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Research Article

FORMULATION AND EVALUATION OF POLYMERIC MICROSPHERES USING BOX–BEHNKEN DESIGN

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ABSTRACT

Objectives: The purpose of this research work was to formulate and systemically evaluate *in vitro* performances of polymeric microspheres of nifedipine.

Methods: Nifedipine microspheres containing two polymers, poloxamer 407 and carbopol 934, were prepared by single emulsion cross-linking technique. Glutaraldehyde was selected as the cross-linking agent. A Box–Behnken design was employed to study the effect of independent variables, polymer concentration (X1), stirring speed (X2), and glutaraldehyde concentration (X3) on the dependent variables particle size, drug entrapment efficiency, and flow properties of microspheres.

Results: Results of preliminary trials indicate that the polymer concentration, glutaraldehyde concentration, and stirring speed affected various characteristics of microspheres. The formulated Microspheres were discrete, spherical, and free flowing. The optimized batch exhibited with the narrow particle size of 75 µm, good flow properties, and drug entrapment efficiency of 96%.

Conclusion: The polymeric microspheres of nifedipine with excellent flowability and good entrapment efficiency were successfully developed. These can be useful in improving patient compliance and bioavailability of nifedipine.

Keywords: Poloxamer 407, Carbopol 934, Cross-linking agent, Single emulsification, Entrapment, Microspheres, Nifedipine, Box–Behnken design.

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INTRODUCTION

Hypertension is a public health problem. It is chronic medical condition, in which the systemic arterial blood pressure is elevated. It is classified as primary and secondary hypertension. Almost 90–95% cases considered as "primary hypertension," and no cause can be found. The remaining 5–10% cases termed as secondary hypertension that affects the organs such as kidney, heart, and endocrine system. In general way, blood pressure is classified as systolic and diastolic blood pressure. The blood pressure in vessels during a heartbeat called as systolic blood pressure and the blood pressure between the heartbeats called as diastolic blood pressure. Diagnosis of blood pressure is usually measured with a device called sphygmomanometer and expression of blood pressure in mm of Hg [2].

There are so many drugs called as antihypertensive drugs for the treatment of high blood pressure. These drugs are very efficacious and lower the blood pressure by decreasing peripheral resistance without compromising cardiac output. Nifedipine belongs to BCS class-II with low solubility and high permeability. It has short biological half-lives of 2–3 h. Researchers have formulated oral controlled release products of nifedipine by various techniques. Nifedipine belongs to calcium channel blockers, another class of first line antihypertensive drugs [3]. Thus, an attempt was made in this research using the polymers poloxamer 407 and Carbopol 934 and formulates microspheres. The microspheres were characterized by particle size determination, drug entrapment efficiency, angle of repose, Carr's index, Hausner's ratio, and *in vitro* tests, and for the surface response, the Box–Behnken design is used.

METHODS

Nifedipine was obtained as gift sample from Zydus Cadila, Gujarat. Poloxamer 407, Carbopol 934, and other chemicals were used of analytical grade.

Preformulation studies

Organoleptic properties

Physical appearance of the drug was examined by various organoleptic properties. The organoleptic properties of drug such as color, odor, and appearance play an important role to identify the sample.

Solubility studies

The solubility of nifedipine was determined in different solvents. For solubility studies, a known amount of drug was dissolved in various solvents and the solubility was determined. The drug nifedipine belongs to BCS type II, having poor solubility and high permeability. Solubilities study of the drug plays an important to know about the characteristics of a drug in aqueous systems. Bioavailability of the drug completely depends on the aqueous solubility. Solubility of nifedipine was determined by shaking flask method. The absorbance is measured by UV spectroscopy and solubility is calculated.

Determination of melting point

Capillary fused method and differential scanning calorimetry method both were employed for determination of melting point of nifedipine. Differential scanning calorimetry has become most widely used thermal analysis technique. In this technique, the sample and reference materials are subjected to precisely temperature change. DSC was employed to determine the melting point of drug sample used in the present investigation.

Procedure

Indium samples were used to calibrate the differential scanning calorimetry instruments. The analysis was carried out over 50–250°C at 5°C/min using sample of 5 mg in crimped aluminium pans.

Determination of absorption maxima (λmax)

A UV absorption maxima was determined by scanning 10 µg/ml solution of nifedipine in phosphate buffer pH 7.4 between 200 and 400 nm.

Procedure

The standard stock solution of nifedipine was prepared using 7.4 pH phosphate buffer. Accurately weighed 100 mg of drug was dissolved in 100 ml of phosphate buffer pH 7.4 in 100 ml volumetric flasks with aid of sonication in bath sonicator for 20 min.

100 µg/ml of nifedipine and for the analytical purpose concentration of nifedipine were diluted to obtain 10 µg/ml. This sample was scanned under UV-Vis spectrophotometer range from 200 to 400 nm. From this spectrum of nifedipine drug, the wavelength with maximum absorbance was chosen for further analysis.

Construction of standard calibration curve of nifedipine

Preparation of standard curve

Preparation of phosphate buffer pH 7.4

An accurately measured 50 ml of 0.2 M potassium dihydrogen orthophosphate was transferred to a 200 ml volumetric flask and 39.14 ml of 0.2 M sodium hydroxide was added to it. The volume was made up with distilled water and mixed and pH was adjusted to 7.4 with 0.2 M sodium hydroxide.

Preparation of standard curve of nifedipine

1st stock solution: 100 mg of nifedipine dissolved in 10 ml of methanol and volume made up to 100 ml with 7.4 Phosphate buffer. This stock solution is further diluted.

2nd stock solution: Form stock solution 1st, 10 ml pipette out and transferred in another 100 ml of volumetric flask and the volume mark with the 7.4 pH phosphate buffer. This solution having concentration of 100 µg/ml. The second stock solution was serially diluted with 7.4 pH buffer to get the final concentrations of 2, 4, 6, 8, 10, and 12 µg/ml.

