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Full Length Research Paper

Formulation optimization of nifedipine containing microspheres using factorial design

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Nifedipine is a calcium channel blocker which is used in the treatment of hypertension angina pectoris. The aim of this study was to formulate and optimize nifedipine containing microspheres in an attempt to prepare a suitable sustained release delivery system using factorial design. Drug loaded microspheres were prepared using Eudragit RL100, through solvent evaporation technique. In the next step, the effect of different formulation variables, including the amount of polymer (1 - 2 g), stabilizer (0.1 - 0.5 g) and drug/polymer ratio (0.05:1 – 0.1:1) on the appearance, physical properties of particles, and the amount of loaded drug was investigated. Based on the type and the variables studied, 8 formulations were designed using factorial design method, and were then prepared and their drug contents were determined. In order to detect the precise effect of the formulation variables and their interactions, design expert software was used. Data analysis showed that microspheres with optimum drug loading could be prepared using 1 g polyvinylalcohol, 1 – 2 g polymer and 0.07:1 drug/polymer ratio. Among the formulations suggested and based on the predicted responses and their desirability indices, 6 formulations were selected as the optimum formulations. Finally, selected microspheres were evaluated from the view points of morphology and release pattern. Results revealed that microspheres obtained from the formulations S^{19}, S^{20} and S^{24} could be selected as the best and optimized formulations due to their high drug contents, appropriate invitro drug release after 12 h and desired morphology.

Key words: Nifedipine, microsphere, solvent evaporation, eudragit RL100, factorial design.

INTRODUCTION

Drug delivery systems (DDS) that can precisely control the release rates or target drug to a specific body site have had an enormous impact on the health care system. The last two decades there has been a remarkable improvement in the field of novel drug delivery systems. Carrier technology offers an intelligent approach for drug delivery by coupling the drug to a carrier particle such as microspheres, nanoparticles, liposomes, etc, which modulates the release and absorption characteristics of the drug. Microspheres constitute an important part of these particulate DDS by virtue of their small size and efficient carrier characteristics (Vasir et al., 2003). Solvent evaporation technique is one of the several methods that is used for production of microspheres. Although this way may not be the main method, but it is the simplest one that several variables can affect the outcome, as well. Kilicarsan and Baykara (2003) investigated the effect of the drug/polymer ratio on properties of the verapamil loaded microspheres that were made by solvent evaporation method and found that the drug release profile could be sustained by increasing polymer amount, and the particle size and surface characterization of microsphere could be modified through the variation of drug/polymer ratio. Diaminopyridine microparticles by solvent evaporation method were prepared by Gibaud et al. (2002).

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Mentioned study showed that the modified solvent evaporation method in an oily phase could be a useful way to prepare Eudragit RS-based slow-release microcarriers for oral adminstration. Other studies on making microspheres by this method have been carried out previously (Yuksel et al., 2000; Dinarvand et al., 2001; Mateovic et al., 2002; Freitas et al., 2005).

Eudragit RL100 were selected based on its high water permeability (or hydriphilic) properties. Coaservates containing salicylic acid and Eudragit RL100 and RS100 (Okor, 1990), coevaporates of indomethacin with Eudragt RL100 and RS100 (Oth and Moes, 1989) and microspheres of indomethacin with Eudragit RL100 and RS100 by emulsion solvent diffusion (Malamataris and Avgerino, 1990) have been reported. Moreover, yang et al. (2003) prepared the sustained-rekease nitrendipine microsphers with Eudragit RS and indicated that the release profile of nitrendipine microspheres was modulated with adjusting the ratio of the retarding agent to the dispersing carrier.

Nifedipine has been widely used as a calcium channel blocker for chronic diseases such as hypertension, various angina and other non-vascular deseases. Therefore, preparation of a sustained release formulation may be deirable.

Factorial design is an efficient tool to obtain an appropriate mathematical model with minimum experiments for optimization of formulation design. Studies based on factorial design allow all the factors to be varied simultanously, thus enabling the evaluation of the effects of each variable at each level and showing interrelationship among them. Most important variables which affect the system function are selected and systemic experiments are then performed to be specified factorial design.

The number of independent variables selected decides the number of experiments that are to be performed (Bhavar et al., 2006).

The objective of this study is preparation of sustained-release microspheres system for a poorly water-soluble drug, nifedipine, using Eudragit RL100 and solvent evaporation technique. Furthermore, in this study factorial design based on response surface method was adopted to optimize effective factors for encapsulation of the drug with polymer. A 2^3 full factorial design was employed to evaluate the effect of each of the selected variables and their interactions on the response.

