Formulation and Characterization of Combination therapy based Double Layered Microspheres of Antihypertensive Drugs

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Abstract
Objectives: Combination of amlodipine besylate/losartan potassium has shown to significantly lower blood pressure as compared to monotherapy. Present work describes development of double layered microspheres where rapid onset of action is achieved by initial burst of amlodipine besylate from the immediate release Eudragit E100 layer followed by the sustained release of losartan potassium from the core.
Method: The core microspheres consisting of losartan potassium, prepared by ionotropic gelation method, were coated with Eudragit E100 and amlodipine besylate by solvent evaporation method. The microspheres were evaluated for particle size, drug loading efficiency, mucoadhesion, swelling index and drug release.
Results: The particle size, % yield, % drug loading efficiency of double layered microspheres was found 593±0.82nm, 89.9±0.86% and 72.42±0.23% respectively. The drug release study of double layered microsphere revealed that 95.30±0.12 % % amlodipine besylate was released in 2 hours and 95.12 ± 1.26% losartan potassium was released in next 24 hours.
Conclusion: It can be concluded that the developed multiparticulate system may prove to be an effective combination dosage form to treat hypertension for longer period.
Keywords: Double layered microspheres, Ionotropic gelation, Losartan potassium, Amlodipine besylate, Hypertension

Introduction
Hypertension is a cardiac chronic medical condition in which the systemic arterial blood pressure is elevated. Blood pressure involves two measurements, namely systolic and diastolic. Normal blood pressure is at or below 120/80 mmHg. High blood pressure is anything above 140/90 mmHg [1]. Hypertension is classified as either primary (essential) hypertension or secondary hypertension. Current treatments of hypertension mainly include lifestyle modifications and medications. Recent clinical trials suggest that the approach of using monotherapy for the control of hypertension is not likely to be successful in most patients.
Combination therapy may be theoretically favoured by the fact that multiple factors contribute to hypertension.

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Achieving control of blood pressure with single agent acting through one particular mechanism may not be possible [2]. Regimens can either be fixed dose combinations or drugs added sequentially one after other. Combining the drugs makes them available in a convenient dosing format, lower the dose of individual component, thus, reducing the side effects and improving compliance. Often multiple medications are needed to be combined to achieve the goal blood pressure. Amlodipine besylate belongs to group of drugs called calcium channel blockers. It relaxes blood vessels and improves blood flow. Losartan potassium belongs to group of drugs called angiotensin II receptor antagonists [3,4]. Problems of conventional combination dosage forms includes poor patient compliance, increased chances of missing the dose of a drug with short half-life for which frequent administration is necessary, typical peak-valley plasma concentration-time profile is obtained which makes attainment of steady-state

condition difficult. The present investigation was undertaken with an objective to develop a multiparticulate system consisting of combination of antihypertensive drugs, amlodipine besylate and losartan potassium, which was achieved by formulating double layer microspheres wherein inner layer would contain losartan potassium (for sustained release action) with mucoadhesive polymer and outer layer would contain amlodipine besylate (for immediate action) with pH dependent polymer.

Materials and Methods

Materials

Amlodipine besylate was obtained as a gift sample from Cadila Pharmaceuticals. Losartan potassium was obtained as a gift sample from Kentreck Lab Pvt. Ltd. Sodium alginate, chitosan and Eudragit E100 were obtained from Chemdyes Corp. Vadodara. All other chemicals used were of analytical grade.

Methods

Preparation of Chitosan-alginate Core Microspheres [5]

Chitosan-alginate microspheres were prepared by ionotropic gelation method. Briefly, chitosan (2-4% w/v) was dissolved in 0.5%w/v solution of acetic acid. 5%w/v solution of calcium chloride was added to chitosan solution. Sodium alginate (0.5-1.0 % w/v) /losartan potassium solution was added drop wise to calcium chloride chitosan solution with the help of syringe to form the microspheres. These microspheres were left suspended in the solution for 30mins to complete the reaction. Later they were filtered, washed thrice with 100 ml distilled water and air dried overnight.