The absorbance of each concentration was measured using UV-Visible spectrophotometer at 236 nm as λmax and the calibration curve was plotted against the concentration and absorbance.

a) Drug and excipients compatibility study

Excipients can affect the stability of drugs in various ways, by direct chemical interaction, absorption of moisture, or catalysis. Drug polymer interaction studies were carried out to check the compatibility between the drug and various polymers. Apart from physical characteristics, compatibility between a drug and polymer is a factor in determining the effectiveness of polymeric delivery systems [6,7]. Hence, it is necessary to confirm that drug is not interacting with polymers under experimental conditions and shelf life. The possible drug polymer interaction was studied by Fourier-Transform Infrared spectroscopy.

Preparation of polymeric microspheres of nifedipine

Polymeric microspheres of nifedipine were prepared using single emulsion cross-linking technique. Poloxamer 407 and Carbopol 934

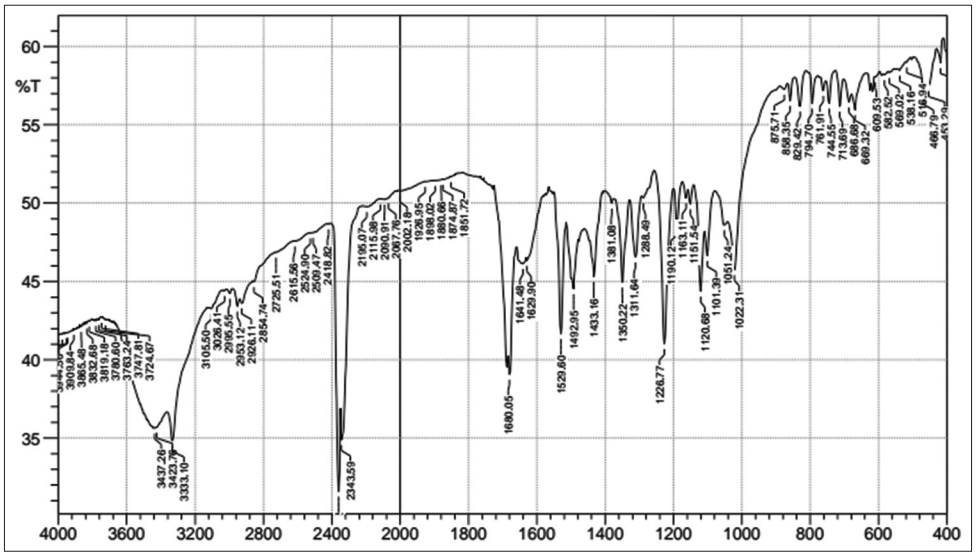

Fig. 1: FT-IR spectrum of nifedipine

Table 1: Box–Behnken design layout for optimization of polymeric microspheres of nifedipine

Formulation code	Run order	Factor A (polymer concentration) (mg)	Factor B (stirring speed) (rpm)	Factor C (Glutaraldehyde) (ml)
F1	1	100	750	0.75
F2	2	200	750	0.75
F3	3	100	750	0.75
F4	4	200	750	0.75
F5	5	100	500	0.50
F6	6	200	500	0.50
F7	7	100	1000	1.00
F8	8	200	1000	1.00
F9	9	150	500	0.50
F10	10	150	500	0.50
F11	11	150	1000	1.00
F12	12	150	1000	1.00
F13	13	150	750	0.75
F14	14	150	750	0.75
F15	15	150	750	0.75
F16	16	150	750	0.75
F17	17	150	750	0.75

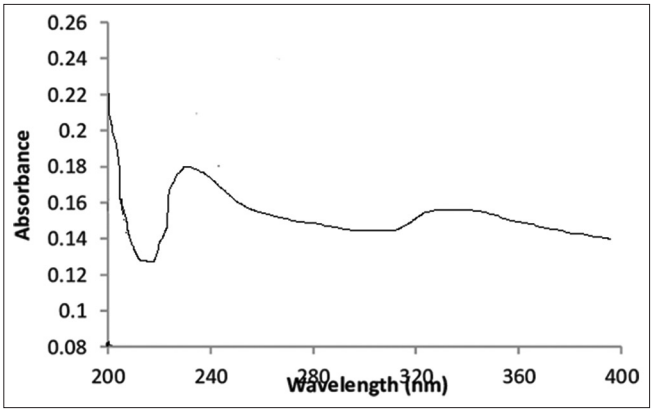

Fig. 2: Absorption maxima of nifedipine

Table 2: Different levels of variable parameters

Independent variables	Levels	
	Low	High
Polymer concentration (mg)	100	200
Stirring speed (rpm)	500	1000
Glutaraldehyde amount (ml)	0.50	1.00

Table 3: Absorbance of nifedipine at different concentration

S. No.	Concentration (µg/ml)	Absorbance
1.	2 µg/ml	0.254
2.	4 µg/ml	0.462
3.	6 µg/ml	0.666
4.	8 µg/ml	0.869
5.	10 µg/ml	1.092
6	12 µg/ml	1.292

were used as polymers and glutaraldehyde was used as cross-linking agent as per method described by Thanoo *et al*. [17].

Polymers were dissolved in 150 ml of 1% v/v aqueous acetic acid solution. 300 mg of drug was dispersed in this polymer solution. The resultant mixture was extruded through a syringe (No. 20) in 1000 ml of liquid paraffin (heavy and light, 1:1 ratio) containing 0.2% DOSS and stirring was performed using the magnetic stirrer. After 30 min, glutaraldehyde was added and the stirring was continued. There was an experimental design with three factors polymer concentration, stirring speed, and cross-linking agent at two levels (low and high). In all batches of Box–Behnken, these independent variables were at different levels. Microspheres thus obtained were filtered and washed several times with petroleum ether to remove the traces of oil. Then, microspheres were finally washed with water to remove excess of glutaraldehyde. The obtained microspheres were dried at room temperature for 24 h.