MATERIALS AND METHODS

Materials

Eudragit RL100 were purchased from Rohm GmbH (Darmstadt, Germany). PVA, dichloromethane, and normal hexane were obtained from Merck (Darmastadt, Germany). Nifedipine was a gift from Zahravi Pharmaceutical Company (Tabriz, Iran). Other chemicals and solvents were of pharmaceutical or analytical grades, and used as received.

Preparation of nifedipine microspheres

Microspheres were prepared by solvent evaporation method. 100 mg nifedipine and 1.5 gr Eudragit RL100 were dissolved completely in chloroform (10 ml) using mechanical stirrer at 800 rpm as the internal phase. The solution was then added dropwise to a solution of PVA in water (1% w/v), which acts as the external phase. The mixture was stirred for 6 h until all chloroform was evaporated and microspheres were obtained. The formed microspheres were separated with paper filter, then rinsed three times with normal hexane and dried in room temprature.

SEM analysis

The morphology of microspheres were evaluted using SEM technique (S360, USA). The dried samples were gold sputter-coated before observation by the microscope.

Entrapment efficacy of microspheres

Micropheres containing approximately 100 mg nifedipine were accurately weighted and dissolved in chloroform used as a common solvent of drug and polymer. Drug loading was determined spectrophotometrically (n = 6) at 320 nm.

In-vitro dissolution studies

The release of nifedipine from microspheres was investigated using basket method (Appartus 1) at 50 rpm and in 900 ml of both an acidic solution (pH=1.2 for 2 h.) and a phosphate buffer solution (pH = 7.4 for 10 h.) at 37 ± 0.5°C (Ph. US 24th edn). At set intervals, 5 ml of sample were removed and replaced with equal volumes of the same solution. The amount of nifedipine released was measured spectrophotometrically at 342.5 nm for acidic phase and 340 nm for the buffer phase. The amount of nifedipine released was plotted against time.

Statistical analysis

First, a screening approach based on factorial design was used to select the factors displaying the most effects on the microspheres properties. The three obtained factors were: Eudragit concentration, PVA concentration, and drug/polymer ratio, which were selected as independent variables (Table 1). Then, these factors were investigated according to a response surface, the drug entrapment efficacy, to optimize preparation of nifedipine microsopheres. The experimental results were analysed using Design Expert® software.

RESULTS AND DISCUSSION

The aim of present work was to achieve optimized formulations for nifedipine-loaded microspheres by determining the effects of some important factors and their interactions during the process of preparation on microparticles physicochemical properties. Meanwhile the microspheres were being processed; the impact of different factors had been evaluated by making changes in their quantity. Finally, three of the most significant factors had been chosen as the independent variables. In the next step, for determining the low and high levels of

Table 1. Low and high levels for each of the factors.

Level	Factor A polymer (gr)	Factor B PVA (gr)	Factor C drug/polymer ratio
Low level	1	0.25	0.05:1
High level	2	0.50	0.10:1

Table 2. Experimental design of the optimization step.

Experiments	Formulation	Factor A (polymer)	Factor B (PVA)	Factor C (drug/polymer ratio)
1	D_1	1	0.25	0.05:1
a	D_2	2	0.25	0.05:1
b	D_3	1	0.50	0.05:1
ab	D_4	2	0.50	0.05:1
c	D_5	1	0.25	0.10:1
ac	D_6	2	0.25	0.10:1
bc	D_7	1	0.50	0.10:1
Abc	D_8	2	0.50	0.10:1

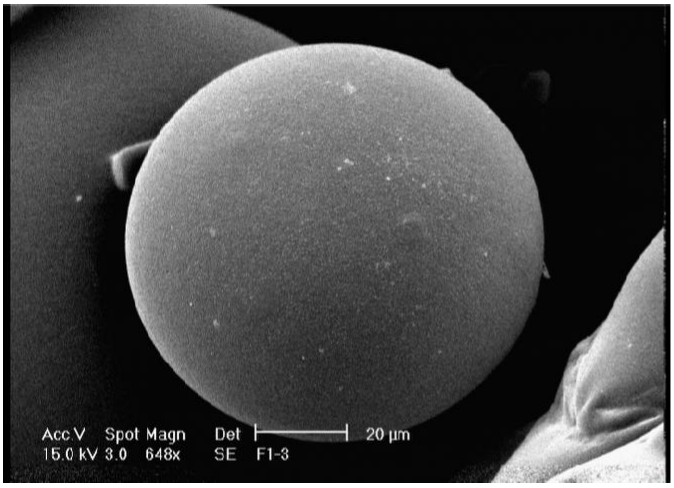

Figure 1. SEM photograph of formulation D_1.N.

