INNSPUB

Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 24, No. 5, p. 140-152, 2024
http://www.innspub.net

RESEARCH PAPER OPEN ACCESS

Design, development and optimization of carvedilol microspheres using 3^2 factorial design

Aisha Rahman[1], K. Latha[*1], Aymen Fatima[1], Vyshak R. Menon[1], T. Shailaja[2]

[1]*Department of Pharmaceutics, G. Pulla Reddy College of Pharmacy, Osmania University, Hyderabad, Telangana, India*

[2]*K.D.R. Government Polytechnic Wanaparthy, Mahabubnagar, India*

Article published on May 13, 2024

Key words: Carvedilol, Solubility, Solid dispersion, Sodium alginate, Microspheres

Abstract

Carvedilol being a BCS class II drug has low solubility and it has short half-life of 2-6 hrs. The objective of the study is to design, develop and optimize carvedilol microspheres using factorial design. Solubility was enhanced by formulating into solid dispersion using solvent evaporation technique and solid dispersion with enhanced solubility was incorporated into sodium alginate microspheres prepared by ionic gelation method and were optimized by 3-level, 2-factor full factorial design. Solid dispersion formulation SD3 showed 15.3 folds enhanced solubility compared to the pure drug. Powder X-ray Diffractogram patterns revealed that the crystalline drug was transformed into an amorphous form. SD3 formulation was incorporated into sodium alginate microspheres and the microspheres were optimized. The optimized microsphere formulation had an entrapment efficiency of 79.41% and showed 80% drug release at 6 hrs. Scanning electron microscopic images proved, that optimized microspheres were spherical in shape and had rough surface.

***Corresponding Author:** K. Latha ✉ lathakukatil@gmail.com

Introduction

The goal of any drug delivery system (DDS) is to provide a therapeutic amount of drug to the appropriate site in the body in order to achieve and maintain therapeutic concentration within range and to demonstrate pharmacological action with the least amount of adverse effects (Adepu and Ramakrishna, 2021; Verma *et al.*, 2010).

To achieve this goal, the dosing frequency and route of administration should be maintained. Despite significant advancements in DDS, the oral route remains the preferred route for administration of therapeutic agents due to several advantages including convenient administration, fewer aseptic constraints, and ease of manufacturing of the dosage form (Siddiqui *et al.*, 2011; Remington, 2021). To address the drawbacks of traditional DDS, several technological advances have resulted in the controlled DDS (CRDDS) development, which could revolutionise medication and provide diverse therapeutic benefits (Chein, 1992).

The attractiveness of the extended release dosage form is the ability to ensure safety, improve drug efficiency, reduce frequency of dose, and hence reduce adverse effects and improve bioavailability. The failure to expand the residence time of the dosage form in the stomach of the small intestine is a major concern of dosage forms with extended release. Therefore, it is advantageous to design formulations that extend the release and retain at the absorption site for an extended time period (Sompur *et al.,* 2011). Microencapsulation techniques and the formation of microspheres and microcapsules is one among such approaches. The possibility of achieving a longer lasting and more dependable release of medication at the desired rate is made possible by the multiunit microparticulate oral drug delivery systems, which can be widely distributed throughout the gastrointestinal tract.

Carvedilol is a non-selective β-adrenergic antagonist with α-1 blocking activity. It is used to treat mild to severe chronic heart failure, hypertension, and ventricular dysfunction following myocardial infarction in clinically stable patients. Carvedilol is a BCS class II drug that has low solubility and has a short biological half-life of 2-6 hrs. Therefore, in the present work, solubility was enhanced by formulating into solid dispersion using solvent evaporation technique and to sustain the drug release, the solid dispersion with enhanced solubility was incorporated into sodium alginate microspheres prepared by ionic gelation method

Material and methods

Carvedilol was obtained from Micro Labs Ltd. PVPK-30, methanol, and sodium alginate were purchased from SD Fine Chem Ltd, Mumbai, India. Calcium chloride was obtained from Thermo Fisher Scientific India Pvt. Ltd, Mumbai, India. All other chemicals and reagents were of analytical grade.

Drug-excipient compatibility studies

Fourier transform infra-red spectroscopy

Fourier Transform Infra-Red (FTIR) spectroscopy was used to examine the spectra of carvedilol and excipients used in the preparation of formulation. Potassium Bromide (KBr) disks were prepared by mixing sample with potassium bromide in ratio of 1:100 and placed in a suitable holder in an IR spectrophotometer (Shimadzu 8400S), and the IR spectra from 400 cm^{-1} to 4000 cm^{-1} were recorded (Yuvaraja and Khanam, 2014).

Differential scanning calorimetry

The differential scanning calorimetry (DSC) thermograms of carvedilol, sodium alginate, and pure drug-sodium alginate blend were obtained using a differential scanning calorimeter (Mettler Toledo STAR, Model DSC 822e) at a heating rate of 10°C/min from 25°C to 250°C (Sharma and Jain, 2010).

Method of preparation of solid dispersion

The solvent evaporation technique was utilised for the preparation of solid dispersion (Chhater and Praveen, 2013).

Required amount of carvedilol was dissolved in methanol to produce a clear solution.

↓

Required amount of PVPK-30 was dispersed in the above solution by stirring.

↓

The solvent was evaporated by mild heating to 40°C with constant stirring to get uniform mass.

↓

Dried mass was crushed using a mortar and pestle.

↓

Passed through sieve #40 and stored for further use.

Carvedilol solid dispersions were prepared using PVPK-30 in the drug: carrier ratio of 1:1, 1:3 and 1:5.

Evaluation of solid dispersion

Percentage yield

The prepared solid dispersion was collected and weighed accurately. The percent yield of solid dispersions was determined using the following Eq. 1 (Chhater and Praveen, 2013).

$$\text{Percentage yield} = \frac{\text{Weight of prepared solid dispersion}}{\text{Weight of drug+carrier}} \times 100 \quad \text{Eq. 1}$$

Drug content

Solid dispersion equivalent to 20 mg drug was dissolved in 10 mL methanol and diluted to 100 mL using 0.1 N HCl, pH 1.2. Required dilutions were done using 0.1 N HCl, pH 1.2 and absorbance was checked at 242.6 nm using UV Spectrophotometer (Lab India Analytical UV 3200). The study was performed in triplicate (Chhater and Praveen, 2013).