Parameter such as polymer concentration, stirring speed, and glutaraldehyde concentration was changed as predicted by the 3 – factor at 2 level Box–Behnken design and the polymeric microspheres of nifedipine were formulated by single emulsion cross-linking technique with different ratio, as shown in the Table 1.

A 3 – factor and 2 – level factorial design was used to explore quadratic response surfaces and constructing second-order polynomial model with design expert software (version 7.0). The design gave 17 runs with five center points, for which responses, such as particle size, entrapment efficiency, angle of repose, Hausner's ratio, and Carr's Index, were calculated.

Characterization [9-12]

Particle size determination

Samples of microspheres were analyzed for particle size by optical microscopy. A stage micrometer is simply a microscope slide with a scale attached on the surface.

Table 4: Experimental design for optimization of polymeric microspheres of Nifedipine using Box–Behnken design

Run	Coded formulation	Particle size(µm) (Y1)	% drug entrapment (Y2)	Angle of repose (°) (Y3)	Carr's Index (Y4)	Hausner's ratio (Y5)
1	F1	89±1.22	76±1.02	22.5±0.02	19.08±0.21	1.19±0.20
S	F2	110±1.23	47±1.02	24±0.01	17.69±0.11	1.08±0.18
3	F3	154±1.20	45±0.56	22±0.02	22.14±0.61	1.08±0.23
4	F4	116±1.12	38±0.54	22.4±0.03	23.57±0.04	1.09±0.30
5	F5	78±1.23	83±1.02	24.3±0.02	9.02±0.41	1.07±0.24
6	F6	96±0.82	77±1.21	24.12±0.12	21.6±0.02	1.18±0.84
7	F7	97±0.23	75±0.86	22.9±0.02	17.39±0.72	1.17±0.09
8	F8	117±0.45	37±1.62	23.64±0.02	25±0.81	1.24±0.26
9	F9	75±1.12	96±1.34	22±0.13	5.92±0.23	1.06±0.54
10	F10	115±1.14	70±1.40	24±0.13	16.36±0.45	1.16±0.67
11	F11	116±1.22	72±1.03	24±0.04	12.5±0.43	1.12±0.03
12	F12	155±1.2	72±1.03	22±0.04	7.14±0.52	1.25±0.28
13	F13	101±1.23	82±1.13	24.21±1.21	9.09±0.55	1.21±0.54
14	F14	78±0.61	90±1.24	24±0.21	8.06±0.71	1.09±0.13
15	F15	134±0.82	82±1.23	23.83±0.11	20.96±0.06	1.21±0.03
16	F16	80±1.22	86±1.11	22±0.12	8.14±0.71	1.07±0.43
17	F17	96±1.21	89±1.05	24.9±0.03	6.97±0.23	1.22±0.87

Table 5: Results of regression analysis for responses Y1, Y2, Y3, Y4, and Y5

Quadratic model	R^2	Adjusted R^2	Predicted R^2	Adequate precision	SD	C.V%
Response (Y1)	0.8281	0.6070	0.1441	7.283	15.44	14.53
Response (Y2)	0.9408	0.8648	0.2826	10.095	6.81	9.52
Response (Y3)	0.8475	0.6514	0.0487	7.131	0.62	23.38
Response (Y4)	0.8485	0.6536	–0.2978	5.743	3.93	14.75
Response (Y5)	0.8760	0.7165	–0.0435	7.814	0.036	3.11

Calibration of Micrometer

Stage micrometer has scale of stage=100 µm

Ocular piece covers the stage=73 µm

One division covers 1.37 parts of the stage.

Drug entrapment efficiency

The practical drug loading can be calculated by taking a weighed amount of polymeric microspheres dissolved in 10 ml of phosphate buffer [11]. After shaking the suspension, vigorously, it was left for 24 h at room temperature with intermittent shaking. Supernatant was collected by centrifugation and drug content in supernatant was determined by UV- spectrophotometer. This solution was filtered through a 0.2 µm filter, suitably diluted, and assayed spectrophotometrically at 236 nm for nifedipine microspheres against a blank.

Drug entrapment efficiency of drug entrapment for each batch can be calculated in terms of % drug entrapment (PDE) as per the following formula:

PDE=(Practical drug loading/Theoretical drug loading)*100

Theoretical drug loading was determined by assuming that the entire drug present in the polymeric solution used gets entrapped in microspheres and no loss occurs at any stage of preparation of microspheres.

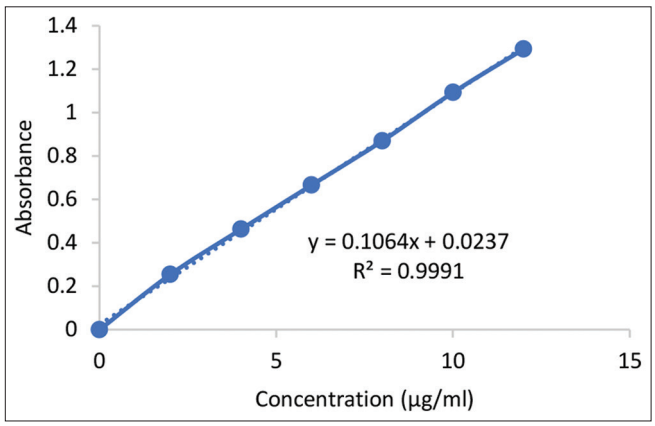

Fig. 3: Calibration curve of nifedipine in 7.4 pH buffer

Scanning electron microscopy (SEM) analysis

The shape and surface morphology of microspheres samples were studied by SEM technique. Microspheres were dusted onto double-sided carbon dust which was placed onto sample carrier (aluminium stubs having double adhesive tape) in the shape of a cylinder with 5 mm of height and 10 mm of diameter and were coated with gold palladium mixture under vacuum with sputter coater to thickness of 50 mm [14,15].

