FORMULATION AND OPTIMIZATION BY APPLYING 3² FULL FACTORIAL DESIGN OF
MUCAOADHESIVE MICROSPHERES OF NIFEDIPINE

CHIMAN LAL¹*, RAJEEV GARG², GHANSHYAM DAS GUPTA³

¹Department of Research and Development, I. K. G. Punjab Technical University, Kapurthala, Punjab, India. ²Department of Pharmaceutical Research, Amar Shaheed Baba Ajit Singh Jujhar Singh Memorial College of Pharmacy, Bela, Ropar, Punjab, India. ³Department of Pharmaceutics, I.F.S College of Pharmacy, Moga, Punjab, India. Email: chimanberi122@gmail.com

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ABSTRACT

Objective: The purpose of this research work is to formulate and optimize mucoadhesive microspheres of nifedipine using Carbopel 934P as mucoadhesive and ethyl cellulose as a carrier polymer for controlling the release of nifedipine.

Methods: The emulsion solvent evaporation technique was used for the preparation of microspheres and the 3² full factorial designs were employed for optimization of microspheres. The developed microspheres were characterized for percent yield, entrapment efficiency, particle size, in vitro release study, percent mucoadhesion, surface morphology, and stability study.

Results: Evaluating outcomes of preliminary batches indicated that 100 ml volume of processing medium, 5 h stirring time and 2% concentration of emulsifying agent were suitable for spherical, free-flowing microspheres and high percentage drug entrapment efficiency. The optimized batch exhibited 94.35% drug entrapment efficiency, 61.78% mucoadhesion and drug release were also sustained for more than 12 h. Scanning electron microscopy study revealed that produced microspheres were spherical in shape.

Conclusion: Experimental responses of the optimized batch have close proximity with the predicted value and stability study of the optimized formulation proved the formulation is stable for a long period of time; hence, it is an excellent alternative over the conventional delivery system.

Keywords: Mucoadhesive microspheres, nifedipine, Carbopol 934P, ethyl cellulose, factorial design.

INTRODUCTION

Nifedipine [Dimethyl 1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl) pyridine–3,5–dicarboxylate] is one of the drugs which is most effective and extensively used in the management of angina (especially in Prinzmetal’s angina) and hypertension. It is also commonly helpful to control the blood pressure in an effective manner during pregnancy [1,2] and in a small subset of pulmonary hypertension patients whose symptoms respond to calcium channel blockers [3]. Nifedipine tablet is eliminated rapidly due to its short biological half-life about 2 h [4] and shows irregular bioavailability due to its high first pass effect [5]. The conventional dosage form has several side effects such as an increase in heart rate, palpitation, and flushing [6], that is, why it is requisite to develop a new kind of dosage form for the nifedipine to overcome these side effects and multiple dose intervals.

In the design of drug delivery systems, mucoadhesive microspheres are a contemporary topic of interest to prolong the residence time of the dosage form on the site of application or absorption [7]. For the enhancement of bioavailability of the drug, it facilitates the intimate contact of the dosage form with the underlying absorption surface [8]. The interactions of bioadhesive polymers to the mucin layer of the mucous membrane are referred to as mucoadhesion [9]. It can be achieved by the specific and nonspecific interaction of bioadhesive polymer with a mucus layer [10]. When the mucoadhesive microspheres of poorly soluble drugs are prepared, they are highly dispersed in the inner part of the microspheres or adsorbed at the surface of microspheres, which may assist to enhance the bioavailability of drugs [11]. The complex phenomenon of mucoadhesion is based on the different types of theories such as electronic, wetting, adsorption, and diffusion theory [12]. The adhesion of microspheres with a mucus layer achieved by two stages, first wetting of the microspheres after that, the establishment of adhesive interaction [13,14].

The purpose of this work is to formulate and optimize the mucoadhesive microspheres of nifedipine using the Carbopel 934P as mucoadhesive and ethyl cellulose as carrier polymer. A 3² full factorial design was employed to study the effect of independent variables such as the amount of ethyl cellulose and amount of Carbopel 934P on the physicochemical characteristics drug entrapment efficiency, %Qh (% cumulative drug release at 1 h), and t₅₀ and mucoadhesion of the mucoadhesive microspheres of nifedipine.