In-vitro dissolution of solid dispersion

Dissolution of pure drug and the prepared solid dispersions were performed using USP Type-II apparatus (Electro lab TDT-08L USP Disso Tester). 20 mg pure drug and solid dispersion equivalent to 20 mg of the drug were weighed and placed in dissolution vessel containing 900 mL 0.1 N HCl, pH 1.2 at 37±1.0°C, stirring speed of 50 rpm. 5 mL of sample was collected periodically using a syringe connected to a tube containing cotton and replaced with the same amount of fresh medium. Samples were filtered through Whatman filter paper and concentration was determined using UV-Spectrophotometer (Lab India Analytical UV 3200). The study was performed in triplicate (Chhater and Praveen, 2013).

Solubility studies of selected solid dispersion formulation

Saturation solubility of carvedilol and solid dispersion was determined in 0.1 N HCl, pH 1.2, and distilled water. Excess amount of carvedilol and solid dispersion was added to 20 mL of each media. Samples were shaken for 24 hrs at room temperature in an orbital shaker bath (Remi RSB-12). Samples were filtered through Whatman filter paper. The filtrate was suitably diluted and the absorbance was measured using UV spectrophotometer (Lab India Analytical UV 3200) (Sharma and Jain, 2010).

Powder x-ray diffraction analysis

The powder X-ray diffraction analysis (PXRD) analysis for pure drug, PVPK-30 and solid dispersion containing 1:5 ratio of drug: PVPK-30 was performed to check crystallinity by using X-ray diffractometer (Smart Lab 9 kW by Rigaku).

Preparation of microspheres

The ionotropic gelation method was used for preparation of sodium alginate microspheres incorporated with solid dispersion. The detailed procedure is explained in the flow chart given below (Chhater and Praveen, 2013).

Required amount of sodium alginate was dissolved in distilled water.

↓

Solid dispersion was dispersed in sodium alginate solution.

↓

Required amount of calcium chloride was dissolved in distilled water in a separate beaker.

↓

Sodium alginate solution containing solid dispersion was dropped into calcium chloride solution using 23 gauge needle.

↓

Microspheres were filtered, washed with distilled water and air dried for 24 hrs.

Optimisation of sodium alginate microspheres using 3^2 full factorial design

Trials were done to obtain the concentration range of sodium alginate and calcium chloride. A two factor with three levels, full factorial design was applied for the optimization of microspheres incorporated with solid dispersion (Kshirsagar *et al.*, 2011).

The concentration of sodium alginate (Y_1) and concentration of calcium chloride (Y_2) were chosen as independent variables. These variables were varied at three levels, low level (-1), medium level (0), high level (+1). Percent entrapment efficiency and percent drug release were selected as dependent variables to optimize the response data. The levels of the process parameters are given in Table 1. The coded and actual values of the batches prepared by applying 3^2 full factorial design are given in Table 2.

Table 1. Factors and factor levels of factorial design

Independent variables	Level		
	-1	0	+1
X_1= Concentration of sodium alginate (%)	1	1.5	2
X_2 = Concentration of calcium chloride (%)	5	7.5	10
Dependent variables			
Y_1 = % Entrapment efficiency			
Y_2 = % Drug release			

From the factorial design run formulae all the formulations were prepared and assessed for entrapment efficiency and drug release studies for the period of 6 hrs. All the results were evaluated to acquire the optimized formulation of microspheres.

Evaluation of *microspheres*

Entrapment Efficiency

The entrapment efficiency of the microspheres was measured indirectly by determining the amount of unentrapped drug, by measuring the concentration of drug in the preparation medium and in the washing solutions spectrophotometrically at 242.6 nm after suitable dilutions with 0.1 N HCl, pH 1.2 (Patil *et al.*, 2009). The entrapment efficiency was calculated according to the formula given in Eq. 2.

$$\text{Entrapment efficiency} = 1-\left\{\frac{\text{Amount of unentrapped drug}}{\text{Amount of added drug}}\right\}\times 100 \quad \text{Eq. 2}$$

Percentage yield

Prepared microspheres were dried and weighed accurately. The percent yield of microspheres was determined using the following Eq. 3 (Kshirsagar *et al.*, 2011; Meshram *et al.*, 2016).

$$\text{Percentage yield} = \frac{\text{Weight of dry microspheres}}{\text{Weight of solid dispersion + weight of sodium alginate}}\times 100 \quad \text{Eq. 3}$$

Swelling index

The swelling index was determined by measuring the extent of swelling of the matrix in a solution of 0.1 N HCl, pH 1.2 at 37±0.5°C for 6 hrs. The swollen microspheres were weighed using an electronic weighing balance (Manubolu *et al.*, 2015). The swelling index was calculated using the following Eq. 4.

$$\text{Swelling index} = \frac{\text{Mass of swollen microspheres - Mass of dry microspheres}}{\text{Mass of swollen microspheres}}\times 100 \quad \text{Eq. 4}$$

Determination of particle size

The particle size of prepared microspheres was determined by optical microscopy using an ocular micro meter calibrated with stage micro meter. A minimum of 100 microspheres were counted for each formulation using a calibration factor (Manubolu *et al.*, 2015).

Scanning electron microscopy

The surface morphology of the optimized formulation of microspheres (OSM) was examined using a scanning electron microscope (SEM) (JIB 4700F FIB, JEOL Ltd., Tokyo, Japan) (Gadad *et al.*, 2016).