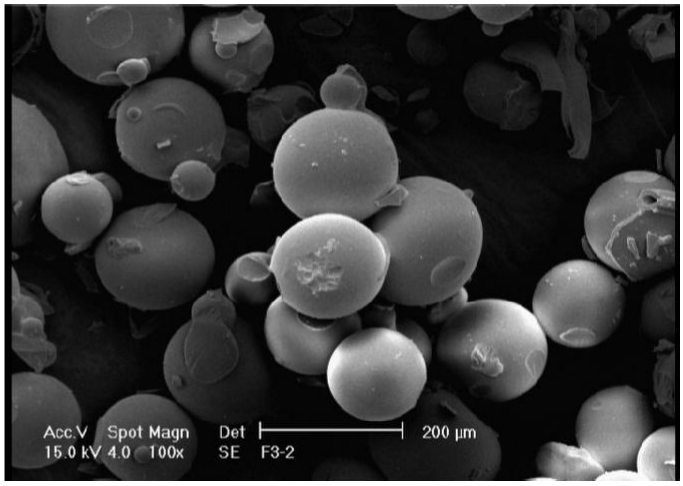

Figure 2. SEM photograph of formulation D_3.

each factor, some formulations were made, and the results are listed in table 1. According to a 2^3 factorial design and considering these three variables, 8 experiments had been performed (Table 2). Each experiment was carried out in triplicate (totally 24 formulations). Amount of the loaded drug in mentioned 24 formulations were obtained. The highest loading was related to formulation D^8 that all three factors had been used in their highest levels. The least response was resulted from D^6 in which although variables "A" and "C" were high, the concentration of PVA was in its low level. Morphological analysis of each of 8 formulations was performed using SEM. Some of the related morphographes are shown in Figures 1 - 4. These photographs showed that in the samples with low ratio of drug/polymer, the particles are spherical possessing smooth surfaces. On the other hand the high drug/ polymer ratio caused a coarse covering, likely due to drug's residue that has not been surrounded by polymer, thoroughly.

The main part of analysis was performed using the Design Expert 7 (DE^7) software. This software is able to evaluate each factor in regarding to its importance in particles characteristics based on the achieved responses. Moreover, it examines the interactions between the variables affecting the amount of drug-loading in microspheres. At last, according to the final results, this program suggested some formulations and also predicted their responses containing a probability factor named "Desirability" that ranged between 0 - 1.

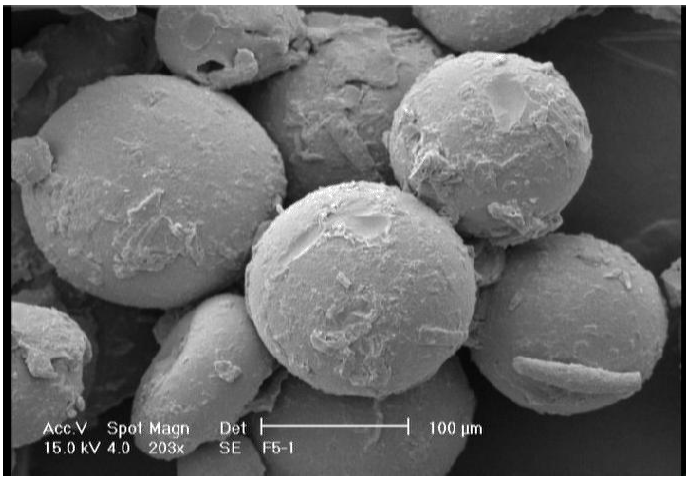

Figure 3. SEM photograph of formulation D_5.

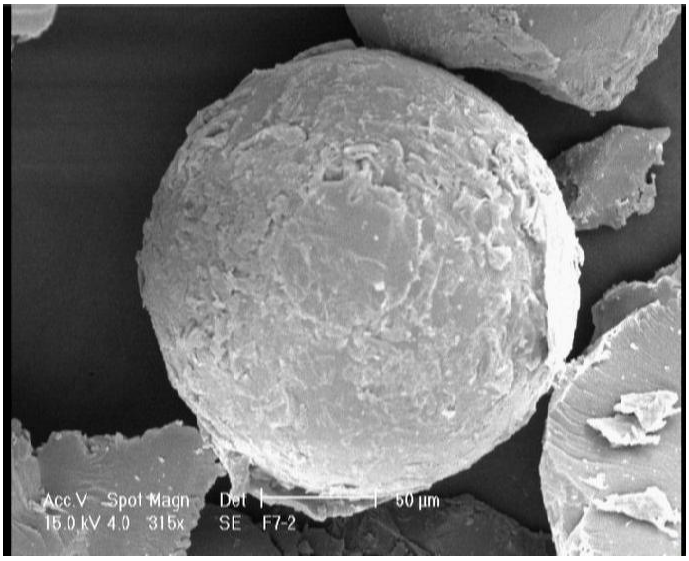

Figure 4. SEM photograph of formulation D_7.