The samples were imaged using a 5–15 kV electron beam. The microphotographs of suitable magnifications were obtained for surface morphology.

Flow properties of microspheres

Angle of repose

Weighed quantity of microspheres (10 gm) was passed through a funnel fixed on a stand at a specific height on the graph paper. A static heap of powder with only gravity acting on it was tending to flow form a conical mouth. The height of heap (h) and the radius of the lower part of the conical were measured.

The angle of repose was calculated using the following formula:

$\tan\theta = h/r$

Carr's index

It is a simple test that has been evaluate the flowability of a powder by comparing the poured (fluff) density (ρ_{Bmin}) and tapped density (ρ_{Bmax}) of a powder and the rate at which it packed down. Carr's index is determined by taking a small quantity of microsphere sample in 10 ml measuring cylinder. The height of sample was measured before and after tapping indicates poured and tapped density, respectively. It was calculated using following formula:

Carr's index (%)=Tapped – poured (bulk) density/Tapped density*100

Hausner's ratio

Hausner defined a similar index in 1967. Same method was employed for determination of poured and tapped density as in case of Carr's index. It was calculated using following formula:

Hausner's ratio=Tapped density/Bulk density

In vitro drug release study

Release of nifedipine from the prepared polymeric microspheres was studied in phosphate buffer pH 7.4 (900 ml) using USP Type II

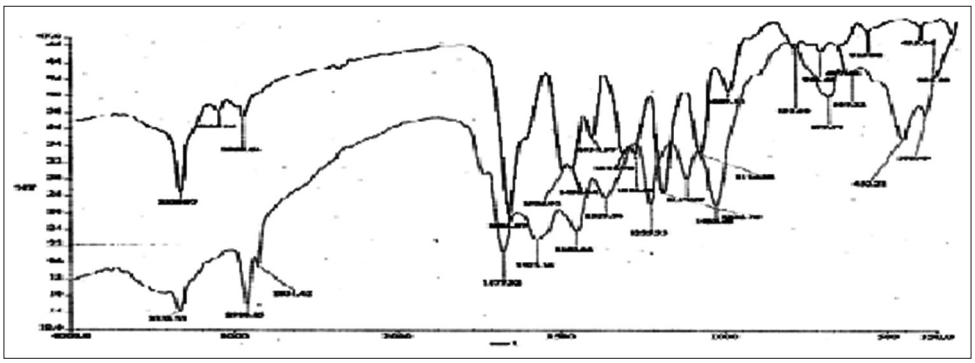

Fig. 4: FT-IR of nifedipine microspheres (nifedipine and polymer [poloxamer 407 and carbomer 934])

Table 6: Optimized formulation

Formulation code	Polymer concentration (mg)	Stirring speed (rpm)	Concentration of glutaraldehyde (ml)	Particle size (Y1) (µm)	Drug entrapment efficiency (Y2) (%)	Angle of repose (Y3) (o)	Carr's index (Y4)	Hausner's ratio (Y5)
F9	150	500	0.50	75±1.12	96±1.34	22±0.13	5.92±0.23	1.06±0.54

six station dissolution test apparatus (paddle type) at 50 rpm at the temperature of 37°C. Samples of polymeric microspheres filled in capsule shell were used in each test. Samples were withdrawn through a filter (0.2 micron) at different time interval and were assayed at 236 nm for nifedipine.

RESULTS AND DISCUSSION

Preformulation studies

The solubility of drug in water, 0.1N HCl, 7.4 pH phosphate buffer, was determined and results are shown in Table 2. The organoleptic properties of nifedipine were performed and physical appearance was good and elegant. The melting point of drug was determined and was found to be 172–174°C.

FT-IR spectrum of pure drug

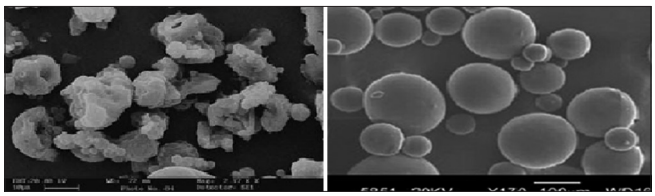

Fig. 5: Scanning electron morphology of Nifedipine microspheres

Determination of absorption maxima

Nifedipine was estimated by simple ultraviolet spectrophotometric method. 10 µg/ml solution of nifedipine in methanolic phosphate buffer pH 7.4 is scanned between 200 and 400 nm. The absorption maximum was observed at 236 nm.

Determination of calibration curve of nifedipine

For the standard plot of nifedipine, the solutions were prepared in phosphate buffer pH 7.4 (2–12 µg/ml) and the absorbance of resulting solutions was measured at 236 nm using UV spectrophotometer. The calibration curve showed a good linearity with correlation coefficient of 0.9991 (Table 3).

FT-IR spectroscopy of nifedipine microspheres

The FT-IR spectra of nifedipine and polymer mixture showed several characteristic peaks. The FT-IR spectrum of pure nifedipine showed the characteristic peaks at wave numbers of 3320 cm^{-1} due to >N–H stretching (>N–H of pyridine), at 1680 cm-1 due (N=O) 2 asymmetric stretching (Aryl-NO)2, at 1220 cm^{-1} is due to (N=O)2 symmetric stretching (Aryl-NO_2), and at 1520 cm^{-1} due to asymmetric carboxylate anion confirming the drug structure. The spectrum of drug and polymer mixture also showed the characteristic peaks for nifedipine indicating no interaction between the drug and polymers used. This indicates that there is no chemical interaction between drug and polymer mixture, that the molecular structure of nifedipine remained completely intact.