In-vitro dissolution of microspheres

The conditions for dissolution test given below:

Temperature: 37 ± 0.5°C

Dissolution media: 0.1 N Hydrochloric acid, pH 1.2

Time Interval: 30 min, 1, 2, 3,4,5,6 hours

Volume of dissolution media: 900 mL

Aliquot withdrawn: 5 mL

Aliquot replaced: 5 mL of fresh 0.1 N Hydrochloric acid Dissolution

Apparatus: USP type II (paddle)

Speed: 50 rpm

Table 2. Runs for 3-level factorial design

Run	Coded value		Actual value	
	Factor 1	Factor 2	Factor 1	Factor 2
	A: Conc. of sodium alginate	B: Conc. of calcium chloride	A: Conc. of sodium alginate (%)	B: Conc. of calcium chloride (%)
1	0	0	1.5	7.5
2	0	0	1.5	7.5
3	1	0	2.0	7.5
4	-1	1	1.0	10.0
5	-1	0	1.0	7.5
6	1	1	2.0	10.0
7	0	-1	1.5	5.0
8	-1	-1	1.0	5.0
9	1	-1	2.0	5.0
10	0	0	1.5	7.5
11	0	1	1.5	10.0

Table 3. Interpretation of FTIR spectra of excipients

Functional group	Observed frequencies (cm^{-1})		
	Carvedilol	Carvedilol + PVPK-30	Carvedilol + Sodium alginate
N-H and O-H Stretching	3345.27	3345.27	3345.27
C-H Stretching (Aromatic)	3056.96	3056.96	3056.96
C-H Stretching (Aliphatic)	2921.96	2920	2923.88
N-H Bending	1607.31	1608.2	1607.95
C=C Stretching (Aromatic)	1502.44	1502.44	1502.44
C-N Stretching	1255.57	1255.7	1255.57
C-O Stretching (Aryl alkyl and alkyl ether)	1097.42	1099.35	1096
$C=CH_2$ Bending	617.18	621.04	619.11

The concentration of carvedilol was measured using a double-beam UV spectrophotometer (Lab India Analytical 3200) at a λ_{max} value of 242.6 nm.

Kinetic mathematical modelling of drug release profile

To understand the mechanism and kinetics of drug release from the formulation, the *in-vitro* data was fitted into zero-order, first-order, Higuchi and Korsmeyer-Peppas models (Patil *et al.,* 2009; Miyazaki *et al.,* 1999; Miyazaki *et al.,* 2001).

Statistical analysis and optimization

Statistical optimization was performed using Design-Expert® software (version 11, Stat Ease, Inc., Minneapolis, MN).

Stability studies

The stability study of the optimised formulation was carried out under different conditions according to ICH guidelines. The optimized microspheres were stored in a stability chamber for stability studies. Accelerated stability studies were carried out at 40°C /75% RH for the optimised microspheres for 3 months. The microspheres were characterized for the percentage yield, entrapment efficiency and % drug release (Kumar and Suresh, 2018).

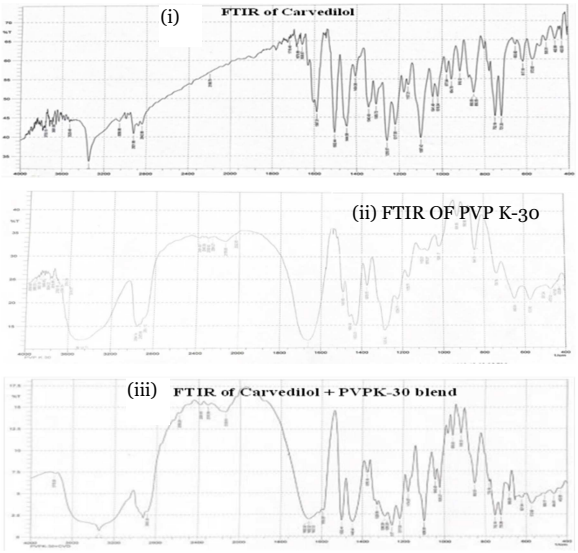
Results and discussion

Drug-excipient compatibility

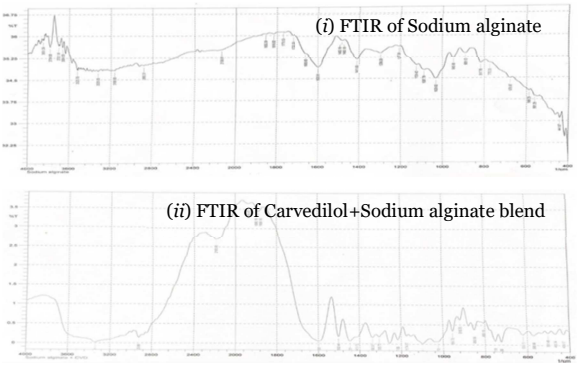
Fourier transform infra-red spectroscopy

The FTIR spectra of carvedilol, individual excipients and combination of carvedilol with excipients are represented in Fig. 1 (i) and (ii) and the interpretation of spectra is given in Table 3.

The FTIR spectrum of drug shows characteristics peaks in close agreement with the standard reference as per IP, indicating carvedilol with high purity. The FTIR spectrum of carvedilol showed typical peaks at 3345.27 cm^{-1} of N-H and O-H stretching (secondary amine) vibrations combined together, at 2921.96 cm^{-1} of aliphatic C-H stretching, N-H bending at 1607 cm^{-1}. Aromatic C=C stretching at 1502.44 cm^{-1}, at 1097.42 cm^{-1} of C-O stretching (aryl alkyl ether and alkyl ether).



