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Central composite design enabled formulation development and characterization of carvedilol polymeric nanoparticles by nanoprecipitation technique for the improved drug solubility

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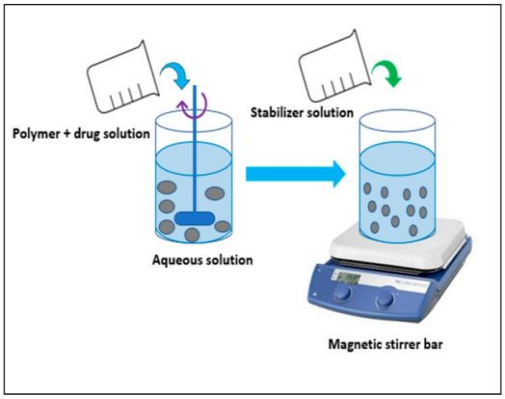
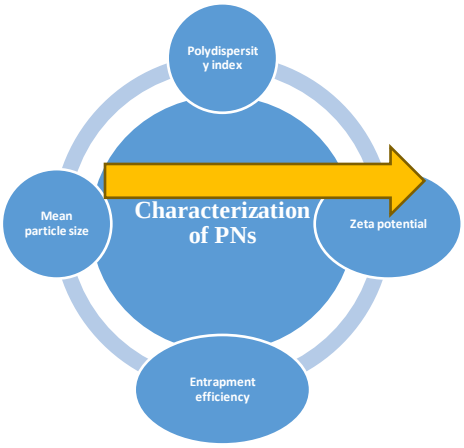
GRAPHICAL ABSTRACT:





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Abstract

~~Carvedilol is a poorly water-soluble anti-hypertensive BCS class-II drug used to prepare a polymeric nanoparticle by nanoprecipitation technique using selected polymers such as chitosan and HPMC K15M and poloxamer 407 as a surfactant for the improvement of drug solubility through the release of drug in a sustained manner.~~ The initial process and formulation variables are screened out based on the selected critical quality attributes such as drug release (%), entrapment efficiency (%), particle size (nm), and zeta potential (mV). The FT-IR and DSC studies reveal that the model drug has no interactions and sharp melting points with the polymers and does not show any additional peaks. The prepared drug-loaded polymeric nanoparticles were characterized for particle size, zeta potential, entrapment efficiency, and in-vitro drug release. The drug release and stability studies indicated that the F6 formulation was more stable, with improved drug solubility and good drug entrapment efficiency along the formation of the nanosized polymeric formulation. The correlation coefficient data and Korsmeyer Pappa's release exponent values showed the formulations followed diffusion-controlled drug release non-Fickian mechanism and Higuchi kinetics. The stability study indicates that the optimized formulation (F6) is more stable for up to 6 months without changes in drug entrapment efficiency and in vitro dissolution rate.

Keywords: Polymeric nanoparticles; nanoprecipitation; HPMC K15M; poloxamer 407; solubility; *In-vitro* diffusion study

Short running title: Formulation design and characterization of carvedilol polymeric nanoparticles

Introduction

Nanoparticles (NPs) are particle sizes between 1 to 100 nanometers, similar to ultrafine particles. Nanoparticles could exist or not demonstrate size-related properties that vary knowingly from those observed in bulk materials and fine particles [1]. Colloidal particles with a 1 to 1000 nm size range are known as polymeric nanoparticles. They comprise pharmaceutically active chemicals incorporated or adsorbed into macromolecules [2]. Polymeric NPs impact the toxicity of oxidative stress, cytotoxicity, and genotoxicity due to the quantum size effect [3]. The nanoprecipitation method requires two miscible solvents. The internal phase consists of a polymer dissolved in a miscible organic solvent, like acetone or acetonitrile. Because of immiscibility in water, they can be easily removed by evaporation. The fundamentals of this method rely on polymer interfacial deposition following the displacement of the organic solvent from a lipophilic solution to the aqueous phase. The polymer is dissolved in a water-miscible solvent with intermediate polarity. The solution is gradually added to an aqueous solution while stirring (dropwise manner) or through a controlled addition rate [4]. The nanoparticles develop instantly in an attempt to escape the water molecules due to the fast spontaneous diffusion of the polymer solution into the aqueous phase. The polymer precipitates as nanocapsules or nanospheres as the solvent diffuses from the nanodroplets [5]. The organic phase is typically introduced to the aqueous phase; however, the technique can be reversed without impacting nanoparticle production. Usually, surfactants can be used to assure the stability of the colloidal suspension, but their presence is not essential for the production of nanoparticles. The resulting nanoparticles have a well-defined size and a restricted size distribution, superior to those produced by the emulsification solvent evaporation procedure [6, 7].