MATERIALS AND METHODS

Materials

Nifedipine was obtained from Macleods Pharmaceutical Ltd. (Mumbai, India). Carbopel 934P was purchased from Sigma-Aldrich Pvt. Ltd. Ethylcellulose, light liquid paraffin, Span 80, Ethanol 95%, petroleum ether, and methanol were purchased from Loba Chemie Pvt., Ltd., Mumbai. Hydrochloric acid (HCl) was purchased from Thermo Fisher Scientific India Pvt., Ltd., Mumbai. All other chemicals were of analytical grade.

Preparation of microspheres

The emulsion solvent evaporation technique was used to prepare mucoadhesive microspheres of nifedipine. Ethyl cellulose was dissolved in 95% ethanol; Carbopel 934P and nifedipine powder were dispersed in ethyl cellulose solution under magnetic stirring for 2 h. Then, this suspension was dispersed dropwise in light liquid paraffin containing span 80 as surfactant with continuous stirring on Rami mechanical stirrer at 1000 rpm. After 5 h of stirring, ethanol was evaporated gradually and microspheres were produced. These prepared microspheres were washed with petroleum ether, filtered and dried at room temperature for 24 h and then stored in the desiccators until used.

RESULTS

Evaluating outcomes of preliminary batches indicated that 100 ml volume of processing medium, 5 h stirring time and 2% concentration of emulsifying agent were suitable for spherical, free-flowing microspheres and high percentage drug entrapment efficiency. The optimized batch exhibited 94.35% drug entrapment efficiency, 61.78% mucoadhesion and drug release were also sustained for more than 12 h. Scanning electron microscopy study revealed that produced microspheres were spherical in shape.
Determination of percentage yield
The percentage of production yield was calculated from the weight of dried microspheres (W1) and the sum of the initial dry weight of starting materials (W2) as the following equation [15,16].

\[
\text{\% yield} = \frac{\text{Total amount of dried microspheres(W1)}}{\text{Total weight of raw material(W2)}} \times 100
\] (1)

Determination of drug content and entrapment efficiency
An appropriate amount of microspheres of nifedipine was crushed to fine powder, extracted with methanol for 2 h and then filtered. After filtration, absorbance measured spectrophotometrically at 235 nm for drug content. The drug entrapment efficiency was calculated using the following equation [17,18].

\[
\text{\% Entrapment efficiency} = \frac{\text{Practical drug loading}}{\text{Theoretical drug loading}} \times 100
\] (2)

Determination of particle size of microspheres
The freshly prepared microsphere was examined with an optical microscope and the size of the microspheres was measured using a pre-calibrated ocular micrometer and stage micrometer. About 200–300 particles of each formulation were observed and counted [19-21].

In vitro release study
USP type II dissolution test apparatus was used for studying the drug release properties of the microspheres. An appropriate amount of microspheres of nifedipine was taken in muslin cloth and tied on the paddle which was suspended in the media under test. The test was carried out in 0.1 N HCl (pH 1.2) (900 ml) equilibrated at 37 ± 0.5°C. The paddles were rotated at 50 rpm. At specific time points, 5 ml of dissolution media was withdrawn and replaced with 5 ml of fresh dissolution medium [22,23]. The collected samples were analyzed spectrophotometrically at 238 nm for their absorbance. Concentrations were calculated using calibration curves developed in respective media. Taking into account, the loss of the drug in aliquot replaced, the correction factor was used, as shown in Equation 3 [24].

\[
C_t = A_t + \frac{A_0}{t} \sum_{i=1}^{n} \frac{V_t}{V_i} \left( \frac{V_t}{V_i - V_s} \right)
\] (3)

Where, \(C_t\) = Corrected absorbance, \(V_t\) = Sample of dissolution media withdrawn, \(V_i\) = Total volume of dissolution media.