(i): FTIR Spectra of (*i*) Carvedilol, (*ii*) PVPK-30, (*iii*) Carvedilol+PVPK-30 blend



(ii): FTIR Spectra of (i) Sodium alginate, and (ii) Carvedilol + sodium alginate blend

Fig. 1. FTIR Spectra

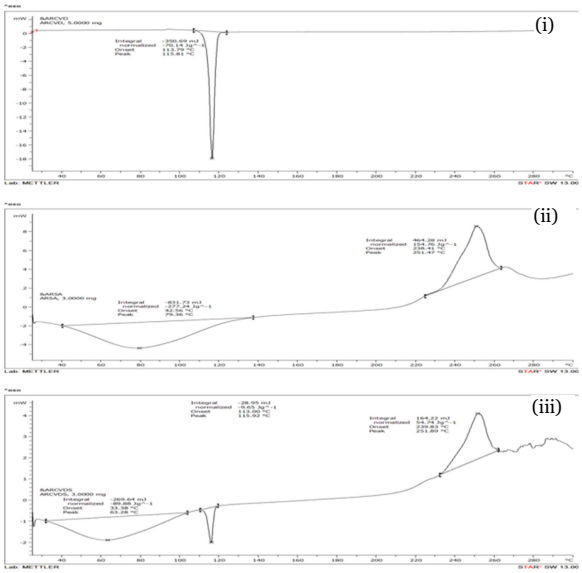


Fig. 2. DSC thermogram of (i) Carvedilol, (ii) Sodium alginate, and (iii) Carvedilol + sodium alginate blend.

Both the drug and excipient peaks were identified and interpreted in the FTIR spectra, which show no evidence of drug-excipient interaction. The spectra demonstrated that the drug and polymers did not interact chemically in any way. Thus, carvedilol is not functionally altered and is compatible with the excipients.

Diffraction scanning calorimetry

DSC thermograms are illustrated in Fig. 2. The pure carvedilol showed a prominent endothermic peak at a temperature of 115.81°C, subsequent to its characteristic melting/transition temperature. The sharpness and high intensity of the peak indicated the purity and high crystallinity of carvedilol. The sodium alginate DSC thermogram showed a wide endothermic peak at 79.8°C and an exothermic peak at 251.47°C. The exothermic peak of sodium alginate relates to its thermolysis, while the endothermic peak is due to the loss of water and moisture content of the polysaccharide. In the thermogram of the physical mixture of carvedilol and sodium alginate, a sharp peak of carvedilol was observed at 115.92°C, a wide endothermic peak of sodium alginate was observed at 63.8°C and an exothermic peak at 251.89°C indicating absence of incompatibility.

Evaluation of formulated solid dispersions

Carvedilol solid dispersions were evaluated for percent yield, drug content, *in-vitro* drug release and solubility.

Percent yield and drug content

The percent yield and drug content of formulated solid dispersions were determined and given in Table 4. The percent yield was found to increase with an increase in the concentration of PVPK-30.

Table 4. Percent yield and drug content of solid dispersions

Formulation	% yield	Drug content (%)
SD1	94.25 ± 0.1	95.06 ± 0.011
SD2	96.90 ± 0.01	92.30 ± 0.02
SD3	97.66 ± 0.02	95.61 ± 0.001

All the values are expressed as mean±SD, n=3

Table 5. *In-vitro* dissolution of carvedilol and solid dispersion

Time (min)	Cumulative % drug release			
	Pure drug	SD1	SD2	SD3
2	4.96 ± 0.01	22.95 ± 0.05	50.185 ± 0.01	76.062 ± 0.03
4	7.15 ± 0.02	27.138 ± 0.01	55.892 ± 0.02	90.742 ± 0.04
6	10.55 ± 0.05	31.642 ± 0.03	60.724 ± 0.1	99.98± 0.01
8	15.05 ± 0.2	33.183 ± 0.1	64.99 ± 0.07	-
10	25.49 ± 0.02	38.082 ± 0.09	68.043 ± 0.1	-
15	29.82 ± 0.5	44.604 ± 0.2	70.289 ± 0.01	-
30	31.36 ± 0.01	48.16 ± 0.03	75.876 ± 0.05	-

In-vitro dissolution of solid dispersion

The dissolution data of the formulation is given in Table 5. The dissolution profile was plotted as depicted in Fig. 3. It is evident that solid dispersions exhibit a faster dissolution rate than the free drug. Solid dispersion SD3 consisting of 1:5 ratio of drug: PVPK-30 showed 99.98% drug release within 6 mins and was considered for further studies.

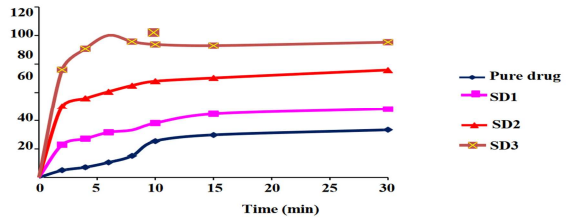


Fig. 3. *In-vitro* dissolution profile of solid dispersions

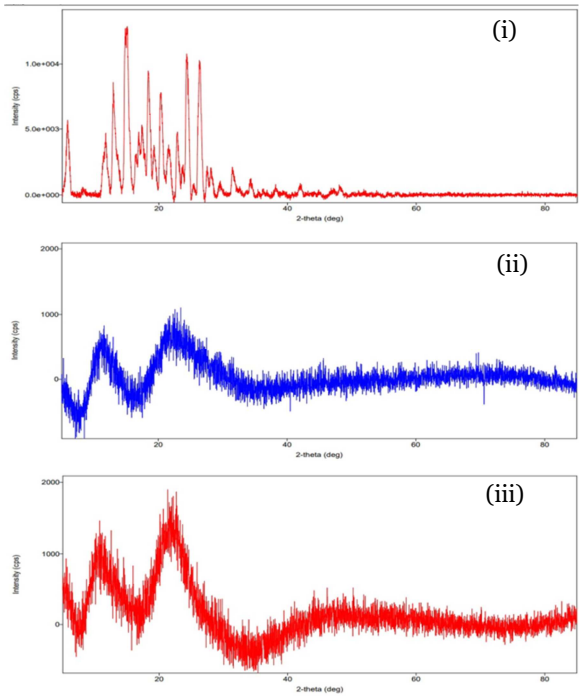


Fig. 4. X-ray diffractograms of (i) Carvedilol, (ii) PVPK-30, and (iii) SD3 formulation

Solubility studies of selected solid dispersion formulation

Solubility of the formulation SD3 was determined in distilled water and 0.1 N HCl, pH 1.2, and compared with that of pure drug. Formulation SD3 showed 79.4 folds enhancement in aqueous solubility and 15.3 folds enhancement in solubility in 0.1 N HCl.as shown in Table 6.