Preparation of Double Layered Microspheres [6, 7, 8]

Double layered microspheres were prepared by solvent evaporation method. Eudragit E 100 (0.5-1.75% w/w of microspheres) and amlodipine besylate were dissolved in dichloromethane and methanol mixture (1:1). Chitosan-alginate microspheres were dispersed in the above organic solution. The aqueous phase of poly vinyl alcohol 0.5% w/v was prepared. Then organic phase was added drop wise to the aqueous phase and stirred at approximate 1500 rpm for 2 hr. The microspheres obtained were then filtered, washed with water and dried.

Optimization of core microspheres using Experimental Design [9, 10]

The 3^2 factorial design was employed for the optimization of core microsphere. Based on preliminary studies, concentration of sodium alginate and chitosan were selected as independent variables and particle size (nm), drug loading efficiency (%) and mucoadhesion (%) were selected as dependent variables as shown in Table 1. The formulation charts for all proposed trial formulations for core and double layered microspheres are presented in Table 2 and Table 6 respectively. Design Expert software (7.0 trial version) was used for generation and evaluation of the statistical experimental design.

Characterization of Chitosan-alginate Core Microspheres [11, 12]

Micromeritic Studies: The particle size of all the formulations was measured by an optical microscope fitted with an ocular and stage micrometer. In all measurements at least 100 particles were examined and each experiment was carried out in triplicate. Bulk density, tapped density, Carr's compressibility index, Hausner's ratio and angle of repose (θ) measurements were carried out in triplicate.

Surface Analysis by Scanning Electron Microscopy (SEM): Morphological examination of the surface of core microspheres was performed by using scanning electron microscope (SEM).

Yield of Microspheres: After drying of microspheres, the microspheres were collected and weighed accurately.

$$\textbf{Yeild of microspheres} = \frac{\text{Total weight of microspheres}}{\text{Total weight of drug} + \text{Total weight of polymer}} X\ 100$$

Determination of Drug Loading Efficiency: Drug loaded microspheres (equivalent to 50 mg drug) were dissolved in 100ml of phosphate buffer pH 7.4 by shaking on a mechanical shaker for 6 hrs. The solution was filtered through Whatmann filter paper. An aliquot following suitable dilution was assayed spectrophotometrically (UV-1800 Shimadzu Corporation, Japan) for losartan potassium at 254nm.

Drug loading efficiency was determined by using the following relationship:

$$\text{Drug Loading Efficiency} = \frac{\text{Experimental Drug Content}}{\text{Theoretical Drug Content}} X\ 100$$

Swelling Ratio Studies:

Swelling ratio of was determined gravimetrically in slightly agitated phosphate buffer solution of pH 7.4. The microspheres were removed periodically from the solution, blotted to remove excess surface liquid and weighed on digital balance (Shimadzu AUW220D Corporation, Japan). Swelling ratio (% w/v) was determined from the following relationship:

$$\text{Swelling ratio} = \frac{\text{Wt} - \text{Wo}}{\text{Wo}} X\ 100$$

Where W_0 & Wt are initial weight and final weight of microspheres respectively.

In-Vitro Wash-off Test [13]: *The* mucoadhesive property of the microspheres was evaluated by in-vitro wash-off test. A 1cm X 1cm piece of chicken intestine mucosa was tied onto a glass slide (3 X 1inch). 50 microspheres was spread onto the wet rinsed tissue specimen, and the prepared slide is hung onto one of the groves of a USP tablet disintegrating test apparatus. The disintegrating test apparatus was operated such that the tissue specimen got regular up and down movements in a beaker containing the simulated intestinal fluid USP. At the end of 10 hours, the number of microspheres adhering onto the tissue was counted.

$$\%\ \text{mucoadhesion} = \frac{\text{N0} - \text{NS}}{\text{Ns}} X\ 100$$

Where No=Initial number of microspheres, N_s=Microspheres remained adhered after 10 hrs.

In Vitro Drug Release: In vitro drug release studies of chitosan-alginate microspheres were carried out at different pH using USP Dissolution Test Apparatus (Apparatus 2). Microspheres loaded with losartan potassium were suspended in dissolution media (900ml) at 37 ± 0.1°C. Samples were withdrawn periodically and compensated with same amount of fresh dissolution media. The samples were analyzed for drug release by measuring absorbance using UV Spectrophotometer. The scheme of using the simulated fluids at different pH was as follows:

First two hrs.: Simulated gastric fluid (SGF) of pH 1.2

Third hr. onwards: Simulated intestinal fluid (SIF) of pH 7.4

Characterization of Double Layered Microsphere [17, 18]:

Double layered microspheres were evaluated for particle size, surface analysis, yield and in *vitro* drug release was estimated as per the procedures mentioned under evaluation of core microspheres.