Dissolution release profiles were plotted with percentage drug released at different time intervals. The average value of \(t_{50}\) for all batches was calculated from the dissolution data.

In vitro wash-off test
In vitro wash-off test was used to evaluate the mucoadhesive property of microspheres. The rats were sacrificed by cervical dislocation. The abdomen was opened and the stomach was excised [25]. A 1-cm piece of rat stomach mucosa was cut and tied on a glass slide (3-inch by 1-inch) with thread. Approximately 100 microspheres were spread onto the wet rinsed tissue specimen and hanging prepared slide onto the grooves of a USP tablet disintegrating test apparatus. After that, the disintegrating test apparatus was switched on, and tissue specimen was given up and down movements for 10 h in a beaker containing simulated gastric fluid (pH 1.2). The microspheres remaining on the surface of the gastric mucosa were counted after 10 h and the percentage mucoadhesion was calculated by the formula shown in Equation 4 [26,27].

\[
\text{Percent mucoadhesion} = \frac{\text{Weight of adhered microspheres}}{\text{Weight of applied microspheres}} \times 100
\] (4)

Release kinetics and mechanism
To know the release mechanism and kinetics of nifedipine, all formulations were attempted to fit into mathematical models. \(r^2\) value of zero-order, first-order, and Higuchi model and \(n\) values for Korsmeyer–Peppas models were calculated [28].

Determination of surface morphology
The surface characteristics of microspheres were studied by scanning electron microscope (JEOL JSM-6100 scanning microscope, Japan). Sample of microspheres was mounted on a stub and coated with a layer of gold using a sputter coater (JFC-1100). The samples were scanned at 5 kV and photomicrograph at different magnification ratio [29,30].

RESULTS AND DISCUSSION

Preliminary studies
During the preliminary trials, the volume of processing medium is the one important factor related to microspheres. Three different volumes of light liquid paraffin 50, 100, and 150 ml were selected. Irregular microspheres are obtained when 50 ml of light liquid paraffin was used and spherical and the free-flowing microspheres are obtained when 100 and 150 ml of processing medium were used, so 100 ml of processing medium was selected for maximum sphericity.

Preliminary batches C1 to C5 were prepared to study the effect of the concentration of emulsifying agents and stirring time on the percent yield, particle size, drug entrapment efficiency, mucoadhesion, and shape of the microspheres.

The concentration of emulsifying agent varied from 1 to 2.5% and stirring time 3-7 h. Spherical and free-flowing microspheres were obtained using more than 2%, and irregular microspheres were obtained using 2% emulsifying agent. The drug entrapment efficiency, mucoadhesion, and particle size were also affected by the concentration of an emulsifying agent. Increases the emulsifying agent was inversely affected the drug entrapment efficiency, however, mucoadhesion and particle size considerably increases. Hence, the 2% emulsifying agent was selected for further studies. Increases the stirring time (3–7 h) were inversely affected the drug entrapment efficiency although, increased the percentage yield and mucoadhesion so that 5 h stirring time was used for the preparation of microspheres.

Experimental design
On the basis of preliminary study, a 3\(^2\) full factorial design was used to determine the effects of independent variables X1 (amount of ethyl cellulose) and X2 (amount of Carbopol 934P) on percent entrapment efficiency, percent mucoadhesion, Qh, and t\(_{50}\). The selection of independent and dependent variables is given in Table 1, although all the batches were prepared according to the experimental design listed in Table 2.

| Table 2. The maximum entrapment efficiency was 86.45% of batch N9, whereas maximum 89.63% for N4 batch. The results are given in Table 3. |
|---|
| Percentage yield and entrapment efficiency |
| The percentage yield of coded batches varies from 64.56 to 89.63%. The minimum percentage yield was 64.56% of batch N4, whereas maximum 89.63% for N4 batch. The results are given in Table 3. |
| The entrapment efficiency of batches varies from 50.23 to 86.45%, as shown in Table 3. The maximum entrapment efficiency was 86.45% of batch N9. It shows entrapment efficiency is increased due to an increase in the concentration of ethyl cellulose and Carbopol 934P. The entrapment efficiency depends on the type and amount of polymers used. It was found that, if increasing the amount of ethyl cellulose and Carbopol 934P the entrapment efficiency was increased. |
| Size of microspheres |
| The microspheres of coded batches are spherical and free-flowing and the size varies from 71.32 to 126.75 um. The size also depends on the concentration of the polymer solution. The results showed that by increasing the concentration of polymers obtained microspheres were more spherical and the size of microspheres was also increased. |