Table 6. Solubility of carvedilol and SD3 in distilled water and 0.1N HCl, pH 1.2

Media		Solubility (mg/mL)
Distilled water	Pure drug	0.0191± 0.0006
	SD3	1.508 ±0.4
0.1 N HCl, pH 1.2	Pure drug	0.439 ±0.0431
	SD3	6.711±0.31

Powder x-ray diffraction analysis

The powder X-ray diffraction analysis was conducted for carvedilol, hydrophilic polymer PVPK-30, and SD3 formulation as displayed in Fig. 4.

The XRD pattern of pure carvedilol demonstrated a series of intense diffraction peaks at 2θ of 12.81°, 15.61°, 17.46°, 18.57°, 20.12°, 24.32°, 26.20° which match with the reported profile and are indicative of the crystallinity of carvedilol. The PXRD of polymer PVP K30 revealed only halo (amorphous) regions with no major intense peaks, indicating that the polymer is amorphous.

X-ray diffraction patterns of SD3 formulation revealed undefined, broad peaks with lower intensity. The formulation exhibits a decrease in reflection intensities. The peak of decreased intensity indicates decreased crystallinity of the drug and a change in its nature to an amorphous form, resulting in improved drug solubility. Therefore, the formulation SD3 showing improved solubility of the drug is used for further study.

Table 7. Evaluation of microspheres

Formulation	% Entrapment efficiency	% Yield	Particle size (µm)	% Swelling index
MS1	85.387 ± 0.1	99.568 ± 0.1	808.34	60 ± 0.1
MS2	85.387 ± 0.01	99.568 ± 0.01	808.34	60 ± 0.1
MS3	87.06 ± 0.01	89.344 ± 0.01	847.47	54.4 ± 0.2
MS4	86.281 ± 0.02	97.463 ± 0.02	844.7	70 ± 0.09
MS5	83.665 ± 0.01	98.439 ± 0.11	788.79	75 ± 0.11
MS6	91.12 ± 0.2	91.253 ± 0.01	804.67	66 ± 0.02
MS7	80.146 ± 0.11	87.886 ± 0.2	754.83	64 ± 0.1
MS8	79.83 ± 0.3	94.588 ± 0.01	718.9	77 ± 0.03
MS9	83.665 ± 0.1	98.360 ± 0.01	720.65	52 ± 0.05
MS10	85.387 ± 0.04	99.568 ± 0.03	808.34	60 ± 0.1
MS11	88.2 ± 0.2	95.642 ± 0.2	860.73	40 ± 0.01

Table 8. Fit summary of responses

Source	Sequential p-value	Lack of Fit p-value	Adjusted R^2	Predicted R^2	
Response 1: Entrapment efficiency					
Linear	< 0.0001		0.9638	0.9428	Suggested
2FI	0.4584		0.9620	0.9325	
Quadratic	0.1762		0.9735	0.8856	
Cubic	0.3650		0.9774	0.1387	Aliased
Response 2: Drug release					
Linear	0.2585		0.1087	-0.7149	
2FI	0.3147		0.1275	-2.4948	
Quadratic	0.0031		0.8796	0.5591	Suggested
Cubic	0.7769		0.8304	-5.4654	Aliased

Table 9. Fit Statistics of responses

Response 1: Entrapment efficiency			
Std. Dev.	0.6242	R^2	0.9711
Mean	85.10	Adjusted R^2	0.9638
C.V. %	0.7335	Predicted R^2	0.9428
		Adeq Precision	34.7976
Response 2: Drug release			
Std. Dev.	1.90	R^2	0.9398
Mean	67.75	Adjusted R^2	0.8796
C.V. %	2.81	Predicted R^2	0.5591
		Adeq Precision	12.9069

Table 10. ANOVA Summary of the responses

ANOVA for linear model						
Response 1: Entrapment Efficiency						
Source	Sum of Squares	Degree of freedom	Mean Square	F-value	p-value	
Model	104.65	2	52.33	134.30	< 0.0001	Significant
A-Conc. of Sodium alginate	24.28	1	24.28	62.31	< 0.0001	
B-Conc. of $CaCl_2$	80.37	1	80.37	206.29	< 0.0001	
Residual	3.12	8	0.3896			
Lack of Fit	3.12	6	0.5195			
Pure Error	0.0000	2	0.0000			
Cor Total	107.77	10				
ANOVA for quadratic model						
Response 2: % Drug release						
Model	282.92	5	56.58	15.61	0.0045	Significant
A-Conc. of Sodium alginate	68.01	1	68.01	18.76	0.0075	
B-Conc. of $CaCl_2$	18.37	1	18.37	5.07	0.0742	
AB	30.80	1	30.80	8.50	0.0332	
A^2	12.74	1	12.74	3.51	0.1198	
B^2	120.34	1	120.34	33.19	0.0022	
Residual	18.13	5	3.63			
Lack of Fit	18.13	3	6.04			
Pure Error	0.0000	2	0.0000			
Cor Total	301.05	10				

Evaluation of microspheres

Microspheres were evaluated for entrapment efficiency, percent yield, particle size, *in-vitro* drug release, and swelling index. The results of all the evaluation parameters are shown in Table 7.

In-vitro dissolution of microspheres

The dissolution profile of the formulations is represented in Fig. 5 (*i*) and (*ii*). The microspheres matrix is having swellable polymer, after addition of them to 0.1N HCl, drug available on the surface of matrix will be release immediately before the imbibition of water. Hence, burst release of around 30% was observed.