Determination of Drug Loading Efficiency: Drug loaded microspheres (equivalent to 50 mg drug) from each batch were dissolved in 100 ml of 0.1 N HCL by shaking on a magnetic stirrer for 1hr. The solution was filtered through Whatmann filter paper. An aliquot following suitable dilution was assayed spectrophotometrically (UV-1800 Shimadzu Corporation, Japan) for amlodipine besylate at 237.5 nm. Drug loading efficiency was determined by using the following relationship for drug loading efficiency:

Results and Discussion

Characterization of Chitosan-alginate Core Microspheres:

Micromeritic Studies of Core Microspheres:

Table 3 records the results of micromeritic studies of core particles. Carr's index, Hausner's ratio and angle of repose were found to be in the range of 9.6 ± 0.058 – 29.2 ± 0.794, 1.11 ± 0.006 –1.42± 0.011 and 22± 0.65 – 37 ± 0.921 respectively. Hence it can be concluded that all the formulation batches had good flow properties.

Surface Analysis by Scanning Electron Microscope:

Scanning electron microscopic study of the chitosan-alginate core microspheres (Figure 1a) showed that the microspheres had uniform spherical shape. Surface examination of uniform spheres under higher magnification showed rough surface of the microspheres.

% Yield and swelling index:

Results of % yield and swelling index are reported in table 3. Yield was found to be lower when the total concentration of polymers is high. This may be attributed highly viscous polymer dispersion which might be lost during manufacturing process. The swelling index of the microspheres found to be higher when the total concentration of polymers is high which may be due to maximum water uptake and more hydrophilicity.

In Vitro Drug Release from Core Microspheres:

Drug release was dependent on polymers concentration. At low concentration of sodium alginate and chitosan, drug release rate was very high in phosphate buffer pH 7.4 (>80% drug released after 10 h) despite low drug release in 0.1 N HCl (<15% drug released after 2 h). At high concentration of sodium alginate and chitosan, drug release rate was slow in phosphate buffer pH 7.4 (<70% drug released after 10 h) despite low drug release in 0.1 N HCl (<10% drug released after 2 h). It has observed that drug release decreases with increasing concentration of polymer. The reason attributed to this fact is formation of dense matrix. The drug release profile from the core microspheres is depicted in figure 2.

Experimental responses of the batches prepared by applying factorial design:

A total of 9 core microsphere formulations were proposed by the 3^2 factorial design for two independent variables viz sodium alginate concentration (X_1) and chitosan concentration ($X_{2)}$ which were varied at three different levels. The effects of these independent variables on particle size (μm) (Y_1), % drug loading efficiency (Y_2) and % mucoadhesion (Y_3) were investigated as optimization response parameters. The results of the ANOVA indicated that these models were significant for all response parameters as shown in Table 4.

Three-dimensional response surface plots and their corresponding contour plots to estimate the effects of the independent variables (factors) on each response investigated are presented in figures 3, 4, and 5. The three-dimensional response surface plots and corresponding contour plots indicate the increased values of particle size and % drug loading efficiency with the increment of two independent variables (conc. of sodium alginate and chitosan), while the increased values of % mucoadhesion with the increment of one independent variables (conc. of chitosan) and decreased values of % mucoadhesion with the increment of another independent variables (conc. of sodium alginate). This is also evident from the polynomial equations for particle size, % mucoadhesion and % drug loading efficiency, mentioned below

Polynomial equation for particle size (Y1) =+ 612.11+ 62.50 X1+ 27.83X2

Polynomial equation for % mucoadhesion (Y2) =+ 68.89 - 4.83X1 + 7.83X2

Polynomial equation for % drug loading efficiency (Y3) = + 85.88 + 4.41 X1+ 3.19X2

For validation of the model, an overlay plot exhibiting combined effect of concentration of sodium alginate and concentration of chitosan and selected check point batch was prepared as shown in Figure 6.