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In vitro drug release study

In vitro drug release study carried on all the formulations. Q₉₀ and tᵢ₉₀ were calculated. The values of Q₉₀ of prepared formulations are 21.60–33.81% and tᵢ₉₀ in the range of 464–705 min, as shown in Table 4. The amount of ethyl cellulose inversely affects the Q₉₀, although increasing the concentration of Carbopol 934P Q₉₀ is also increased.

Batch N₁ shows drug release for a long time and batch N₇ shows 90% drug release in 464 min. It shows that by increasing the amount of ethyl cellulose, the microspheres released drug for a long time while increasing the amount of Carbopol 934P the drug release reduced. The drug release curves of all the batches are shown in Fig. 1.

Mucoadhesion

In vitro wash-off test for percentage, mucoadhesion varied from 49 to 76%. Results indicated that the amount of ethyl cellulose has a negative effect on percent mucoadhesion. Batch N₇ has a maximum percent mucoadhesion because of less concentration of ethyl cellulose. The results are depicted in Table 4.

Release kinetics and mechanism

The different kinetics equations are applied on all the formulations of nifedipine microspheres such as zero-order, first-order, Higuchi, Hixon Crownwell, and Korsmeyer–Peppas. The R² values of all formulations are shown in Table 5.

The R² value of all formulations is >0.90 and best fit in Korsmeyer–Peppas model (R² > 0.997). The values of release exponent for the batch N₇, N₈, and N₉ are <0.5 indicate Fickian diffusion, and all the other batches show non-Fickian diffusion.

Data fitting to the model

Full factorial design as the response surface methodology (RSM) requires nine batches with two center point batches (N₁, N₁₁). The responses of all the prepared formulations were simultaneously fit to quadratic modal using Design Expert 11. The positive values of the coefficient stand for an effect in favors and the negative values oppose factors and response. Polynomial equations generated by Design Expert were established on the basis of ANOVA. The statistical analysis suggests that the independent variables significantly affected dependent variables.

Table 1: Independent and dependent variables

| Independent variable          | Low (-1) | Medium (0) | High (1) |
|------------------------------|----------|------------|----------|
| Amount of ethyl cellulose (mg) | 100      | 150        | 200      |
| Amount of carbopol 934P (mg)  | 50       | 100        | 150      |

Table 2: 3² full factorial design matrix

| Batches | X1   | X2   | X12  | X11  | X22  |
|---------|------|------|------|------|------|
| N₁      | -1   | -1   | 1    | 1    | 1    |
| N₂      | -1   | 0    | 0    | 1    | 0    |
| N₃      | -1   | -1   | -1   | 1    | 1    |
| N₄      | 0    | -1   | 0    | 0    | 1    |
| N₅      | 0    | 0    | 0    | 0    | 0    |
| N₆      | 1    | 1    | -1   | 1    | 1    |
| N₇      | 1    | 1    | 1    | 1    | 1    |
| N₈      | 0    | 0    | 0    | 0    | 0    |
| N₉      | 0    | 0    | 0    | 0    | 0    |