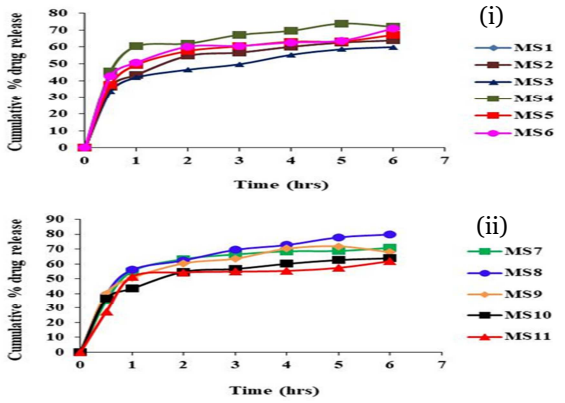


Fig. 5. *In-vitro* dissolution profile of sodium alginate microspheres (*i*) (MS1-MS6), (*ii*) MS7-MS11

After the swelling of the matrix the release was at moderate rate due to diffusion of the matrix followed by sustain release.

The cumulative drug release from the microspheres in six hours was in the range 61.87%±0.02 to 79.83%±0.06. Initially there was high drug release (burst effect) followed by moderate release.

Statistical analysis and optimization

To evaluate responses, a statistical model with interactive and polynomial terms was used. Using the Design-Expert® software, the responses observed for eleven runs were simultaneously fitted to linear, interaction, and quadratic models. The values of R^2, Adjusted R^2, Predicted R^2, % CV, and significant P values ($p<0.05$) were compared.

Fit summary of responses

The Table 8 shows fit summary for responses. For response 1 i.e., entrapment efficiency, fit summary suggested a linear model and the cubic model was aliased. Adjusted R^2 was 0.9638 and Predicted R^2 was 0.9428. For response 2 i.e., drug release, fit summary suggested quadratic model and cubic model was aliased. Adjusted R^2 was 0.8796 and predicted R^2 was 0.591.

Fit statistics

Response 1 (Entrapment efficiency): The Predicted R^2 of 0.9428 is in reasonable agreement with the Adjusted R^2 of 0.9638; i.e. the difference is < 0.2.

The signal to noise ratio is measured by Adeq Precision. A ratio >4 is desirable. Ratio of 34.798 indicates an adequate signal. Therefore, this model is used to navigate the design space.

Response 2 (Drug release): The Predicted R^2 of 0.5591 is not as close to the Adjusted R^2 of 0.8796 as expected; i.e., the difference is > 0.2. This may indicate a large block effect. Things to consider are model reduction, response transformation, outliers, etc. Adeq Precision ratio of 12.907 indicates an adequate signal. Therefore, this model is used to navigate the design space as shown in Table 9.

ANOVA summary

The ANOVA summary for response parameters for 3^2 full factorial designs for sodium alginate microspheres is given in Table 10. The *p*-value for entrapment efficiency and drug release were found to be <0.0001 and 0.004 respectively, which is <0.05 indicating the significance of model terms.

Response 1: The model is apparently significant as the Model F-value is 134.30. There is only a 0.01% chance that an F-value this large could be due to noise. A, and B are significant model terms in this case as P-value is <0.05.

Response 2: The model is apparently significant as the Model F-value is 15.61. There is only a 0.45% chance that an F-value this large could be due to noise. A, AB, B^2 are significant model terms in this case.

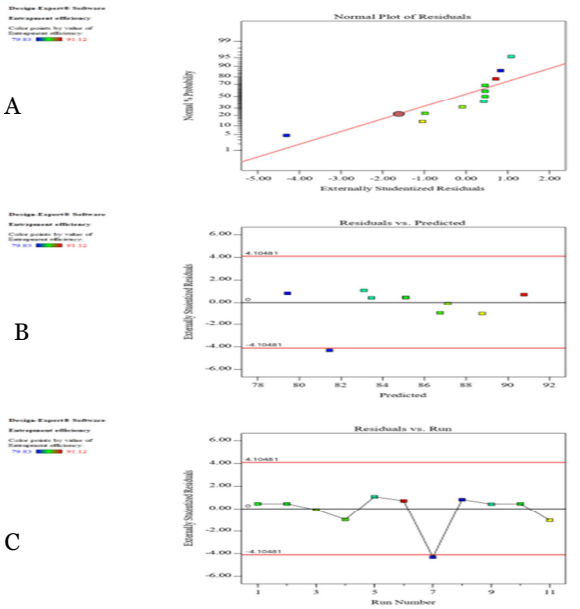


Fig. 6. (i): Entrapment Efficiency A) Normal probability plot of the residuals, B) Residuals vs predicted response plot, C) Studentized residuals vs run number plot

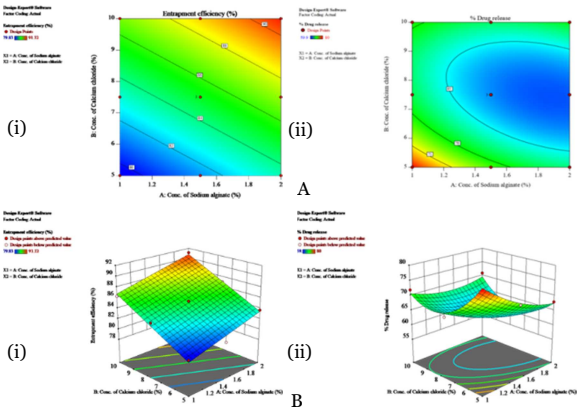


Fig. 7. A) 2D Counter plot and B) 3D response surface plot showing influence of X1 and X2 on (i) % entrapment efficiency, (ii) % drug release

Diagnostic analysis of formulation characteristics

The model's adequacy can be confirmed further by using the diagnostic plots provided by the software. Fig. 6 (i) and (ii) show the diagnostic plots for the two response parameters.

The normal probability vs studentized residual plot demonstrated a cluster of points around the straight line, indicating that the errors are distributed normally for all the responses and the normality assumption was satisfied. Using residual vs predicted response plot, the constant variance assumption was tested. There was no discernible pattern, and the residual was dispersed randomly. Thus, the assumption of variance homogeneity was fulfilled for this work. Therefore, the models were accurate in describing their respective responses.