That the most presumable answer would be the nearest to 1. Therefore, the program considering the obtained data (Table 3), distinguished the efficacy of each factor, and their interrelationship, as well. Parameter B can be considered to have the highest effect in drug loading of the yield microparticles as shown in Figure 5; thus, the amount of polymer (factor A) has the least influence. In the next step, significance of this influence was also statistically confirmed by ANOVA Test ($P < 0.05$). According to Table 4 all of the variables and their interactions had significant effects except the amount of polymer as factor A.

Design Expert 7 then evaluate the effects of variables that were plotted in some diagrams. In each plot, two factors remained constant and the other factor was in the

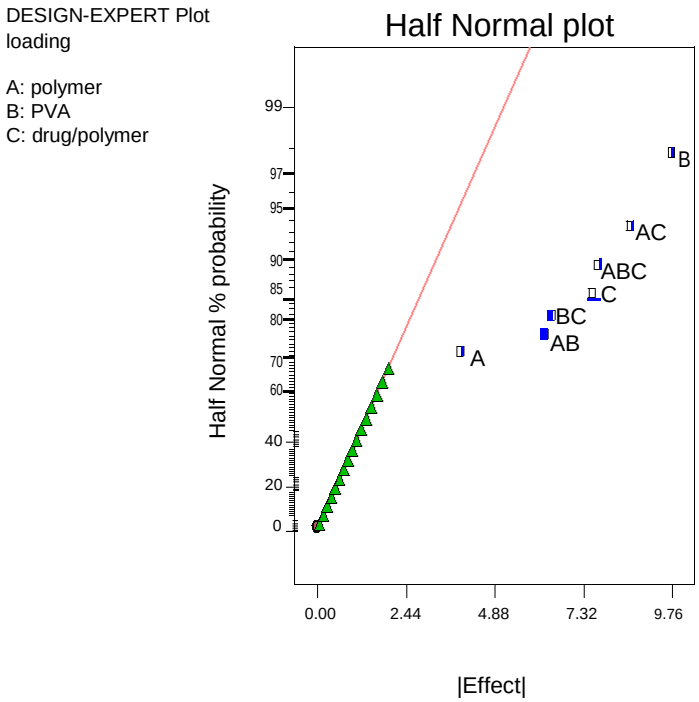

Figure 5. Half-Normal plot obtained by D.E.7 related to the given data.

Table 3. The experimental data for design and obtained responses (mean values ± S.D.) for each formulation.

Formulation	Percent of drug-loaded in microspheres
$D_{1(1)}$	38.33 ± 2.08
$D_{1(2)}$	49.33 ± 1.15
$D_{1(3)}$	54.37 ± 1.53
$D_{2(1)}$	52.36 ± 0.59
$D_{2(2)}$	67.63 ± 2.76
$D_{2(3)}$	64.13 ± 1.58
$D_{3(1)}$	51.93 ± 3.56
$D_{3(2)}$	52.70 ± 2.46
$D_{3(3)}$	51.70 ± 2.04
$D_{4(1)}$	64.67 ± 1.53
$D_{4(2)}$	63.03 ± 0.63
$D_{4(3)}$	61.93 ± 2.77
$D_{5(1)}$	64.73 ± 1.70
$D_{5(2)}$	60.20 ± 3.02
$D_{5(3)}$	69.50 ± 3.96
$D_{6(1)}$	43.37 ± 1.89
$D_{6(2)}$	42.67 ± 1.53
$D_{6(3)}$	52.37 ± 1.40
$D_{7(1)}$	64.97 ± 1.79
$D_{7(2)}$	68.47 ± 5.66
$D_{7(3)}$	67.67 ± 3.21
$D_{8(1)}$	77.13 ± 1.03
$D_{8(2)}$	76.97 ± 2.00
$D_{8(3)}$	74.93 ± 1.53

Table 4. ANOVA test for determining the significance of the variables.