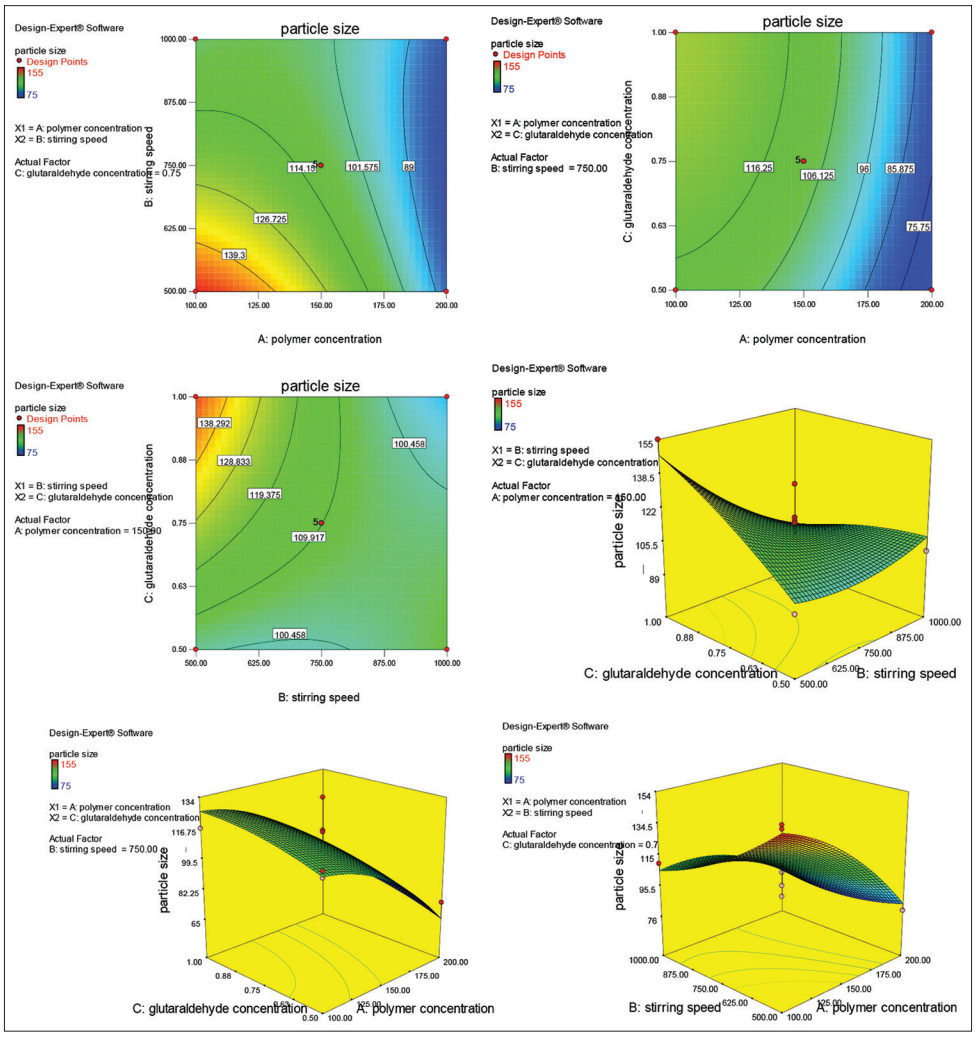

Fig. 6: Response Surface Graphs and Contour Plots for Particle Size Response 2 (Y2) Drug entrapment efficiency

