density, Tapped density and Hausner's ration were determined to assess the flow ability of the prepared granules.[14,15]

Angle of repose

Angle of repose was determined by using funnel method. Accurately weighed amount of microspheres were taken in funnel. The height of the funnel was adjusted in such a way that the tip of the funnel just touches the heap of the pile. The microspheres were allowed to flow through the funnel freely on to the surface. The diameter of powder cone was measured and the angle of repose was calculated using the following formula:

$$\text{Tan } \theta = h/r$$

Where, h is the height of the pile; θ is the angle of repose; and r is the radius of the heap

Bulk Density

Bulk density is the ratio between a given mass of powder and its bulk volume. Apparent bulk density was determined by pouring the weighed granules into a graduated cylinder via funnel and measuring the volume. Density was calculated using the formula:

$$\rho_b = M/V_b$$

Where, ρ_b is the bulk density; M is the mass of the microspheres and V_b is the volume occupied by the microspheres.

Tapped Density

Tapped density is the ratio between a given mass of powder and the constant or final volume of powder after tapping. It was determined by tapping a graduated cylinder containing a known mass of granules for a fix number of taps until the powder volume has reached a constant value. The tapped density was computed using the formula:

$$\rho_t = M/V_t$$

Hausner's Ratio

Hausner's ratio was calculated from the bulk and tapped density using the formula:

Hausner's Ratio = Tapped density/ Bulk density

Percent compressibility (Carr's Index)

Compressibility is an important measure that can be obtained from the bulk and tapped densities. The flow ability of the granules was measured by the application of compressibility index given by the equation:

$$I = (1 - Vf/Vo)\ X\ 100$$

Where Vf = volume of the sample after tapping; V_0 = volume before tapping

In Carr's Index, the value below 15% indicates good flow properties whereas a value above 25% indicates poor flow characteristics.

Yield

The formed microspheres were weighed accurately and the yield of the microspheres was determined by comparing the weight of the microspheres against the combined weight of the copolymer and drug using the equation:

$$\boldsymbol{Yield}\ (\%) = \frac{\boldsymbol{Weight\ of\ microspheres}}{\boldsymbol{Weight\ of\ drug + polymers}}\ \boldsymbol{X\ 100}$$

Drug content

The simvastatin content of the microspheres was determined using UV spectrophotometery. 10 mg of microspheres were dispersed in 10 mL of simulated intestinal fluid (SIF, pH 7.2). The dispersion was allowed to stand for 2 h, vortexed for 5 min and then centrifuged at 4,000 rpm for 10 min. The amount of simvastatin contained in each batch of the formulations was determined by the UV at 238 nm. The drug-loading efficiency was then determined by the equation:

$$Drug\ loading\ efficiency\ (\%) = \frac{Weight\ of\ drug\ in\ microspheres}{Weight\ of\ microspheres}\ X\ 100$$

Particles Size

The average particle size was calculated using calibrated optical micrometer by dispersing the prepared formulation in distilled water and counting the size of individual particles under an optical microscope.

In-vitro release

The USP type II paddle apparatus with a paddle speed of 50 rpm was used for studying the release of the drug from the microspheres. The dissolution media used consisted of 900 mL of phosphate buffered saline (pH 6.8) and maintained 37±0.5°C. 5 mL of samples were collected at time points of every hour until 12 h and the media was replenished with the same volume of fresh media. The free simvastatin concentration was estimated using a UV spectrophotometer at a wavelength of 238 nm. The release kinetic was studied by various kinetic models like zero order, first order, Higuchi plot and Korsemeyer-Peppas model. The best fit model was confirmed by the value of correlation coefficient.

Swelling Index

100 mg microspheres were allowed to swell for 24 h in 6.8 pH phosphate buffer. After 24 hr excess liquid were removed by blotting paper and microspheres were weighed. The degree of swelling was then calculated by the following formula

$$Degree\ of\ Swelling = (M0 - Mt)/M0) * 100$$

Where, S.I = swelling index, M_0 = weight of microsphere at the end and M_t = weight of microsphere at start.