Response surface analysis

To determine the effect of independent variables on dependent variables, 2D contour plot and 3D response surface analysis were done using Design-Expert® software.

Effect on entrapment efficiency

2D counter plot for effect on entrapment efficiency is presented in Fig. 7 (*A*) (*i*). From the plot it can be perceived that as the level of X_1 (conc. of sodium alginate) and X_2(conc. of calcium chloride) was increased the % entrapment efficiency increased from 80% to 90%.

3D response surface plot for the effect on entrapment efficiency is shown in Fig. 7 (*B*) (*i*). The plot was flat indicating a linear relationship between X_1 and X_2 with no interactions between the variables.

Effect on % drug release

2D counterplot for the effect on drug release is shown in Fig. 7 (A) (ii). From the plot, it can be seen that as the level of X_1 (conc. of sodium alginate) and X_2 (conc. of calcium chloride) was increased the % drug release decreased from 75% to 65%.

3D plot for effect on drug release Fig. 7 (B) (ii) was curved showing a non-linear relationship between X_1 and X_2. There was a decrease in drug release with an increase in conc. of X_1 and X_2 and then increased up to some extent with a further increase in X_1 and X_2. It was observed that the maximum drug release at 6 hrs was 79.85% with a minimum concentration of X_1 and X_2.

Validation of 3^2 full factorial designs

The optimum variables were obtained by the numerical analysis depending on the criterion of

desirability. Predicted and actual responses of all the formulations are noted in the form of a graph (Fig. 8 (i) and (ii)). As the outcome of actual responses is close to the predicted responses, it is proved that the design applied has significantly fitted the data and thus the design is validated.

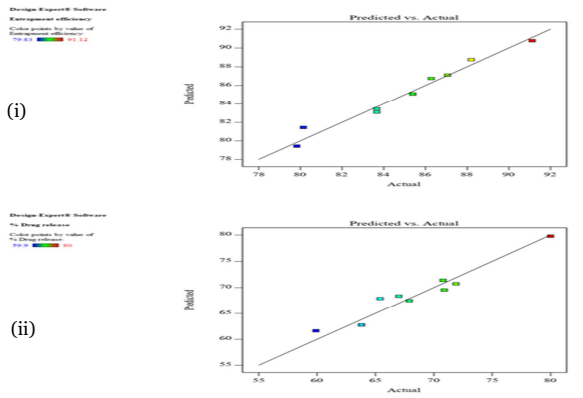


Fig. 8. Predicted vs actual responses for (*i*) Entrapment efficiency and (*ii*) % drug release

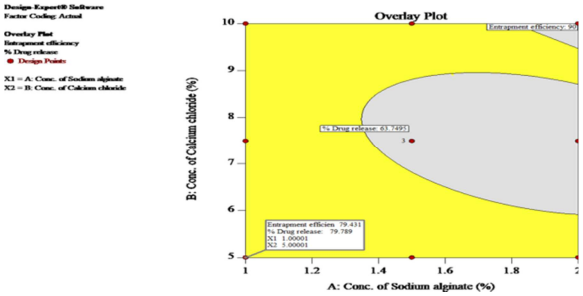


Fig. 9. Overlay plot for optimized formulation (OMS)

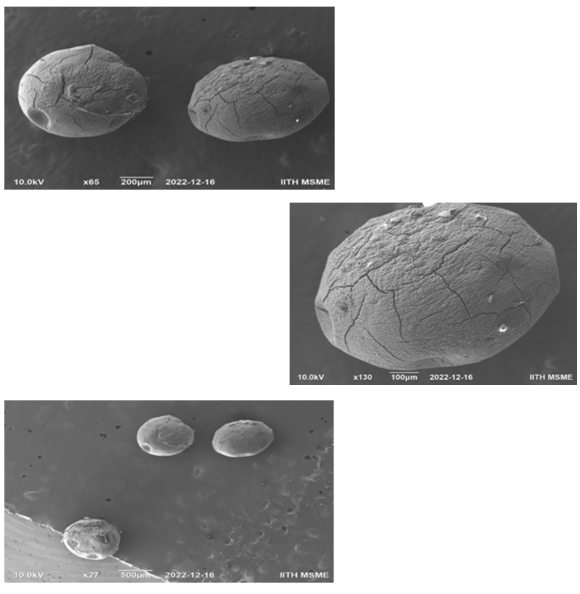


Fig. 10. SEM images of OMS formulation

Optimized formulation

The optimized microsphere formulation was obtained by applying constraints on independent variables. Constraint for the % entrapment efficiency was in the range of 79-90% and the % drug release was targeted for 79% at 6 hrs. Fig. 9 shows the suggested optimized formulation code as X1=1.000% and X2=5.000%. The values of predicted responses of R1 and R2 were 79.43% and 79.78% respectively. From the graph, the predicted formulations' composition matched that of MS8 formulation. The experiment was repeated to reconfirm the result and validate the design.

The overlay plot (Fig. 9) gives the regions not meeting the specifications as greyed out, leaving an operating window or sweet spot in yellow color.

Characterization of optimized microsphere formulation

Entrapment efficiency, percent yield and swelling index were found to be 80.89%, 87.886%, 77% respectively. Particle size was found to be 718.9 µm.

Scanning electron microscopy

Scanning electron microscopy (SEM) images of optimized batch of microspheres (OMS) were taken to acquire the topographical information about the formulation. Fig. 10 revealed that the formulated sodium alginate microspheres are spherical in appearance and have rough surfaces.

In-vitro dissolution of optimized microspheres

The in-vitro dissolution profile of the optimized microspheres (OMS) is shown in Fig. 11 and drug release was found to be 78.67% at 6 hrs.

Model dependent kinetics

Model-dependent kinetics was done for all the 11 formulations obtained by the design to determine the release kinetics, release mechanism, and drug transport mechanism. The optimized formulation OMS follows first-order drug release kinetics (R^2 =0.9243). From the value of the release component (n=0.258), it can be presumed that the mechanism of drug release from OMS formulation was Fickian diffusion.