Table 5 depicts the results of predicted values obtained from the mathematical model and practically observed (actual value). It was found that relative error was less than 0.5% for

all responses for all the levels of polymers. So equation obtained for selected responses are validated in selected ranges of variables. The close resemblance between the observed and predicted response value assessed the robustness of predictions. These values indicate the validity of generated model. The optimized core microsphere formulation (A-10) showed particle size 660±0.09 nm, % mucoadhesion 69.12±0.72%, and % drug loading efficiency 92.31±0.16%.

Characterization of Double Layered Microspheres

Size, % yield of microspheres and determination of % drug loading efficiency:

The result summarized in Table 6 indicate that there was a proportional increase in the mean particle size of microspheres with increased amount of Eudragit E100 in the formulation, which could be due to the increased thickness of the coat.

The product yield was found to decrease with increase in polymer concentration, due to the formation of high viscous polymer dispersion which might have been lost during manufacturing process. Also when Eudragit E100 concentration increased, drug entrapment efficiency was found to increase, due to more amount of coating on the core microspheres, till the core: coat ratio was 1:1. Further increasing the polymer did not result in any increase in the entrapment efficiency.

Surface Analysis by Scanning Electron Microscope

Scanning electron microscopic study of the double layered microspheres (Figure 1b) showed that the microspheres to be spherical in shape. Surface examination of spheres under higher magnification showed rough surface of the microspheres.

In Vitro Drug Release from Double Layered Microspheres:

Percentage Drug (Amlodipine Besylate) release was found to be dependent on Eudragit E100 concentration. Drug release rate decreased with increasing Eudragit E100 concentration. At lower concentrations of polymer used (Table 6; till 1:1 core: coat ratio), decrease in the amount of drug released in 2 hrs, was not significant, but at higher concentrations (1:1.5 and1:1.75 core: coat ratio), drug release dropped down. Hence looking at the results, 1:1 core: coat ratio was taken to be the optimized concentration.

Table 1. Variables of experimental design

Code	Independent Variable	Lower Value (-1)	Middle Value (0)	Higher Value (1)
X1	Concentration of sodium alginate (%w/v)	2	3	4
X2	Concentration of chitosan (%w/v)	0.5	0.75	1.0
	Dependant variables			
Y1	Particle Size (nm)			
Y2	Drug loading efficiency (%)			
Y3	Mucoadhesion (%)			

Table 2. Composition and responses of 3^2 full factorial design batches of core microsphere

Batch no.	Concentration of sodium alginate (%)	Concentration of chitosan (%)	Particle Size (nm) ± SD	Drug loading efficiency (%) ± SD	Mucoadhesion (%) ± SD
A1	2	0.75	528± 0.22	84.12± 0.36	80.42± 0.66
A2	4	0.75	651± 0.02	90.20± 0.81	64.67± 0.82
A3	3	1	643± 0.46	92.70± 0.44	76.18± 1.09
A4	**4**	**1**	**660± 0.09**	**92.31± 0.16**	**69.12± 0.72**
A5	2	0.5	488± 0.67	79.16± 0.58	78.92± 0.67
A6	4	0.5	644± 0.88	88.42± 0.92	59.54± 0.12
A7	3	0.5	568± 0.92	82.61± 0.18	62.18± 0.57
A8	3	0.75	617± 0.61	85.09± 0.54	69.11± 1.06
A9	2	1	564± 0.82	90.13± 1.06	82.23± 0.66

All values are represented as mean ± standard deviation (n=3)

Table 3. Evaluation/characterization of core microspheres

Batch no.	Carr's index ± SD	Hausner's ratio ± SD	Angle of repose ± SD	% Yield ± SD	Swelling index ± SD
A1	14.2 ± 0.784	1.16 ± 0.001	31.5 ± 0.865	89 ± 0.420	5.13 ± 0.33
A2	20 ± 1.08	1.25 ± 0.002	32 ± 1.06	86 ± 0.156	4.51 ± 0.46
A3	26.3 ± 0.144	1.36 ± 0.009	33 ± 0.782	78 ± 0.743	4.47 ± 0.56
A4	**29.2 ± 0.794**	**1.42 ± 0.011**	**34 ± 0.754**	**76 ± 0.584**	**4.56 ± 0.21**
A5	11.8 ± 1.0	1.14 ± 0.004	26 ± 0.398	82 ± 0.386	4.07 ± 0.34
A6	14.9 ± 0.69	1.18 ± 0.008	25 ± 0.647	86 ± 0.812	4.1 ± 0.22
A7	8.2 ± 0.93	1.09 ± 0.002	22 ± 0.65	89 ± 0.464	3.71 ± 0.31
A8	9.6 ± 0.058	1.11± 0.006	29 ± 0.915	87 ± 0.378	4.12 ± 0.27
A9	24.5 ± 0.138	1.34 ± 0.005	37 ± 0.921	91± 0.182	5.02 ± 0.36