***In-vitro mucoadhesion wash-off test*[16]**

Mucoadhesive property of microspheres was determined by *in-vitro* adhesion test. Eggshell membrane was used for this purpose. A 2x1 cm piece of eggshell membrane were taken and fixed on a glass slide (kept at an angle of 45°C). About 100 mg microspheres were spread on rinsed, tissue specimen and hung onto one of the groves of a USP tablet disintegrating test apparatus containing 6.8 pH phosphate buffer. The disintegrating test apparatus was started, the tissue specimen showed regular up and down movements in a beaker. The time required for detaching of microspheres from mucosal surface membrane was recorded by visual inspection.

3. Results and Discussion

Calibration Curves of simvastatin

The standard calibration curve of simvastatin was constructed in methanol to obtain different concentrations ranging from 2.5 to 45 µg/ml, for which the absorbance readings were determined spectrophotometrically at λmax 238 nm (Figure 5.3). The standard calibration curve was linear over the concentration range studied and obeys Beer-Lambert's law with a correlation coefficient (R^2) 0.998. The corresponding regression equation was found to be Y = 0.018X – 0.005.

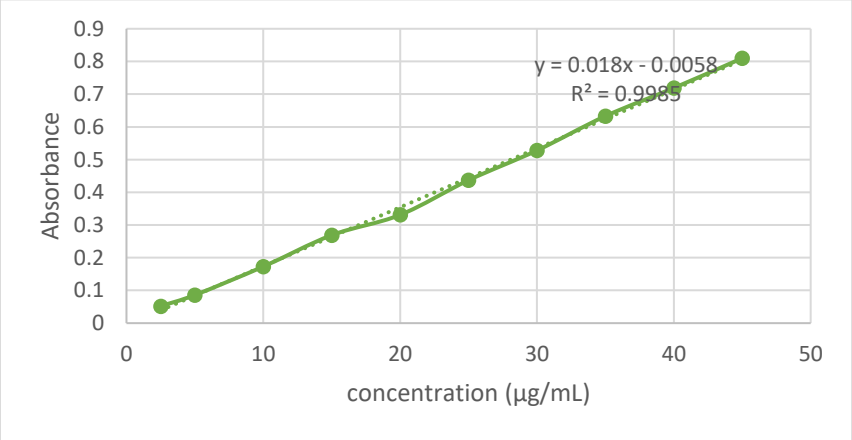


Fig 1: Calibration curve of simvastatin

Characterization of Formulation Blends

Bulk and Tapped Density

The bulk and tapped density of the formulations ranged from 0.382 ± 0.010 to 0.612 ± 0.003 g/cm^3 and 0.411 ± 0.004 to 0.738 ± 0.006 g/cm^3 respectively. The bulk and tapped density play a vital role in pharmaceuticals as it reflects processing ability of the blend. It also reflects flow ability of the blend using various calculative ratios. The bulk and tapped density of the formulation blends is presented in table 2.

Table 2: Micromeritic features of the formulation blends

Formulation code	Bulk density (g/cm^3)	Tapped density (g/cm^3)	Angle of Repose (°)	Carr's Index	Hausner's Ratio
F1	0.382 ± 0.010	0.446 ± 0.002	23.57 ± 0.121	14.35	1.17
F2	0.451 ± 0.006	0.525 ± 0.005	24.15 ± 0.060	14.10	1.16
F3	0.558 ± 0.007	0.695 ± 0.005	25.31 ± 0.146	19.71	1.25
F4	0.612 ± 0.003	0.738 ± 0.006	25.62 ± 0.297	17.07	1.21
F5	0.395 ± 0.004	0.411 ± 0.004	27.44 ± 0.235	3.89	1.04
F6	0.393 ± 0.004	0.416 ± 0.006	28.54 ± 0.078	5.53	1.06
F7	0.408 ± 0.003	0.426 ± 0.003	28.71 ± 0.055	4.23	1.04
F8	0.389 ± 0.005	0.432 ± 0.005	30.01 ± 0.833	9.95	1.11

Results are represented as mean ± standard deviation; n =3

Angle of Repose

Angle of repose is a measure of the ability to powder to flow through the hopper of the tablet punching machine. The angle of repose was measured using the fixed funnel method and was found to be ranging from 23.57 ± 0.121 to 30.01 ± 0.833. Angle of repose of less than 30° is considered to be good for the flow of the powder. The angle of repose of all the formulation blends is presented in table 2.