*Center point batch

Table 3: Results of percentage yield, entrapment efficiency, and particle size of microspheres

| Batch number | Percent yield | Percent entrapment efficiency | Particle size (μm) |
|--------------|---------------|-------------------------------|-------------------|
| N₁           | 64.56         | 50.23±2.36                   | 71.32±3.88        |
| N₂           | 69.24         | 52.64±3.24                   | 72.65±2.54        |
| N₃           | 76.14         | 53.94±1.12                   | 74.41±2.98        |
| N₄           | 72.21         | 54.36±3.14                   | 95.21±1.96        |
| N₅           | 81.45         | 61.23±3.08                   | 98.16±2.82        |
| N₆           | 85.23         | 63.14±2.66                   | 97.32±3.16        |
| N₇           | 76.21         | 76.23±1.34                   | 112.5±2.76        |
| N₈           | 74.05         | 83.23±4.08                   | 116.98±4.28       |
| N₉           | 89.63         | 86.45±3.78                   | 126.75±1.32       |

*MeansSD, (n=3). SD: Standard deviation

Table 4: Results of Q₁h, tᵢ₉₀, and mucoadhesion

| Batch number | Q₁h (Percent CDR)* | tᵢ₉₀ | Mucoadhesion |
|--------------|--------------------|------|--------------|
| N₁           | 31.00 ± 0.63       | 594  | 60           |
| N₂           | 32.42 ± 1.58       | 558  | 68           |
| N₃           | 33.81 ± 1.36       | 464  | 76           |
| N₄           | 22.38 ± 1.38       | 680  | 55           |
| N₅           | 26.83 ± 0.97       | 634  | 64           |
| N₆           | 33.15 ± 1.16       | 471  | 71           |
| N₇           | 21.60 ± 1.36       | 705  | 49           |
| N₈           | 22.00 ± 1.22       | 671  | 57           |
| N₉           | 22.70 ± 1.02       | 514  | 68           |
| N₁₁          | 27.17 ± 0.55       | 648  | 60           |
| N₁₁          | 25.83 ± 1.10       | 641  | 65           |

*Mean ± SD, (n = 3). SD: Standard deviation, CDR: Cumulative drug release

![Fig. 1: Percent cumulative drug release of batches (a) N₁ – N₉, (b) N₇ – N₁₁](image-url)
Factorial equation for drug entrapment efficiency

Assessing the drug loading capacity of microspheres, the drug entrapment efficiency is an important variable. The following polynomial equation was derived from multiple regression analysis of the data.

\[ Y_1 = 6.1.07 \times 14.85X_1 + 3.75X_1 + 7.27X_1^2 - 1.82X_1^2 \]

The percentage drug entrapment of all the 11 batches varied from 50.23% to 86.45% and showed good correlation coefficient 0.9980. \( Y_1 \) is strongly affected by the independent variables. The equation of drug entrapment efficiency reflects the wide range of values of various coefficients. Out of two independent variables \( X_1 (14.85) \) and \( X_2 (3.75) \), the \( X_2 \) has a lower value of the coefficient; \( p \text{-value of } X_1 \) and \( X_2 \) is 0.0001 found to be significantly affected \( Y_1 \). The interaction of both the independent variables has significantly affected with positive value (+1.63). This showed that the amount of ethyl cellulose has a prominent effect on entrapment efficiency as compared to the amount of Carbopol 934P. Contour plot and response surface graph for drug entrapment efficiency is shown in Fig. 2.

Factorial equation for percent cumulative drug release in 1 h

\[ Y_2 = 27.37 - 5.15X_1 + 2.82X_2 - 0.9896X_1X_2 - 0.3735X_1^2 + 0.3962X_2^2 \]

The values of \( Q_1 \) for all the batches varied from 21.60 to 33.81%. \( Y_2 \) is affected by the independent variable with good correlation coefficient 0.9123. The value of \( X_1 \) and \( X_2^2 \) interaction has a negative value indicating undesirable effects on \( Y_2 \). The value of \( X_2 \) (+2.82) has a positive effect indicating that the \( X_2 \) has favorable effects on \( Y_2 \). Both the independent variables \( (X_1, X_2) \) were also significantly \( (p < 0.05) \) affected the \( Y_2 \). All the results of \( Q_1 \) showed that the Carbopol 934P has a positive effect on dissolution after 1 h. Contour plot and response surface graph for percentage mucoadhesion are shown in Fig. 3.