All values are represented as mean ± standard deviation (n=3)

Table 4. Results showing ANOVA study of core microspheres

Response model	F value	P value	R2 value	Adequate precision
Y1	53.1	0.004	0.9888	20.874
Y2	22.86	0.0136	0.9744	14.264
Y3	42.86	0.0054	0.9862	19.37

Table 5. Results of experiments to assure optimization capability of core microspheres

Batch code	Concentration	Parameter	Predicted value	Experimental value	Relative error (%)
A10	3.95% sodium alginate and 0.89% chitosan	Particle size (nm)	659.7	660	0.04%
		Mucoadhesion (%)	68.06	69.12	0.15%
		Drug loading efficiency (%)	91.8	92.31	0.05%

Table 6. Evaluation/characterization of Double layer microspheres

Batch no.	Core microspheres : Eudragit E100	Particle size (nm) ± SD	Yield obtained (%) ± SD	Drug loading Efficiency (%) ± SD	% Amlodipine besylate released in 2 hrs ± SD
C1	1:0.5	487 ± 0.12	91.62 ± 1.02	67.09 ± 0.32	94.92 ± 0.03
C2	1:0.75	491 ± 0.56	90.12 ± 0.94	68.76 ± 0.76	95.63 ± 0.16
C3	**1:1**	**593 ± 0.82**	**89.9 ± 0.86**	**71.42 ± 0.23**	**95.30 ± 0.12**
C4	1:1.5	597 ± 0.67	89.22 ± 0.18	71.0 ± 0.12	89.87 ± 0.07
C5	1:1.75	610 ± 0.20	87.80 ± 0.97	69.23 ± 0. 43	83.74 ± 0.10

All values are represented as mean ± standard deviation (n=3)