Hausner's ratio and Carr's Index

The Hausner's ratio and Carr's Index were calculated using the data obtained from bulk and tapped density and are shown in table 2. The values of Hausner's ratio ranged from 1.04 to 1.25 whereas the Carr's Index ranged from 3.89 to 19.71.

All the results of powder characterization indicate that the formulation blends exhibited good to fair ability to flow and compress in to tablets.

Yield

The yield of all the batches of microspheres was found to be reasonably good ranging from 52.96 % to 75.48 % (Table 3). The highest yield was exhibited by the formulation F7. The good yield of the microspheres indicates that the formulation process and variables employed in preparing the microspheres are efficient.

Table 3: Yield, drug loading and size of microspheres

Formulation Batch	Yield (%)	Drug loading (%)*	Size (µm)**
F1	52.96	73.75 ± 0.631	13.34 ± 0.092
F2	66.51	73.58 ± 0.615	14.23 ± 0.293
F3	73.29	74.63 ± 0.535	16.29 ± 0.291
F4	74.13	74.55 ± 0.408	17.17 ± 0.205
F5	69.48	70.46 ± 0.130	17.73 ± 0.217
F6	72.67	71.69 ± 0.364	17.05 ± 0.075
F7	75.48	72.28 ± 1.756	16.67 ± 0.255
F8	74.96	70.94 ± 0.843	16.98 ± 0.218

Results are represented as mean ± standard deviation; *n =3; **n=30

Drug loading

As such the drug loading percentage was almost similar for all the formulations indicating that the concentration of guar gum did not had much effect on drug loading. The highest loading was however found in F3 (Table 3). The high drug loading efficiency in all the formulations was also suggestive of the high efficiency of the process parameters used for microsphere formulation.

Particle Size

The particle size was calculated using calibrated eye piece (n=30). The average particles size of the microspheres ranged from 13.34 ± 0.092 µm to 17.73 ± 0.217 µm.

Swelling index and mucoadhesive property of microspheres

For the evaluation of mucoadhesion eggshell membranes were utilized as the substitute of animal mucosa. The swelling index ranged from 2.95 to 4.27 whereas the time of mucoadhesion was from 2h 43min to 6h 18min (Table 4). The mucoadhesion increased with the higher concentrations of guar gum in the formulations.

Table 4: Swelling Index and Mucoadhesion of the microspheres

Formulation Batch	Swelling Index	Mucoadhesion time (h)
F1	2.96	5.49
F2	3.71	6.18
F3	3.73	3.55
F4	4.27	2.43
F5	2.95	3.16
F6	3.11	5.22
F7	3.26	2.51
F8	2.99	6.01

Optimization of the process variables

The optimization of process variables for formulation with maximum mucoadhesion and minimum particle size was done using the trial version of Design Expert version 7.0.0. A 2^3 full factorial approach with 8 runs was utilized to obtain the solutions for optimized formulation.

The standard error of design was studied and the graphical representation is presented in Figure 2.