Source model	Sum of squares	df	Mean square	F value	p-value probe > F
	2300.01	**7**	**328.57**	**13.89**	**< 0.0001 significant**
A-polymer	93.22	1	93.22	3.94	0.0645
B-PVA	570.96	1	570.96	24.14	0.0002
C-drug/polmer ratio	345.04	1	345.04	14.59	0.0015
AB	234.75	1	234.75	9.92	0.0062
AC	446.52	1	446.52	18.88	0.0005
BC	250.39	1	250.39	10.58	0.0050
ABC	359.14	1	359.14	15.18	0.0013
Pure error	378.50	16	23.66		
Cor total	2678.51	23			

Final equation in terms of coded factors: Loading =+59.79 +1.97* A +4.88 * B+3.79 * C+3.13 * A * B-4.31 *A*C+3.23*B* C+3.87 * A * B * C .

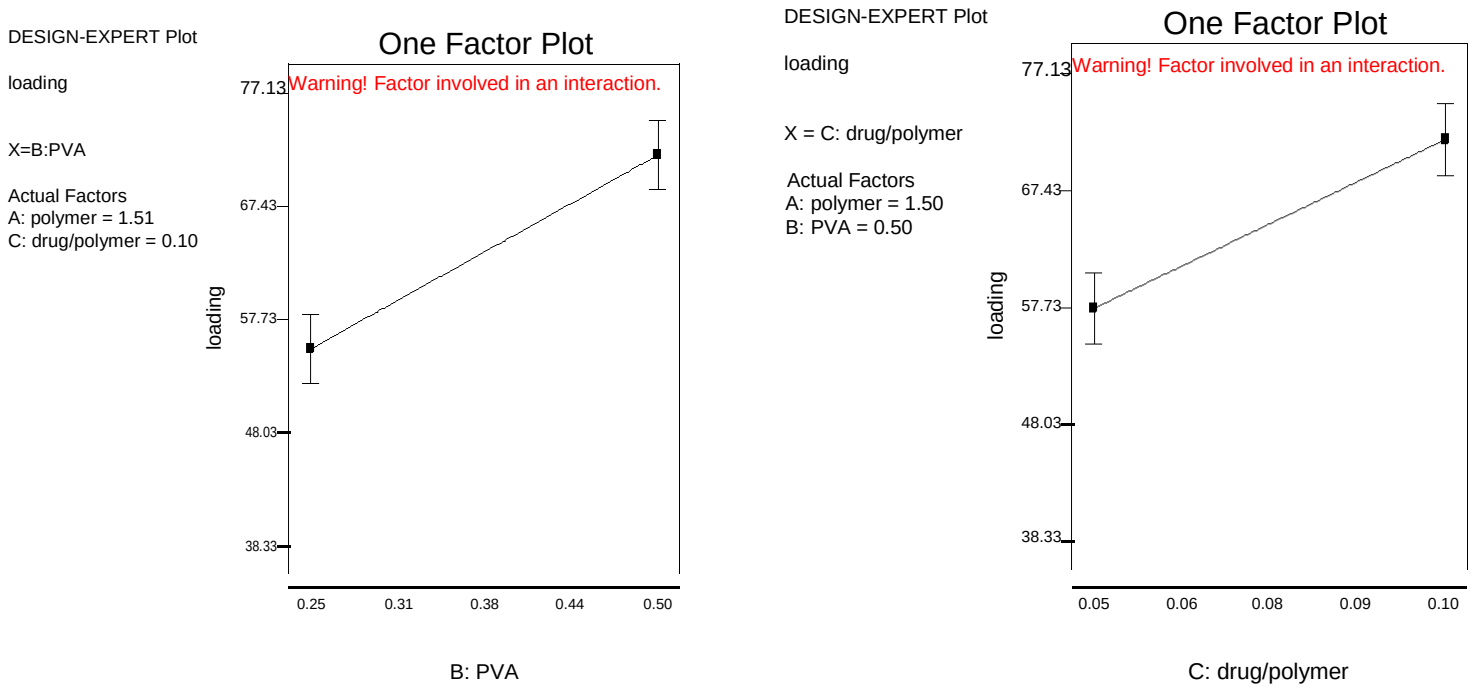

Figure 6 (a). The effect of variable B on nifedipine loading in particles while the amount of polymer (factor A) was 1.5 g and factor C has a 0.1:1 ratio. (b) The effect of variable C on nifedipine loading in particles while the amount of polymer (factor A) was 1.5 g and factor B has an amount equal to 0.5.

given range between its high and low levels; therefore, its influence can be seen as a line that represented the demanded response. The most effective factor, amount of PVA, would have the highest increase in response when the amount of polymer (factor A) was in its medium and the factor C was in its highest level (Figure 6a).