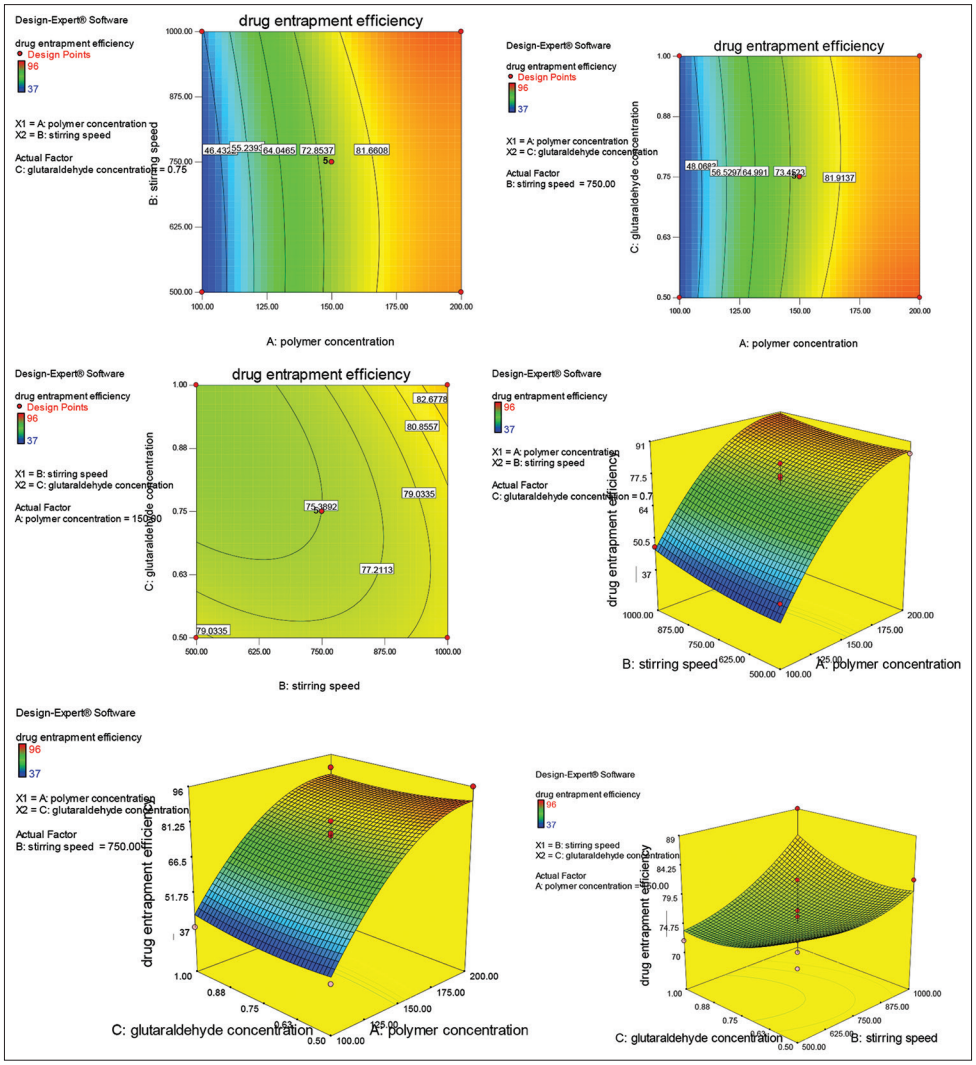

Fig. 7: Response surface graphs and contour plots for drug entrapment efficiency Response 3 (Y3) Angle of repose

SEM

The microspheres prepared by single emulsion cross-linking technique showed a good spherical shape, with smooth surface and the particles are distributed uniformly without any lumps.

From the formulated batches of nifedipine microspheres, the optimized batch was examined for surface morphology using scanning electron microscope. Sample was fixed on carbon tape and fine gold sputtering was applied in a high vacuum evaporator. The surface morphology of polymeric microspheres of nifedipine was studied by SEM. Microphotographs of nifedipine microspheres were taken on different magnification that was used for the surface morphology.

Micromeritic properties

Parameters such as concentration of polymers, stirring speed, and concentration of cross-linking agent were changed as predicted by the Box–Behnken design.

Fitting the model to the data

Total 19 runs with triplicate center points were generated and the responses so observed are shown in Tables 4 and 5. The response ranges Y1, Y2, Y3, Y4, and Y5 for all batches were 75±1.12–155±1.14 µm, 37±1.62–96±1.34%, 22°±0.13–24°.9±0.03, 5.92±0.23–23.57±0.04, and 1.06±0.54–1.24±0.26, respectively.

Response analysis though polynomial equations

Response 1 (Y1): Particle size

The following polynomial equation prevailed from the model for particle size of nifedipine polymeric microspheres.

$$Y1 = 110–23.25\ X1–12.62\ X2+7.13\ X3+10.50X1X2+0.50\ X1X3-15.75\ X2X3-10.12\ X1^2+5.62\ X2^2–3.38\ X3^2$$

A positive value for the coefficient is an indicative of the favorable effect, whereas a negative value for the coefficient indicates an unfavorable effect of that particular factor on the response. In the present research, particle size (Y1) was found to be significantly higher when the concentration of cross-linking agent (X3) increased. The particle size decreased with increase in stirring speed, this is because the higher shearing stress breaks up the molecules to larger extent at higher stirring rates.

The polymer concentration (X1) has negative effect on particle size, but with stirring speed (X2) and glutaraldehyde (X3) having positive effect, (X2) stirring speed, and (X3) glutaraldehyde concentration together negative effect on particle size.