Figure 1a. SEM of Core microspere

Figure 1b. SEM of Double layered MS

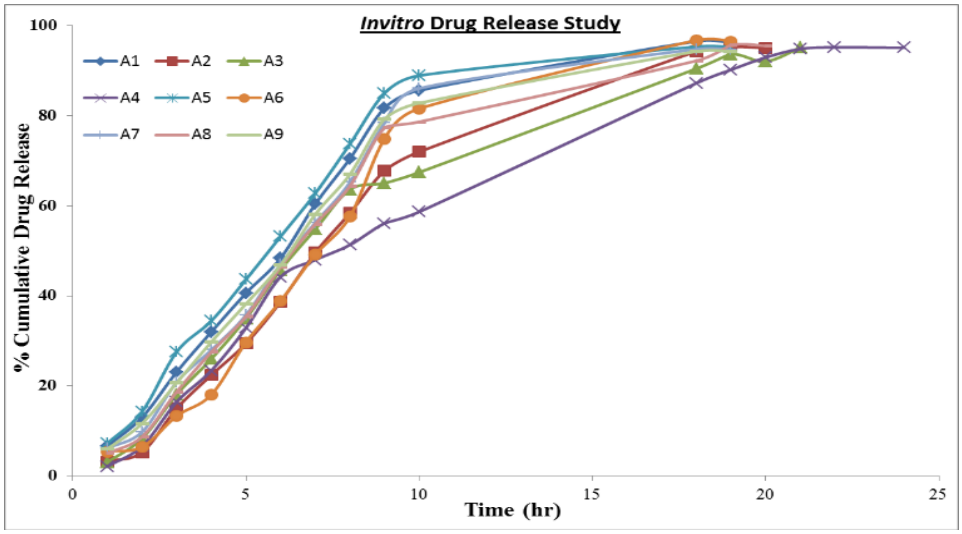

Figure 2. *Invitro* drug release of losartan potassium from core microspheres

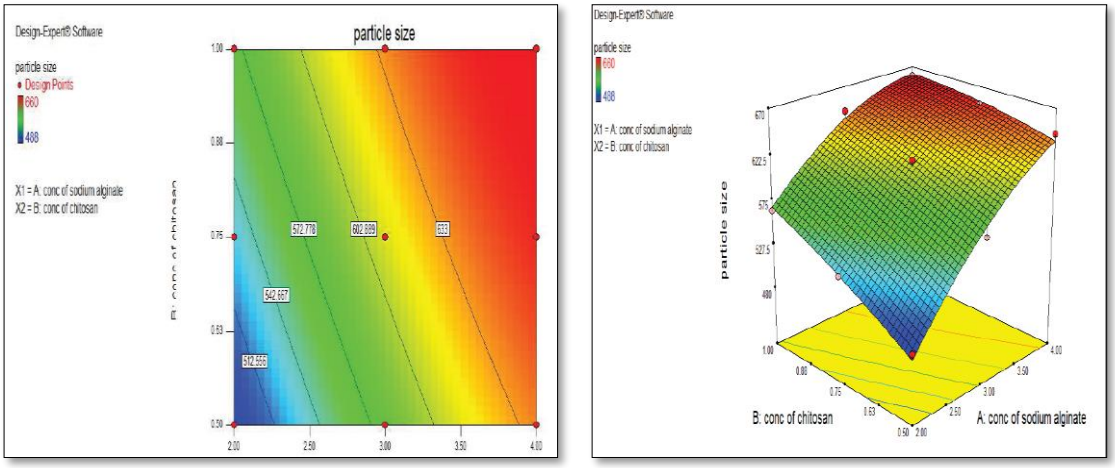

Figure 3. Contour plot and 3D plot eliciting the effect of X_1(sodium alginate concentration) and X_2(chitosan concentration) on Y_1 (particle size of core microspheres)

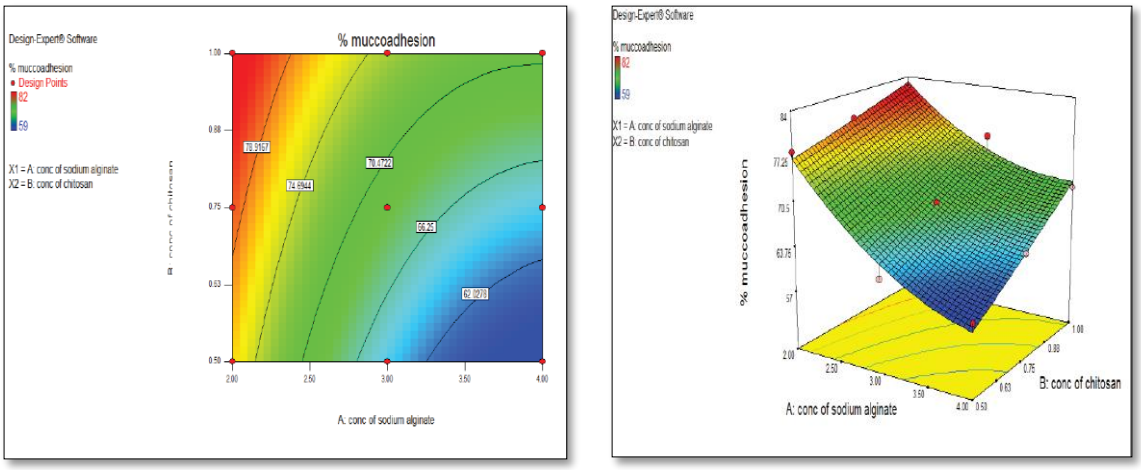

Figure 4. Contour plot and 3D plot eliciting the effect of X_1(sodium alginate concentration) and X_2(chitosan concentration) on Y_2 (% mucoadhesion of core microspheres)