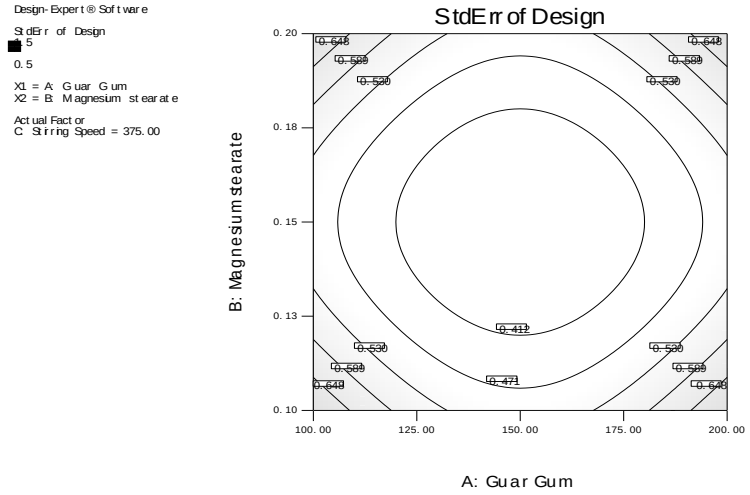


Fig 2: Contour plot for standard error of design

The particle size and mucoadhesion were statistically analyzed by ANOVA and the equations defining the effect of variables is presented in equation 1 and 2 respectively.

$$\text{Particle Size} = 16.18 + 0.78*A + 0.12*B - 0.93*C \quad \text{------ Eq (1)}$$

The standard deviation, and R-squared for Eq 1 were 1.12 and 0.7023 respectively suggesting that the process variables did not significantly contribute to particle size as such.

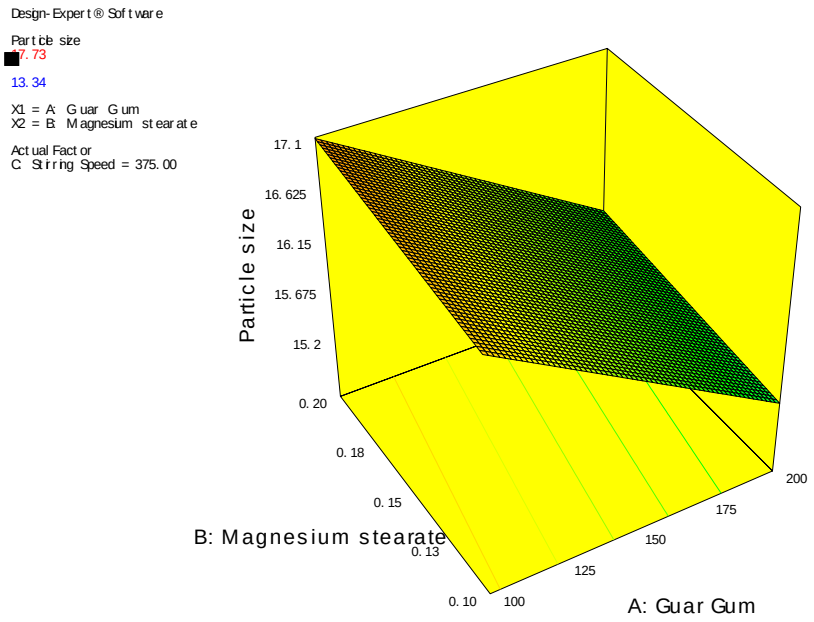


Fig 3: 3D surface plot depicting effect of process variables on particle size

Mucoadhesion Time = 4.32 + 1.41*A + 0.41*B +0.094*C ------- Eq (2)

The standard deviation, and R-squared for Eq 1 were 0.13 and 0.9959 respectively, suggesting a highly significant correlation of the process variables with mucoadhesion time. The Eq 2 was able to predict the mucoadhesion of the formulation with minimum residuals.