Response 2 (Y2): Drug entrapment efficiency

$$Y2 = 75.40+23.50\ X1+2.88\ X2–0.37X3–1.25\ X1X2-1.25\ X1X3+2.50\ X2X3-12.20\ X1^2+2.05\ X2^2+2.05\ X3^2$$

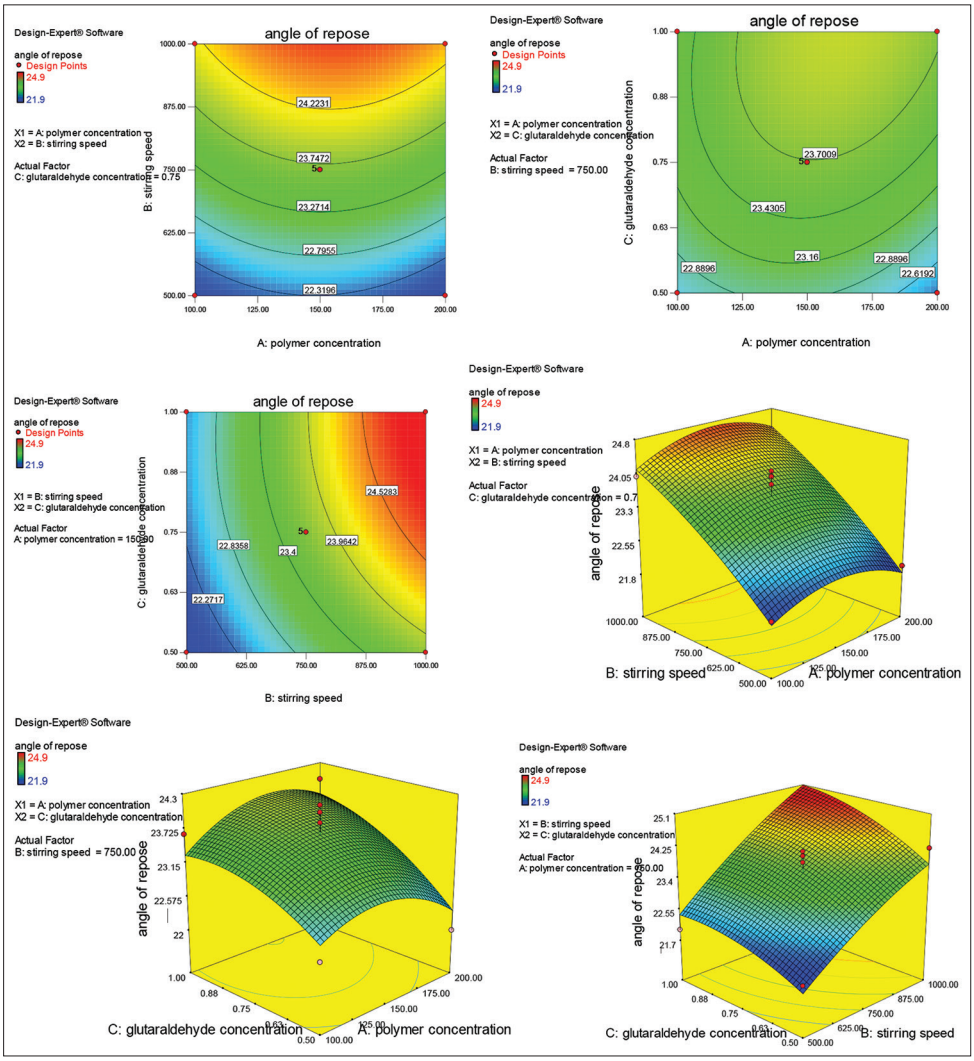

Fig. 8: Response surface graphs and contour plots for angle of repose Response 4 (Y4) Carr's index

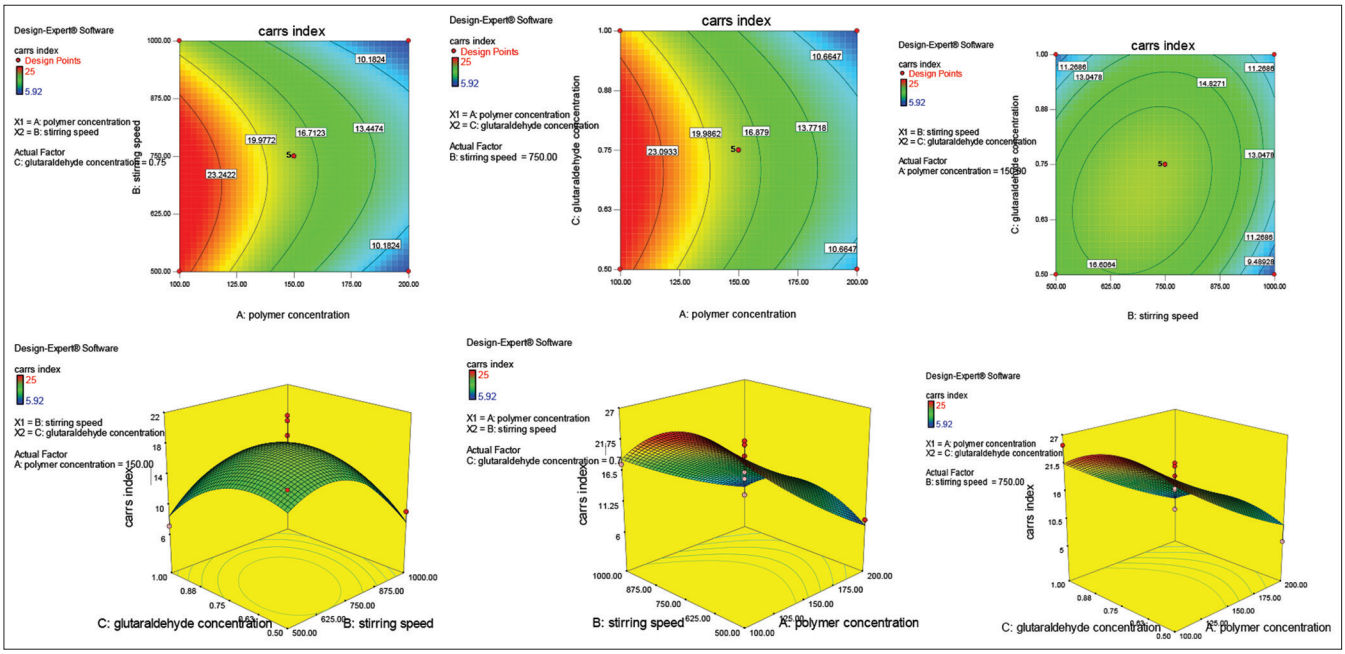

Fig. 9: Response Surface Graphs and Contour Plots for Carr's Index Response 5 (Y5) Hausner's ratio