Polymeric nanoparticles that respond to internal or external stimuli are absorbed because they enable more efficient therapeutic delivery to diseased regions. Stimulus-sensitive polymeric nanoparticles have been created based on various nanostructures, such as micelles, vesicles, crosslinked nanoparticles, and hybrid nanoparticles [8]. Polymeric nanoparticles' chemical or

physical properties alter in response to single, dual, or multiple stimuli. This allows them to retain cargoes during circulation, target the diseased region, and release their contents following cell internalization [9].

Carvedilol is (RS)-I-(9H-carbazol-4-yloxy)-3-[[2-(2-methoxyphenoxy) ethyl] amino] propan-2-ol an antihypertensive drug sold under the brand name Coreg among others, is a medication used to treat high blood pressure, congestive heart failure, and left ventricular dysfunction in people who are otherwise stable. This drug shows an absolute bioavailability of approximately 25% to 35% due to a significant degree of first-pass metabolism and poor water solubility [10].

This study aimed to develop and optimize carvedilol-loaded polymeric nanoparticles prepared by the nanoprecipitation method to improve the drug solubility in a sustained release mechanism to minimize drug dosing intervals. However, there are other techniques for making nanoparticles, but the nanoprecipitation method is the most extensively used since it is the simplest and requires the fewest stages. To achieve this aim, the following objectives have been selected to carry out pre-formulation studies, including analytical method development to develop the polymeric nanoparticulate systems employing selected polymers to optimize the formulation variables or conditions and to characterize the prepared nanoparticulate formulations.

Materials and methods

Materials

Reddy's Laboratories, Hyderabad, India, provided gift samples of pure drug sample (Carvedilol) and other gift polymer samples such as chitosan, HPMC K 15M, Poloxamer 407 for the academic research purpose. Sigma Aldrich, India, provided acetone. Throughout the study, all essential ingredients used in the research have been of excellent quality.

Methods

Preformulation studies

Solubility Analysis

10 mg of the drug was dissolved in 9 ml of 0.1 N HCl, 1 ml of ethanol was added as a co-solvent, and the volume was increased to 10 ml. The quantitative solubility studies of the drug (Carvedilol) were carried out using different solvents, i.e., water, acetonitrile, phosphate buffer

6.8 and 7.4, 0.1 N HCl, methanol, ethanol, DMSO, PEG 200 and 400, n-octanol. First, 5 ml of each solvent was taken, and a minute amount of the drug was added to its saturation point. Then it was placed in a shaker for about 3 h. After that, the solubility of the drug was noticed for all the solvents. If it is entirely soluble, then again, 2 ml of each solvent was taken, and the drug was added up to its saturation point, which was placed in the shaker for 3 h. Then filtration was carried out for the respective solvents and analyzed under a UV spectrophotometer. The solubility analysis data of pure drugs in different solvents are shown in **Table-1 and Figure-1** [11].