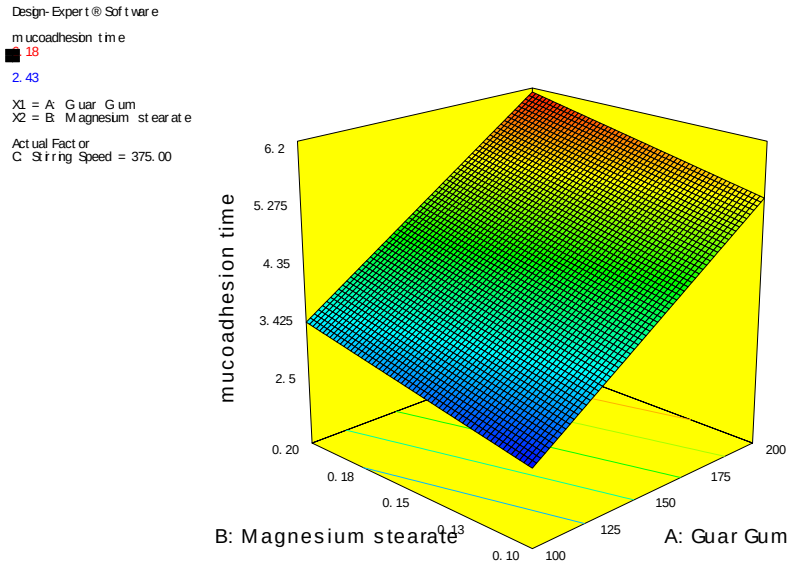


Fig 4: 3D surface plot for effect of process variables on mucoadhesion

A total of 25 solutions were obtained by the above equations for achieving maximum mucoadhesion time and minimum particle size. The highest desirability was found with 200 mg guar gum, 0.19 g magnesium stearate and a stirring speed of 500 rpm. The solution predicted a particle size of 14.5862 µm and mucoadhesion time of 6.18005 h.