**Table 5: Release kinetic data for the batch N1–N11**

| Batch number | Zero-order kinetics \( R^2 \) | First-order kinetics \( R^2 \) | Higuchi kinetics \( R^2 \) | Hixon-Crowell \( R^2 \) | Korsmeyer–Peppas \( R^2 \) | Korsmeyer–Peppas \( n \) |
|--------------|-------------------------------|-------------------------------|--------------------------|-----------------------|-----------------------------|--------------------------|
| \( N_1 \)    | 0.939                         | 0.984                         | 0.989                    | 0.990                 | 0.977                       | 0.519                    |
| \( N_2 \)    | 0.904                         | 0.987                         | 0.991                    | 0.987                 | 0.992                       | 0.485                    |
| \( N_3 \)    | 0.901                         | 0.923                         | 0.995                    | 0.975                 | 0.997                       | 0.461                    |
| \( N_4 \)    | 0.935                         | 0.996                         | 0.990                    | 0.991                 | 0.989                       | 0.610                    |
| \( N_5 \)    | 0.919                         | 0.996                         | 0.993                    | 0.990                 | 0.993                       | 0.545                    |
| \( N_6 \)    | 0.903                         | 0.991                         | 0.966                    | 0.964                 | 0.960                       | 0.486                    |
| \( N_7 \)    | 0.932                         | 0.995                         | 0.991                    | 0.991                 | 0.995                       | 0.604                    |
| \( N_8 \)    | 0.928                         | 0.997                         | 0.989                    | 0.988                 | 0.990                       | 0.603                    |
| \( N_9 \)    | 0.926                         | 0.993                         | 0.977                    | 0.987                 | 0.980                       | 0.648                    |
| \( N_{10} \) | 0.923                         | 0.994                         | 0.994                    | 0.990                 | 0.995                       | 0.525                    |
| \( N_{11} \) | 0.908                         | 0.998                         | 0.993                    | 0.989                 | 0.993                       | 0.554                    |

**Fig. 2:** Contour and three-dimensional surface plot for entrapment efficiency

**Fig. 3:** Contour and three-dimensional surface plot for Q1h
Table 6: Results of the optimized batch for response variables

| Response variables | PV     | EV     |
|--------------------|--------|--------|
| Y<sub>1</sub> = Entrapment efficiency | 85.56  | 84.35  |
| Y<sub>2</sub> = Q<sub>1</sub>h, Percent CDR after 1 h | 22.95  | 23.25  |
| Y<sub>3</sub> = t<sub>90%</sub> | 599.9  | 618    |
| Y<sub>4</sub> = Percent mucoadhesion | 62.87  | 61.78  |

CDR: Cumulative drug release, EV: Experimental value, PV: Predicted value

Factorial equation for t<sub>90%</sub>

\[ Y_3 = +636.11 + 43.50X_1 - 103.83X_2 + 6.00X_1X_2 - 6.76X_1^2 - 66.76X_2^2 \]

The t<sub>90%</sub> of the batches varied from 464 to 705 min. Y<sub>3</sub> is strongly affected by the independent variables with a good correlation coefficient 0.9973. The batches N3, N6, and N9 had the lower time required for achieving the t<sub>90%</sub> (<514 min). From the independent variables, X<sub>1</sub> (43.50) has a positive effect and X<sub>2</sub> (-103.83) has a negative effect on t<sub>90%</sub>. The independent variables significantly (p < 0.01) affecting the drug release. This showed that the amount of ethyl cellulose has a prominent effect, although the amount of Carbopol 934P had unfavorable effects on t<sub>90%</sub>. Batch N7 exhibited a higher t<sub>90%</sub> of 706 min and seems to be a promising candidate for achieving drug release up to 12 h. Contour plot and response surface graph for t<sub>90%</sub> is shown in Fig. 4.