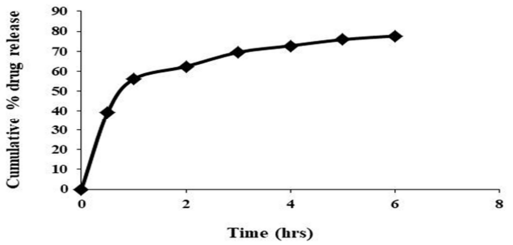


Fig. 11. *In-vitro* dissolution profile of OMS formulation

Stability studies

From the results (Table 11) it was concluded that, the optimised formulation was stable and retained the original properties.

Table 11. Stability studies of optimized formulation (OMS)

Retest time of optimised formulation (days)	% Entrapment efficiency	% Drug release
0	80.89 ± 0.3	78.67 ± 0.01
30	79.96 ± 0.05	77.54 ± 0.06
60	79.62 ± 0.2	78.28 ± 0.02
90	78.36 ± 0.04	78.45 ± 0.01

Conclusion

In the present work, Carvedilol being a BCS class II drug, the solubility was enhanced by formulating into solid dispersion which was further incorporated into sodium alginate microspheres to prolong the drug release and optimized using 3^2 full factorial design. Formulation SD3 consisting of 1:5 ratio of drug: PVPK-30 showed 15.3 folds enhancement in solubility compared to pure drug, which was incorporated into sodium alginate microspheres, prepared using ionic gelation method and optimized by 3^2 full factorial design. For optimization X1 (concentration of sodium alginate), X2 (concentration of calcium chloride) were considered as factors and Y1 (% EE) and Y2 (Drug release at 6 hrs) as responses. The optimized formulation obtained has showed % EE of 79.41% and 80% of drug release at 6 hrs. The particle size of OMS was 718.9 µm and swelling index was 77%. SEM images showed that the OMS were spherical in shape with rough surface.

References

Adepu S, Ramakrishna S. 2021. Controlled drug delivery systems: Current status and future directions. Molecules **26**(19), 5905.

Chein YW. 1992. Novel drug delivery systems. Marcel Dekker Inc., New York, 13.

Chhater S, Praveen K. 2013. Solid Dispersion Incorporated Microcapsules: Predictive Tools for Improve the Half Life and Dissolution Rate of Pioglitazone Hydrochloride. American Journal of Biomedical Research **1**(3), 57-70. DOI: 10.12691/ajbr-1-3-3.

Gadad AP, Naik SS, Dandagi PM, Bolmal UB. 2016. Formulation and evaluation of gastroretentive floating microspheres of lafutidine. Indian Journal of Pharmaceutical Education and Research **50**(2), S76-S81. DOI: 10.5530/ijper.50.2.21

Kshirsagar SJ, Patil SV, Bhalekar MR. 2011. Statistical optimization of floating pulsatile drug delivery system for chronotherapy of hypertension. International journal of pharmaceutical investigation **1**(4), 207–213.
https://doi.org/10.4103/2230-973X.93005

Manubolu K, Sujatha B, Sreenivasulu M, Kumar SR, Kumar SN, Rao KB. 2015. Formulation and in vitro evaluation of gliclazide microbeads. World Journal of Pharmaceutical Research **4**(5), 1970-1982.

Meshram BMS, Mishra A, Pathak A. 2016. Formulation and Evaluation of Microsphere of Rabeprazole Sodium. International Journal of Advances in Pharmaceutics **5**(3), 76-83.
DOI: 10.7439/ijap

Miyazaki S, Aoyama H, Kawasaki N, Kubo W, Attwood D. 1999. In situ-gelling gellan formulations as vehicles for oral drug delivery. Journal of Controlled Release **60**(2), 287–295.
DOI: 10.1016/S0168-3659(99)00084-X

Miyazaki S, Kawasaki N, Kubo W, Endo K, Attwood D. 2001. Comparison of in situ gelling formulations for the oral delivery of cimetidine. International journal of pharmaceutics **220**(1-2), 161–168.
https://doi.org/10.1016/s0378-5173(01)00669-x

Patil SV, Lade PD, Janugade BU, Babar SA, Ghewade YB. 2009. Effect of Concentration of Gellan Gum and Calcium Chloride Solution on Entrapment Efficiency and Drug Release from Calcium Gellan Beads. Research Journal of Pharmacy and Technology **2**(4), 862-865.

Kumar KR, Suresh G. 2018. Development and characterization of alginate microspheres containing olmesartan by ionotropic gelation method. International Journal of Pharmaceutical Science and Drug Research **10**(4), p.335-341.
DOI: 10.25004/IJPSDR.2018.100420

Remington JP. 2021. Remington: The science and practice of pharmacy (Vol. 1). Lippincott Williams & Wilkins.

Sharma A, Jain CP. 2010. Preparation and characterization of solid dispersions of carvedilol with PVP K30. Research in Pharmaceutical Sciences **5**(1), 49–56.

Siddiqui MN, Garg G, Sharma PK. 2011. A Short Review on 'A Novel Approach in Oral Fast Dissolving Drug Delivery System and Their Patents'. Advances in Biological Research **5**(6), 291-303.

Sompur CK, Doijad RC, Patil SM, Maske AP. 2011. An approach for development of oral sustained release suspension. International Journal of Pharma and Bio Sciences **2**(2), 320–329.

Verma P, Thakur AS, Deshmukh K, Jha AK, Verma S. 2010. Routes of drug administration. International Journal of Pharmaceutical Studies and Research **1**(1), 54-59.

Yuvaraja K, Khanam J. 2014. Enhancement of carvedilol solubility by solid dispersion technique using cyclodextrins, water soluble polymers and hydroxyl acid. Journal of Pharmaceutical and Biomedical Analysis **96**, 10-20.
DOI: 10.1016/j.jba.2014.03.019.