High level of the variable C affected the loading the most when A is in its medium amount and B has its highest quantity (Figure 6b). Effects of the interactions were plotted in diagrams almost similar to the ones above that one factor was plotted against the percent of loading and the second variable remained constant. But about the third one, there were two lines that the red one represented high level of this variable and the black one was referred to the low level. Figure 7 shows the interaction between B and C. As it can be observed effect of increasing amounts of B in the formulation prepared with 2 gr of polymer (high level) and 0.05:1 ratio of

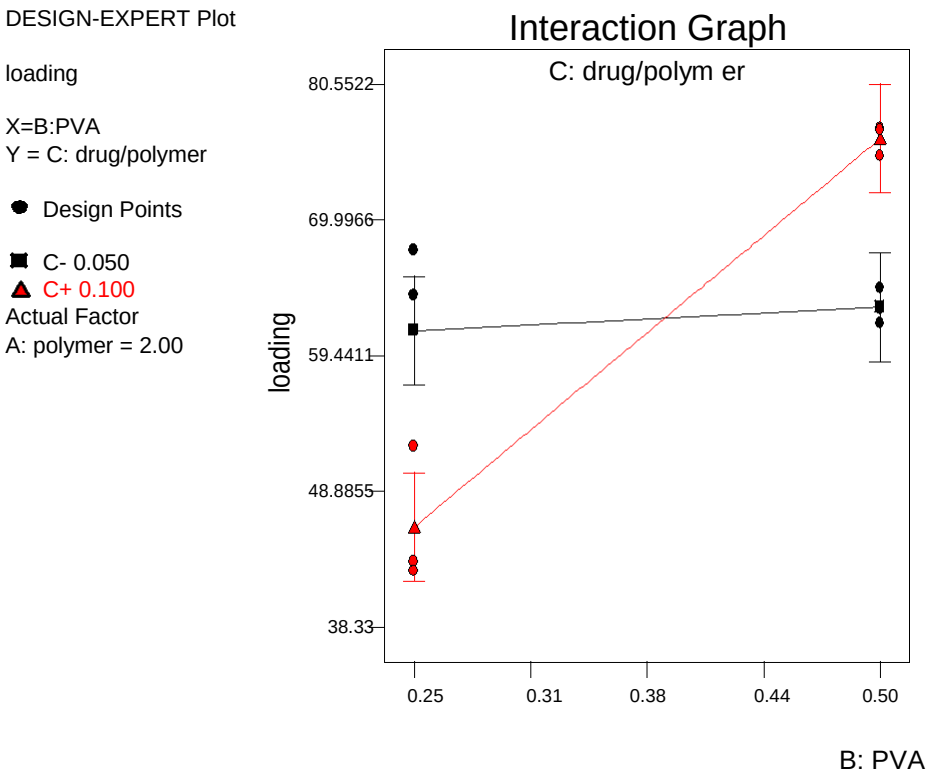

Figure 7. The effect of interaction between variables B and C on the response when polymer amount is 2 g.

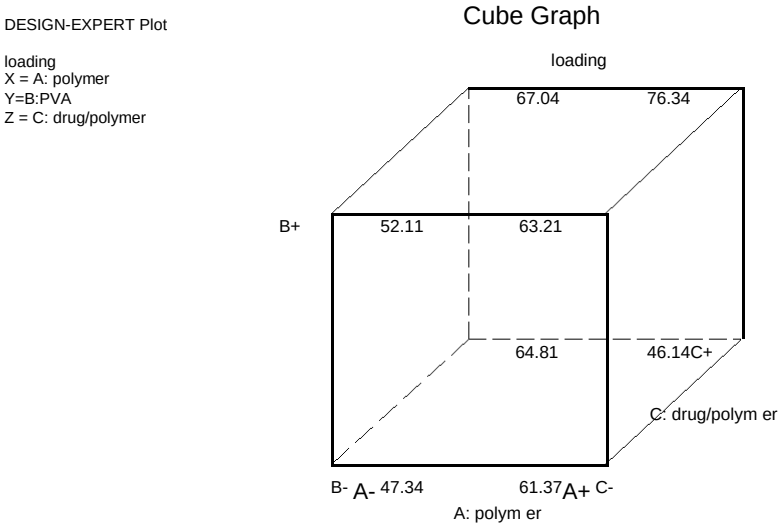

Figure 8. Cube graph related to the variables interaction and their effects on loading percent in the microspheres.

drug/polymer, was not very significant. However this formulation can cause high loading when the ratio of drug/polymer and the variable B reach to their maximum level. According to the applied method, the optimum value for this response (loading) corresponded to formulation D^8 of our experimental design, prepared with the maximum amounts of each variable and this was located in the optimum corner of the cube graph (Figure 8). Data analysis showed that upper levels of PVA always would cause in higher drug-loading percent and in such a

Table 5. Selective formulations that DE.7 predicted out of the specified limit for each variable.