Factorial equation for percentage mucoadhesion

\[ Y_4 = +62.82 - 5.00X_1 + 8.50X_2 + 0.75X_1X_2 - 0.1053X_1^2 + 0.3947X_2^2 \]

The values of in vitro wash-off test for percentage mucoadhesion varied from 49 to 76. Y<sub>4</sub> is strongly affected by the independent variable with a good correlation coefficient 0.9770. The batches N3, N6, and N9 showed higher percentage mucoadhesion (>60%) and the batches N<sub>4</sub> and N<sub>7</sub> showed <60% percent mucoadhesion. The value of X<sub>1</sub> (-5.00) has a negative effect and X<sub>2</sub> (8.50) has a positive effect on percent mucoadhesion, indicating that the X<sub>1</sub> has favorable and X<sub>2</sub> has unfavorable effects on Y<sub>4</sub>. Both the independent variables (X<sub>1</sub>, X<sub>2</sub>) were also significantly (p < 0.01) affecting the Y<sub>4</sub>. All the results of percent mucoadhesion showed that the Carbopol 934P is mainly responsible for mucoadhesion. Contour plot and response surface graph for percent mucoadhesion is shown in Fig. 5.

Optimization of formulation

The optimized formulation was selected based on the criteria of attaining completed and controlled release with highest possible entrapment efficiency and mucoadhesion. On “trading off” various response variables, optimum batch (O<sub>1</sub>) was prepared using 200 mg amount of ethyl cellulose and 127 mg of Carbopol 934P as determined by the optimization technique. The experimental value of drug entrapment efficiency is about 84.35%, Q<sub>1</sub>h about 23.25% t<sub>90%</sub> of about 618 min and the percent mucoadhesion about 61.78%, as shown in Table 6 and the release curve with extrapolation is shown in Fig. 6.

To determine the mechanism of drug release from the optimized batch, the release profile fitted to zero-order, first-order, and Higuchi equation, the “R<sup>2</sup>” value was found to be 0.937, 0.996, and 0.989, respectively. The release profile fitted to Korsmeyer–Peppas equation, the “R<sup>2</sup>” value was found to be 0.993 and “n” value was 0.603 for the optimized batch. It showed that the mechanism of drug release was non-Fickian diffusion. It may be due to swelling of the microspheres.

Surface morphology

Scanning electron microscopy analysis of the optimized batch (O<sub>1</sub>) revealed that the microspheres were discrete and spherical. It appeared to have smooth surfaces at higher magnification, as shown in Fig. 7.

Validation of design

Additional three random batches (V<sub>1</sub>, V<sub>2</sub>, and V<sub>3</sub>) of mucoadhesive microspheres were prepared for the validation of the experiment.
The effects of independent variables on the response parameters were evaluated for the responses, and the experimental values obtained were compared with those predicted by the mathematical models generated. The results of the 3^2 full factorial design of mucoadhesive microspheres of nifedipine using ethyl cellulose as carrier polymer and Carbopol 934P as mucoadhesive polymer (independent variables) significantly affected the dependent variables such as entrapment efficiency, Q_h, t_90%, and percent mucoadhesion. The optimized batch exhibited a high entrapment efficiency and good mucoadhesion. Moreover, drug release of mucoadhesive microspheres >12 h indicates that the microspheres have sustained release. Experimental responses of the optimized batch have close proximity with the predicted value and stability study of the optimized formulation proved that the formulation is stable for a long period of time; hence, it is an excellent alternative over the conventional delivery system.

**CONCLUSIONS**

The results of 3^2 full factorial design of mucoadhesive microspheres of nifedipine using ethyl cellulose as carrier polymer and Carbopol 934P as mucoadhesive polymer (independent variables) significantly affected the dependent variables such as entrapment efficiency, Q_h, t_90%, and percent mucoadhesion. The optimized batch exhibited a high entrapment efficiency and good mucoadhesion. Moreover, drug release of mucoadhesive microspheres >12 h indicates that the microspheres have sustained release. Experimental responses of the optimized batch have close proximity with the predicted value and stability study of the optimized formulation proved that the formulation is stable for a long period of time; hence, it is an excellent alternative over the conventional delivery system.

**CONFLICTS OF INTEREST**

None.

**AUTHORS’ CONTRIBUTIONS**

This work is carried out in collaboration between all authors and all are equally contributed to do this research work.

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