Table-1. Solubility analysis data of pure drug in different solvents

Solvent Name	Dilution Factor (DF)	Absorbance (nm)	Concentration (µg/ml)	Concentration (mg/ml)
Water	100	0.333	14.833	1.483
Acetone	100	0.021	1.833	0.183
Phosphate buffer pH 6.8	100	0.019	1.750	0.175
Phosphate buffer pH 7.4	100	0.036	2.458	0.245
0.1N HCl	100	0.014	1.541	4789.092
Methanol	100	0.563	24.416	2.441
n-Octanol	100	0.167	7.916	0.791
PEG-200	100	0.365	16.166	1.616
PEG-400	10000	0.097	5	50
DMSO	10000	0.249	11.333	113.333
Ethanol	100000	0.570	24.708	2470.833

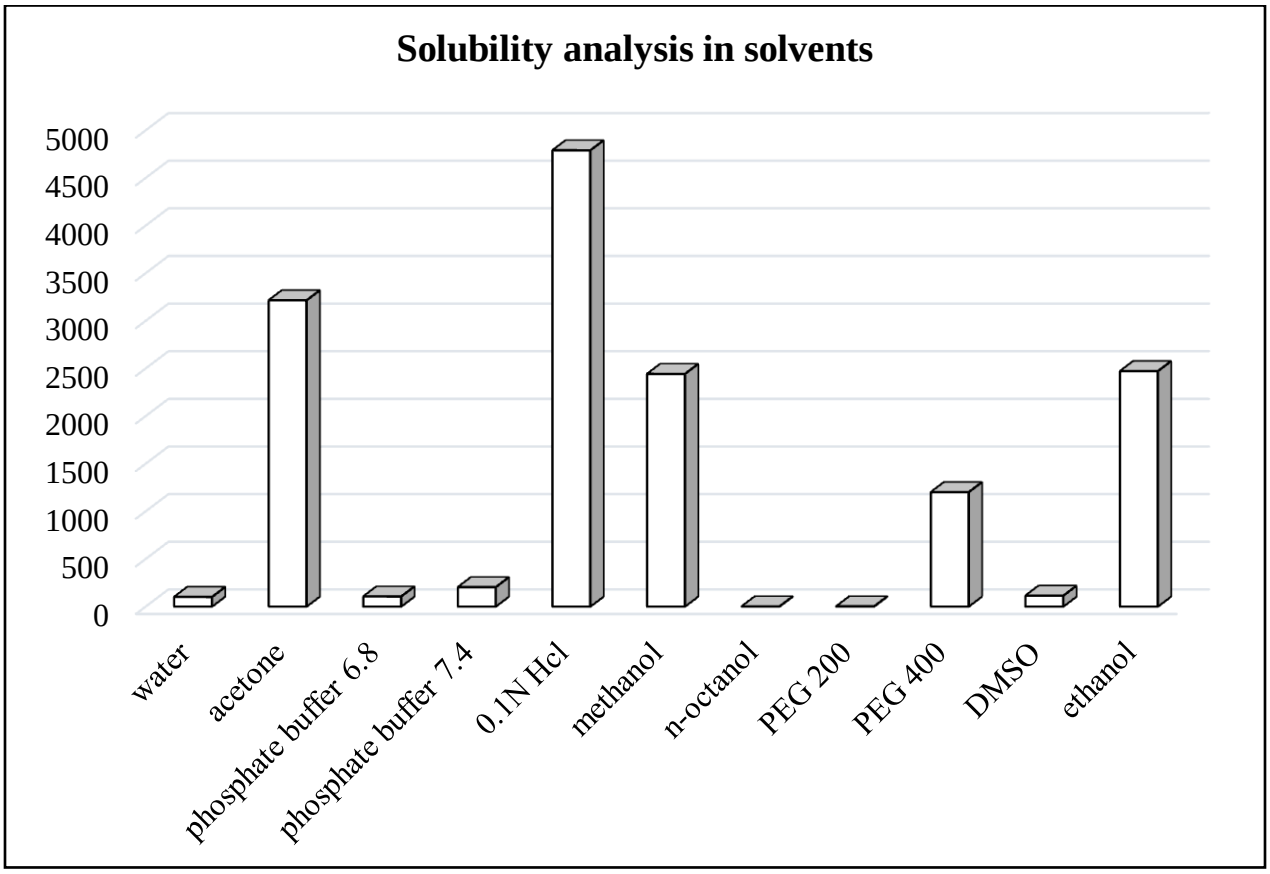

Figure 1. Bar graph showing solubility of pure drug in different solvents

Determination of λ_{max} of carvedilol

To prepare the stock solution, 100mg of carvedilol was dissolved with a mixture solvent of 90ml of 0.1N HCl and 10ml of ethanol. i.e., 1000 µg/liter. Then, 1 ml was taken and diluted to 100 µg /liter. From those, further dilutions were taken as follows 10 µg/ml, 20 µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml, and100 µg/ml. Finally, it was analyzed at λ_{max} 282nm by using the UV method.

Critical quality characteristics (CQAs) and the quality target product profile (QTPP)

In a broader sense, QTPP refers to a drug's predetermined anticipated characteristics, which are necessary to establish the product's intended performance concerning safety, and efficacy further to enable the recognition of product CQAs. The QTPP was determined based on regulatory and scientific requirements as listed in **Table-2**. QTPPs, which regulate the development of goods and processes, create CQAs. They are also coupled to in-process materials like critical material

attributes (CMAs) and process parameters like Critical Process Parameters (CPPs) in synthesizing nanomaterials [12].

Optimization by response surface methodology

It was optimized using Design Expert 12.1.1. (State-Ease Inc., Minneapolis, MN). Two independent factors were considered: drug: polymer ratio (A), Stabilizer concentration (B), and the additional impact of these individual variables on observed responses such as drug release (%), entrapment efficiency (%), particle size (nm), and zeta potential (mV). **Table-2** depicts the optimization design with the two components and three levels. The model described 13 different runs undertaken, and the responses for each run were documented. Finally, the composition with the optimal outcomes was chosen for future research.