Sample	Polymer	PVA	Drug/Polymer Ratio	Percent of drug loaded in particles	Desirability
S_{19}	2.00	1	0.07:1	92.00	0.982
S_{20}	1.97	1	0.07:1	93.87	0.966
S_{21}	1.93	1	0.07:1	92.74	0.959
S_{22}	1.85	1	0.07:1	90.40	0.945
S_{23}	1.70	1	0.07:1	86.00	0.918
S_{24}	1.55	1	0.07:1	81.64	0.889
S_{25}	1.40	1	0.07:1	77.18	0.857
S_{26}	1.89	1	0.07:1	91.08	0.856
S_{27}	1.58	1	0.07:1	80.50	0.845
S_{28}	1.30	1	0.07:1	74.34	0.836
S_{29}	1.99	1	0.07:1	94.55	0.912
S_{30}	1.99	1	0.07:1	94.39	0.909
S_{31}	1.97	1	0.07:1	93.78	0.899
S_{32}	1.92	1	0.07:1	92.37	0.876

Table 6. Predicted drug-loading percent and related obtained response of 8 of suggested samples.

Sample	Polymer	PVA	Drug/Polymer ratio	Predicted response	Obtained response
S_{19}	2.00	1	0.07:1	92.00	86.00
S_{20}	1.97	1	0.07:1	93.87	83.00
S_{21}	1.93	1	0.07:1	92.74	67.00
S_{22}	1.85	1	0.07:1	90.40	81.50
S_{23}	1.70	1	0.07:1	86.00	81.50
S_{24}	1.55	1	0.07:1	81.64	75.50
S_{25}	1.58	1	0.07:1	80.50	68.50
S_{26}	1.30	1	0.07:1	74.34	79.00

Table 7. Mean percent of *in vitro* drug-release from each of final samples after 12 h (n = 4).

Sample	S_{19}	S_{20}	S_{22}	S_{23}	S_{24}	S_{28}
Mean drug released	69.58 ± 5.10	63.20 ± 2.96	60.37 ± 2.94	66.47 ± 3.54	69.40 ± 6.87	62.17 ± 5.04

formulation the drug/polymer ratio had been chosen to be 0.07:1, whereas, the amount of polymer as the less important variable could vary in range of 1 – 2 gr. This software also suggests some formulations out of the range that was given at first, in regard to the results of analysis. Table 5 includes some of the suggested formulations of DE[7] that are out of the specified limitation of each item. Also the desirability of each item could be observed. Eight of these samples were made and the amount of nifedipine embedded in obtained microspheres were calculated and listed in Table 6.

All of the formulations in this table except S21 and S27, had been chosen for release test and morphology evaluation.

All of the samples showed a burst effect in the first hour of drug-release. The initial burst release is sometimes attributed to the rapid release by diffusion of dissolved drug initially deposited inside the pores. The most commonly supported hypothesis for the explanation of the burst is that some drug particles could have migrated at the surface during the drying of microspheres (Cohen et al., 1991; Martinez-Sancho et al., 2004). Furthermore, some of the free drug crystals that had not been surrounded by polymer and remained free in the surface of particles could corporate causing this effect. Release of the drug during the next hours followed a line with a slight slope that showed a slow libration. It is probably dynamically controlled by the diffusion rate of drug through the porosity of polymer matrix (Figure 9). Table 7 showed the final percent of drug release from the 6 samples. Finally, morphologically, SEM revealed that all microsphere obtained from the 6 suggested formulations