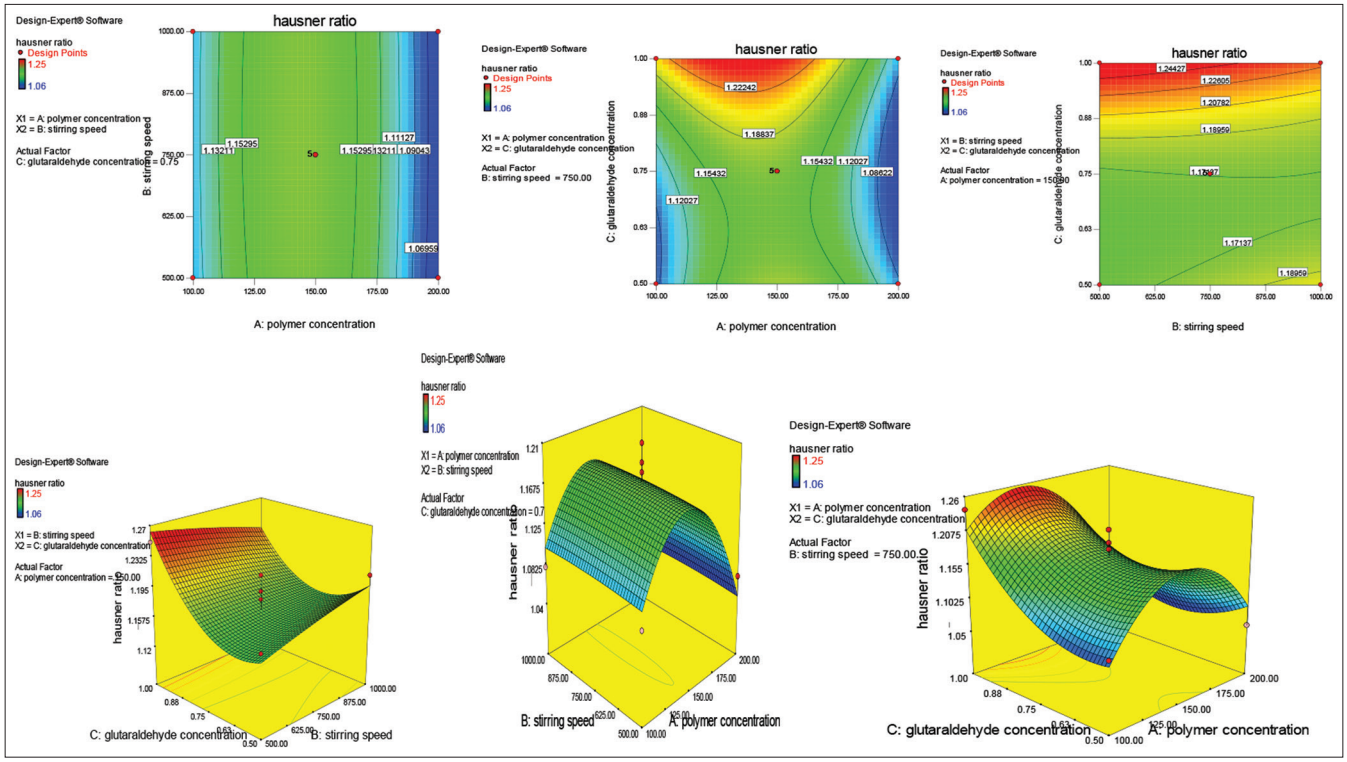

Fig. 10: Response surface graphs and contour plots for Hausner's ratio

Polymer concentration (X1) and stirring speed (X2) similarly have positive effect on drug entrapment. The concentration of glutaraldehyde has negative effect on % drug entrapment.

The polymer concentration has negative effect on drug entrapment with stirring speed (X2) and glutaraldehyde concentration (X3), but stirring speed (X2) and glutaraldehyde concentration (X3) have positive effect on drug entrapment efficiency.

Response 3 (Y3): Angle of repose

$Y3 = 23.69+0.033\ X1+1.19\ X2+0.50\ X3+0.075\ X1X2+0.19\ X1X3+0.15\ X2X3–0.43\ X1^2\text{-}0.19\ X2^2\text{-}0.25\ X3^2$

All the three factors polymer concentration (X1), Stirring Speed (X2), and Glutaraldehyde concentration (X3) have positive effect on angle of repose. The angle of repose increased when these factors increased.

Response 4 (Y4): Carr's index

$Y4 = 18.10–7.16\ X1–1.50\ X2–1.10\ X3+1.33\ X1X2+0.18\ X1X3+2.03\ X2X3–0.82X1^2\text{-}4.67X2^2\text{-}3.28\ X3^2$

All three factors polymer concentration (X1), Stirring speed (X2), and the concentration of glutaraldehyde have negative effect on Carr's index, but polymer concentration (X1) with stirring speed (X2) and glutaraldehyde concentration (X3) together has positive impact on the Carr's index.

Response 5 (Y5): Carr's index

$Y5 = 1.17–0.025\ X1+1.250\ X2+0.034\ X3+0.00\ X1X2\text{-}0.030\ X1X3\text{-}0.018X2X3–0.095X1^2\text{-}2.250X2^2\text{-}0.043\ X3^2$

The stirring speed (X2) and glutaraldehyde concentration (X3) have positive effect on Hausner's ratio, but polymer concentration (X1) has negative effect on this property of microspheres. Together, the polymer concentration and stirring speed have no effect on Hausner's ratio, but with glutaraldehyde has negative effects.

Contour plots and response surface analysis

Two dimensional contour plots and three dimensional response surface plots were prepared for the five responses are shown in Figs. 1 and 2 for responses Y1, Y2, Y3, Y4, and Y5, respectively. An interaction effect of the factors on the responses is clearly evident from the plots.

Selection of the optimized formulation

From the values given in Table 6, it is evident that the model is significant with significant p value ($p<0.0001$), lack of fit value ($p<0.0063$), and R^2 values. Formulation F9 was found to have narrow particle size range, better drug entrapment, and good flow properties. Based on these parameters, F9 formulation was considered to be the optimized.

CONCLUSION

Polymeric microspheres containing nifedipine can be prepared successfully using the single emulsion cross-linking technique. The surface structure of the microspheres was spherical and smooth. Box–Behnken design, ANOVA, and contour plots were used in optimizing formulation variables in the formulation of nifedipine microspheres. The optimized formulation prepared using the predicted levels of factors provided the desired observed responses with Y1, Y2, Y3, Y4, and Y5 values of 75 µm, 96%, 22°, 5.92, and 1.06 for particle size, drug entrapment efficiency, angle of repose, Carr's Index, and Hausner's ratio, respectively.

AUTHORS' CONTRIBUTIONS

All authors have contributed equally.

CONFLICTS OF INTEREST

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