Method of preparation of polymeric nanoparticles

The nanoparticles were fabricated according to the nanoprecipitation with solvent evaporation method. In the first step, the ratio of 1:1, 1:2, 1:3, i.e., the drug: polymer ratio of 20 mg drug:20mg polymer, 40 mg drug:80 mg polymer, and 60 mg of drug:180 mg of polymer, i.e., the polymer chitosan and HPMC K15M was dissolved in 5ml of glacial acetic acid and 5ml of acetone respectively. The pure drug carvedilol was dissolved separately in 10ml of acetone in both the solution, i.e., the polymeric solution, and the drug solution, i.e., polymeric solution and drug solution, were then mixed using a magnetic stirrer. From this, 20ml of the drug-polymer solution and 5ml of the solution was taken and added to distilled water containing 1%, 1.5%, and 2% of the stabilizer poloxamer 407 to obtain the final volume of 20 ml. The acetone was eliminated or evaporated under reduced pressure using a rotary flask evaporator, and the volume was adjusted to 10ml. Add the drug to the polymer solution slowly to dissolve the drug in the polymer solution. The drug and polymer were added slowly using a #27-gauge needle-size syringe with the addition rate, i.e., 1 ml/min. This process of addition should take 1hr at 1000 rpm. The 20ml of final volume obtained was then subjected to evaporation, i.e., rotary flask evaporator to remove the acetone. Finally, the volume was adjusted to 10ml. The prepared nanosuspension was subjected to freeze drying for about 36 h to obtain the dried powder form of polymeric nanoparticles.

Run	Factor 1	Factor 2	Response 1	Response 2	Response 3	Response 4

Table-2. Design matrix for the experimental runs as per the central composite design and their assigned codes to the formulation variables

	A: Drug: Polymer ratio (mg)	**B: Stabilizer concentration (gm %)**	**Drug release (%)**	**Entrapment efficiency (%)**	**Particle size (nm)**	**Zeta potential (mV)**
1	1	0	25.364	42.396	1025.35	-6.23
2	0	0	49.253	62.359	701.05	-10.6
3	0	0	44.225	60.145	733.68	-10.98
4	0	1	70.225	65.224	552.36	-16.88
5	1	-1	67.015	60.987	693.25	-13.94
6	-1	0	96.586	86.225	263.8	-24.2
7	0	0	40.115	56.229	759.25	-9.09
8	-1	-1	79.398	76.696	395.22	-18.09
9	-1	1	86.235	80.296	369.12	-20.45
10	0	0	39.266	54.631	796.22	-7.09
11	1	1	12.365	35.448	1329.35	-5.85
12	0	-1	76.314	72.336	452.01	-17.9
13	0	0	36.598	50.448	839.25	-6.9