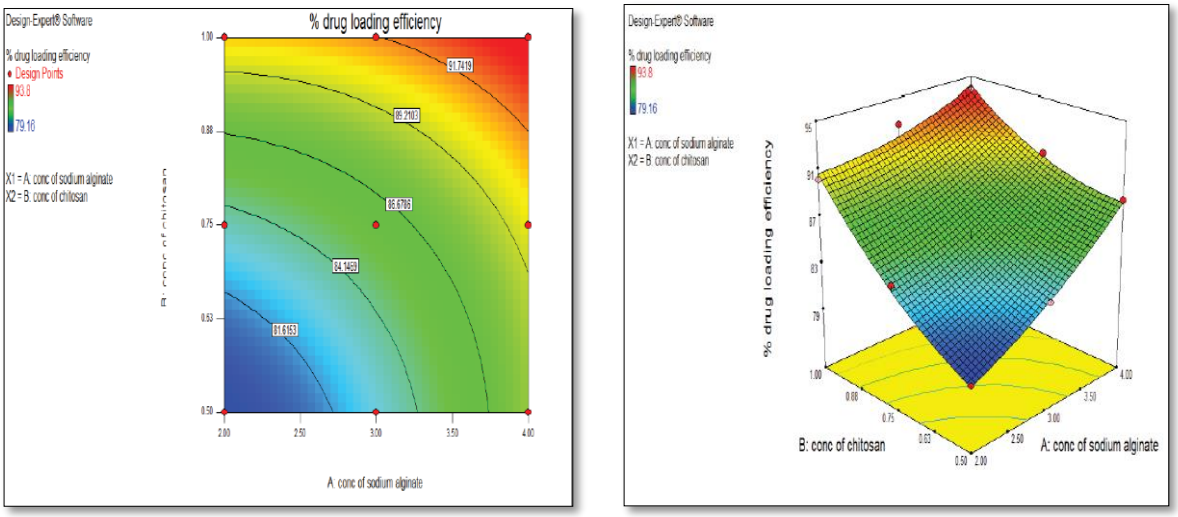

Figure 5. Contour plot and 3D plot eliciting the effect of X_1 (sodium alginate concentration) and X_2 (chitosan concentration) on Y_3 (% drug loading efficiency of core microspheres)

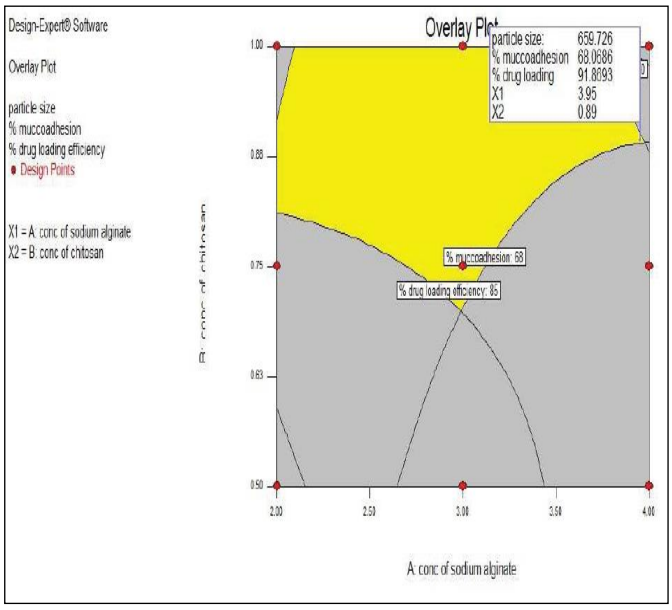

Figure 6. Overlay plot showing combined effect of concentration of sodium alginate and concentration of chitosan and selected check point batch

Conclusion

To achieve the therapeutic goals, there is need of a combination therapy of antihypertensive agents to circumvent risk of side effect associated with high dose of monotherapy. In present study, double layered microspheres containing amlodipine besylate and losartan potassium were successfully prepared using ionotropic gelation technique and solvent evaporation method. Polymer combinations of sodium alginate, chitosan and eudragit polymers resulted in microspheres with good yield and maximum drug loading. Results of present study suggest that optimized formulation with varying polymer ratio shows initial peak release followed by sustained release profile. A4 batch of core microspheres was selected as optimum formulation as it gave best result (95.12 % CDR at 24hrs) among all batches. C3 batch of double layered microspheres was selected as optimum formulation on the basis of point prediction (design expert 7.0 trial).

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Conflict of Interest

The authors declare no conflict of interest.

Declaration of Interest

The authors are responsible for the content and writing of this article.

Disclaimer

The views, thoughts and opinions expressed in this review belong solely to the authors, and not necessarily to the author's employer, organization, committee or other group or individual.

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