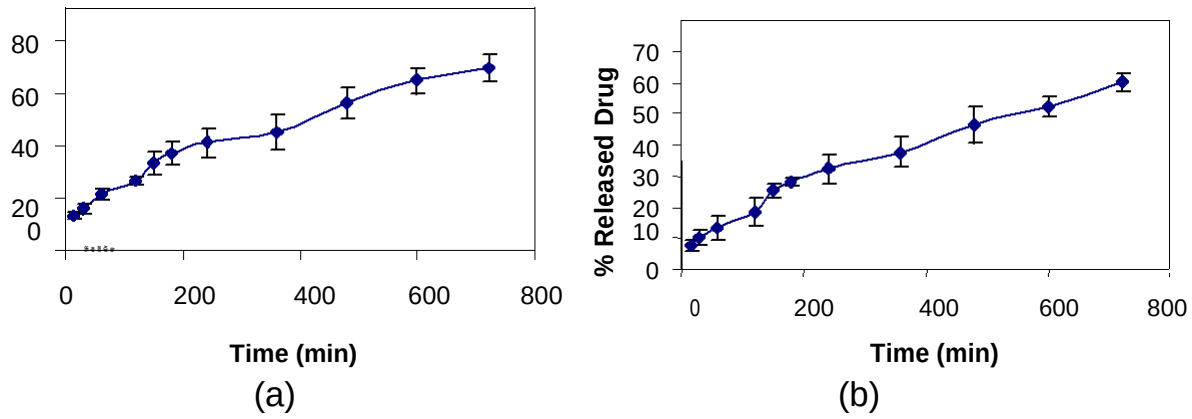

Figure 9. Drug-release profile of nifedipine from the formulations a) S_{19}, and b) S_{22}.

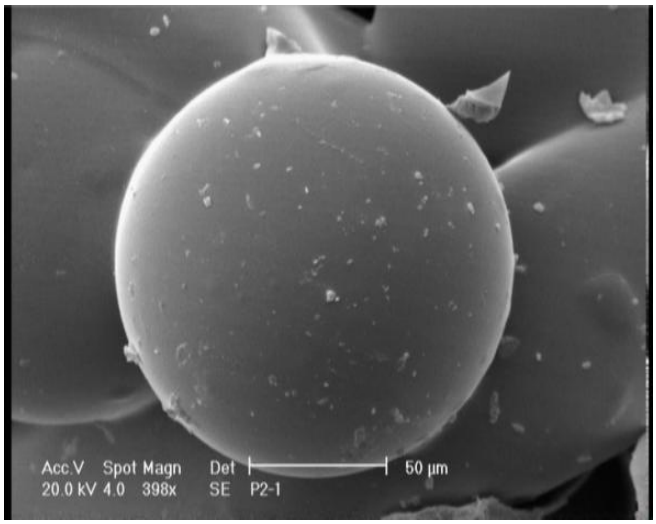

Figure 10. SEM photograph of formulation S_{20}.

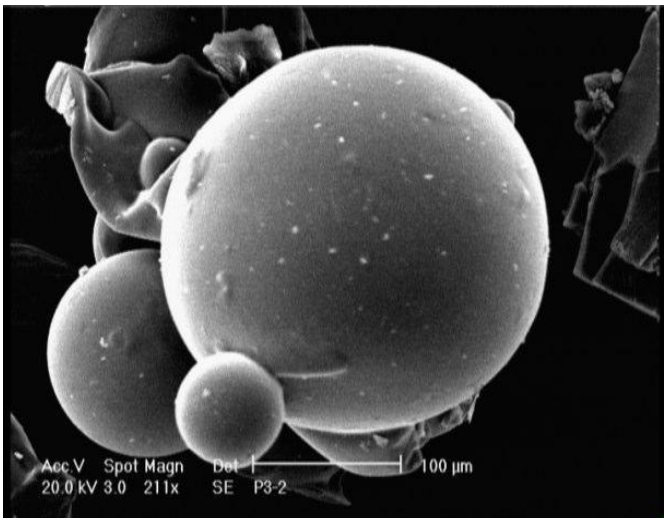

Figure 11. SEM photograph of formulation S_{22}.

resulted in spherical shapes and possessed a smooth surface (Figures 10 and 11).

Conclusion

Results from our study indicates that nifedipine microsphers can be prepared by using DCM, PVA and Eudragit RL100 as organic solvent, stabilizer and desired polymer, respectively, by solvent evaporation technique. Application of factorial design demonstrates a useful method for optimization of microspheres. Furthermore, DE[7] analysis of the obtained results described adequately the influence of the selected variables (amount of polymer and PVA and drug/polymer ratio) at different levels on the response under study (percent of drug-loading) in this work.

According to the studied factors, the obtained microspheres corresponded to formulations S^{19}, S^{20} and S^{24} that were prepared using 2, 1.97 and 1.55 gr polymer in presence of 1 gr PVA and 0.07:1 ratio of drug/polymer, were selected as optimum formulations because of high drug-loading percent, successful retarding drug release over the test period of 12 h in in vitro studies, spherical and smooth surface morphology.

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