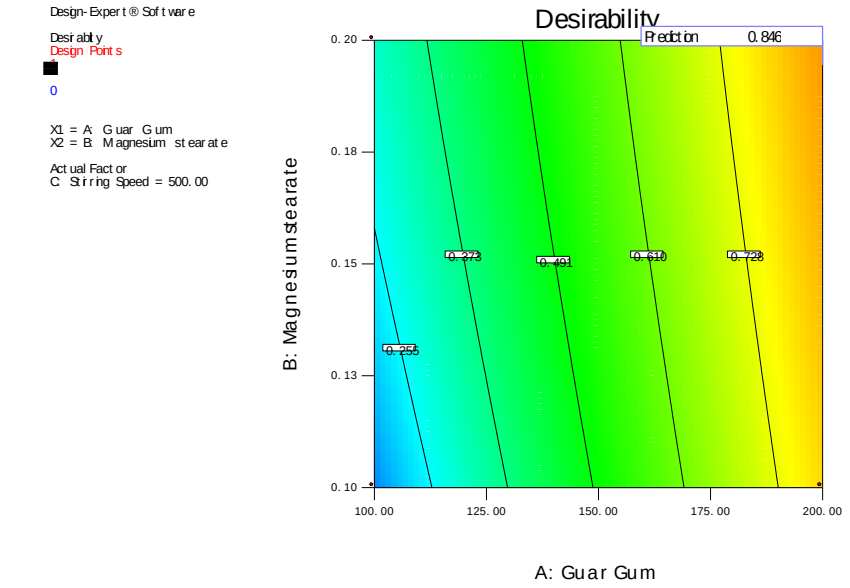


Fig 5: Contour plot for optimized formulation

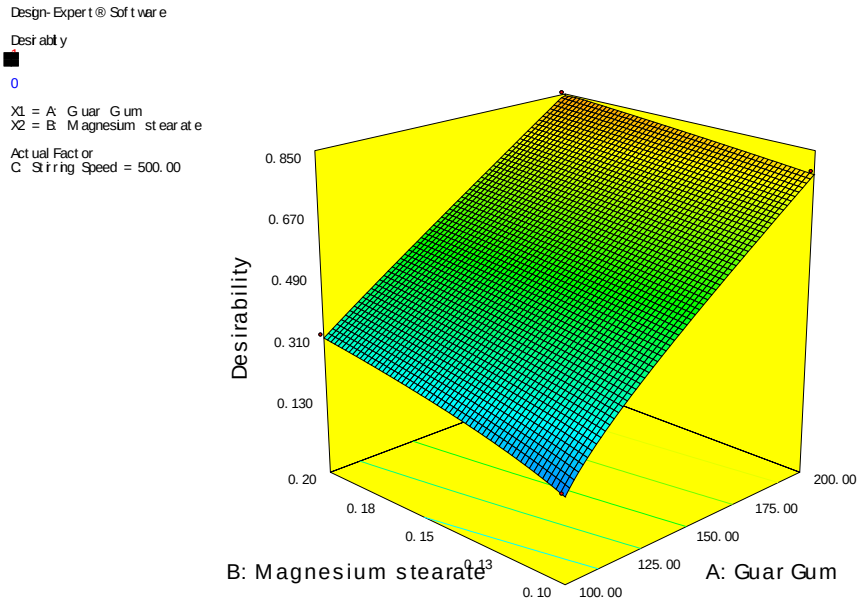


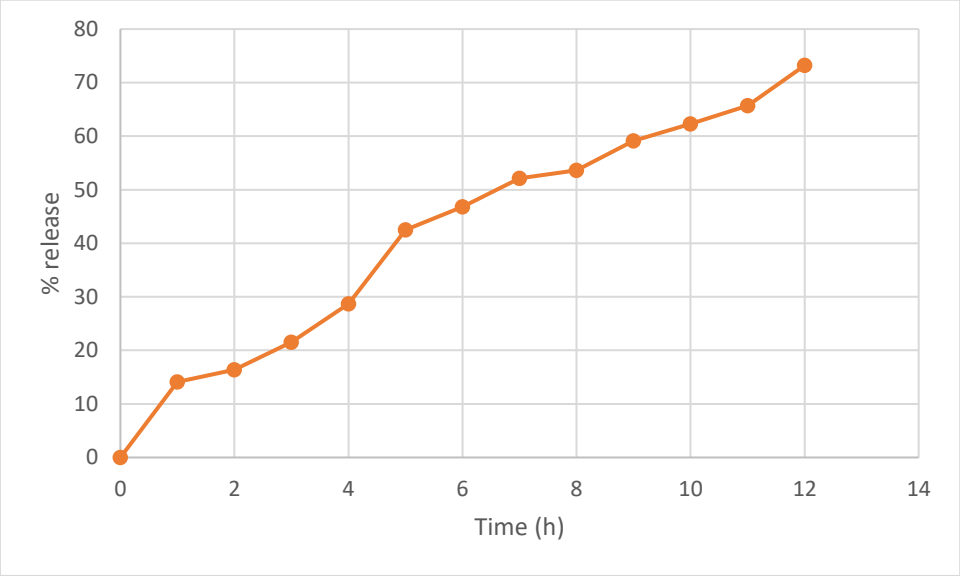
Fig 6: 3D surface plot for optimization of formulation with maximum mucoahesion and minimum particle size

Evaluation of the optimized formulation

A formulation with the optimized process variables was prepared as per the reported procedure and was tested for particle size, mucoadhesion and *in vitro* release.

The particles size of the formulation was found to be 14.04 µm with polydispersity index of 0.701. The mucoadhesion time of the formulation was obtained to be 6 h 19 min.

The *in vitro* release of simvastatin was studied for 12 h and it was found that the microsphere formulation was able to sustain the release of the drug for more than 12 h with less than 80% drug released at the end of the 12th hour.



The correlation coefficient for each formulation was obtained from the statistical software (Excel) and the best fit was determined by the closeness of the correlation value to one (Table 5.10). The best fit model was found to be Korsemeyer-Peppas model suggesting that the sustained release of the drug from the microspheres was due to the degradation of the matrix over time.

Table 5: Correlation coefficients of various mathematical models for optimized formulation

Zero order	First order	Higuchi	Korsemeyer-Peppas
0.9691	0.9691	0.9573	0.9603

The regression coefficient of the kinetic models suggest that the release of simvastatin from the mucoadhesive microspheres followed zero and first order kinetics suggesting a release dependent on the concentration of drug in the solution.

4. Conclusion

The results obtained from the study indicate that use of guar gum as the mucoadhesive polymer could help in achieving sustained release over a longer duration and help in reducing the dose as well as frequency of administration of the medicaments. Further *in vivo* release studies are needed to support for the conclusion of the present investigation.

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