Drug: polymer ratio (X1)	**Stabilizer concentration (X2)**
1:1 (-1) low	1% (-1) low
1:2 (0) mid	1.5% (0) mid
1:3 (1) high	2% (1) high

Characterization

Fourier transform infrared spectroscopy (FT-IR)

Triturate the solid (drug) with dry, finely powdered potassium bromide. The amount taken should be such that the weight of the substance per area of the disc. Insert a portion of the mixture in a special die and subject it under a vacuum to high pressure. Mount the resultant disc in a suitable holder. IR scans potassium bromide 45 times in 1.5 minutes. A blank spectra of air

backdrop was collected before capturing the sample spectrum. A pure drug sample, a pure polymer sample, and formulations, including the drug and the polymer, were scanned (**Figures-2 to 4**) [13].

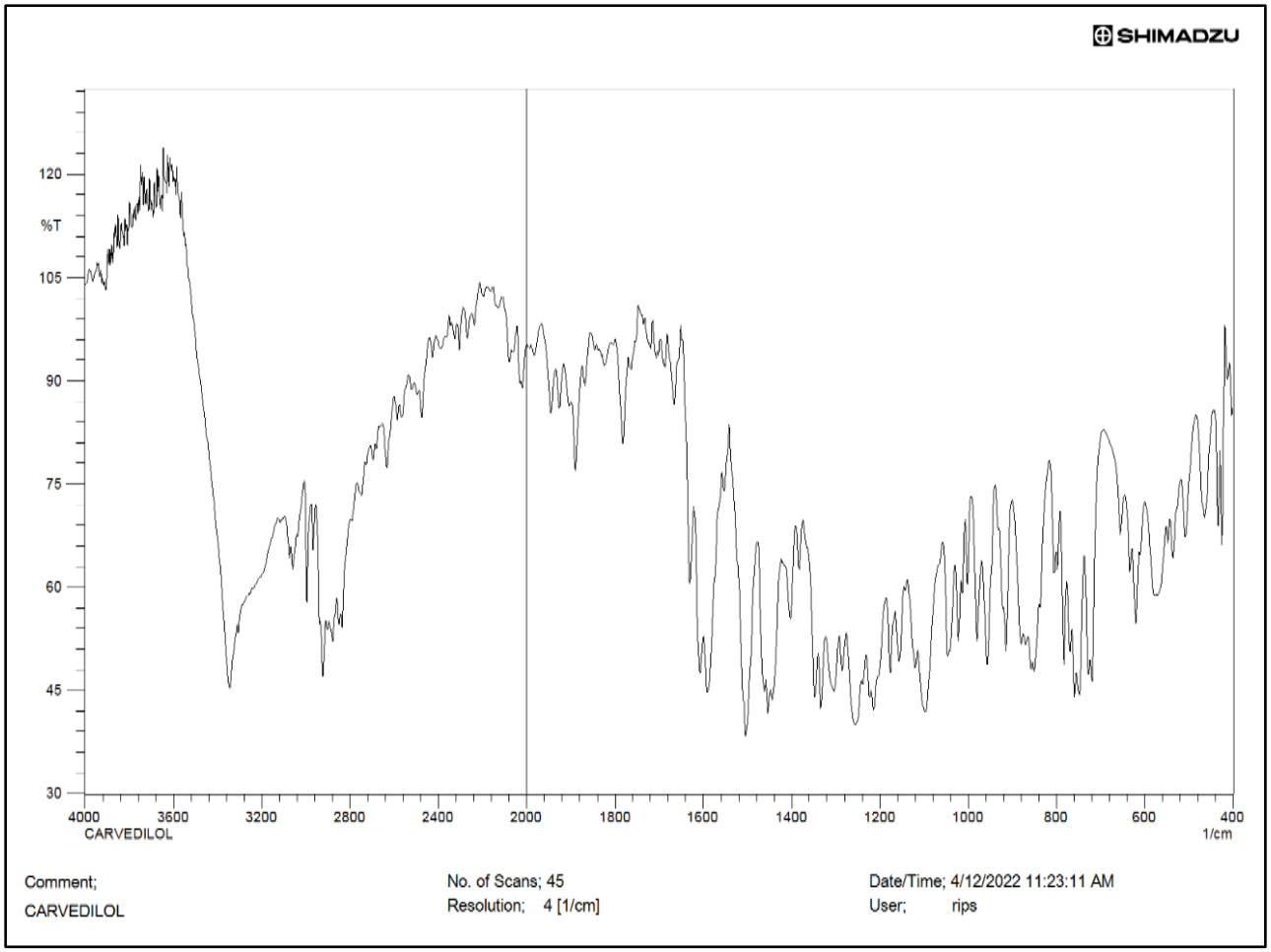

Figure-2. FT-IR spectra of carvedilol

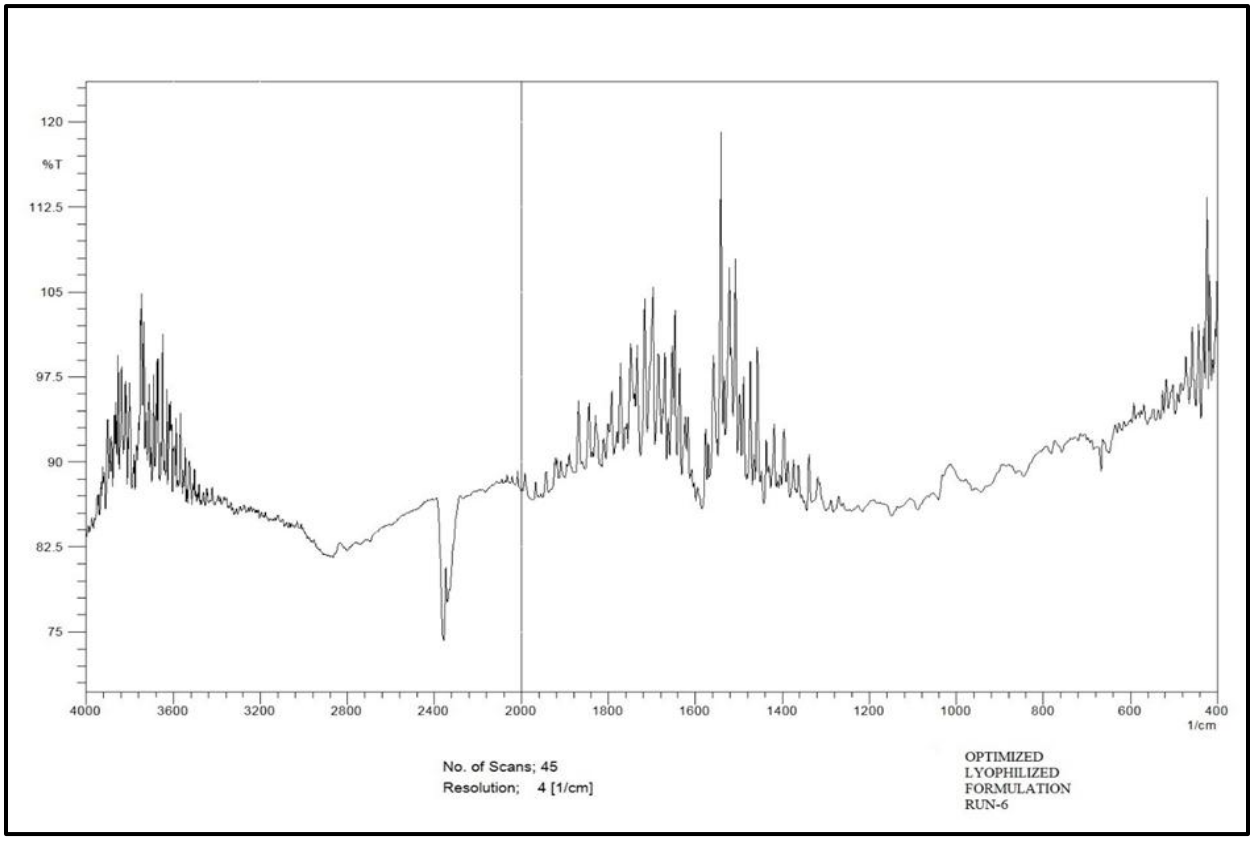

Figure-3. FT-IR spectra of physical mixture of carvedilol + poloxamer 407 + HPMC K15M + chitosan

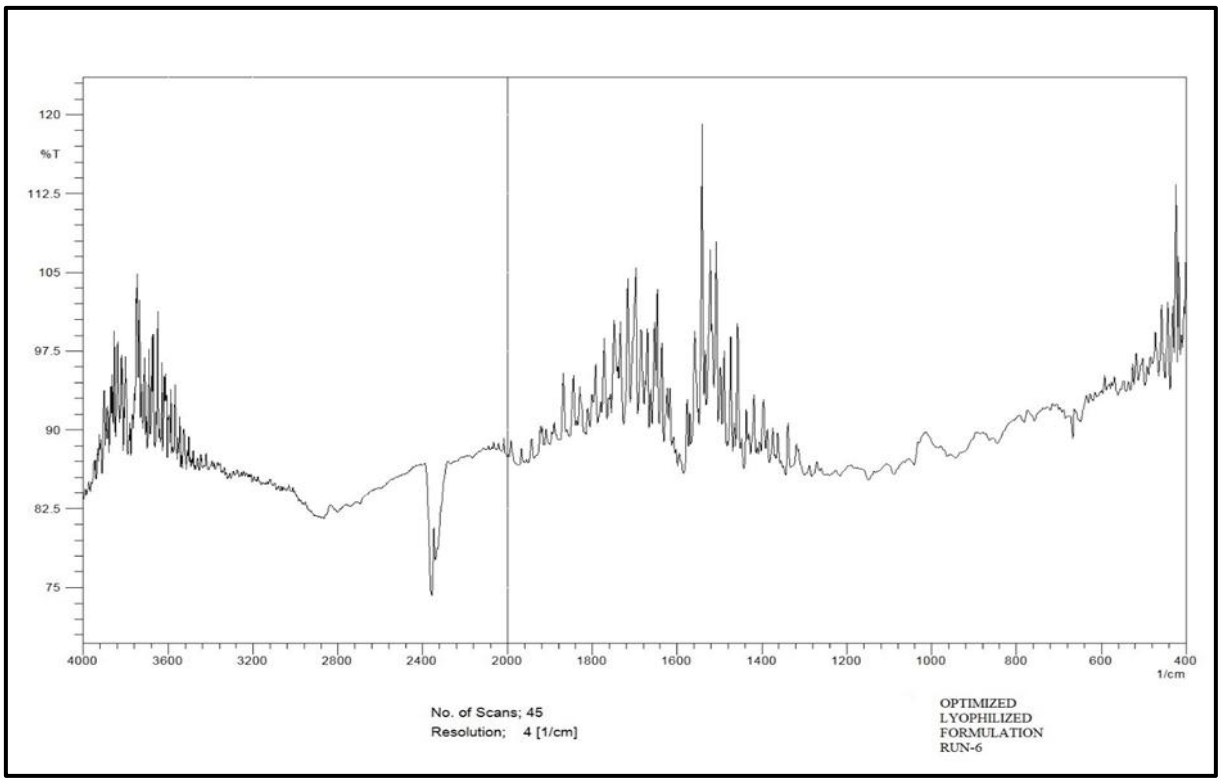

Figure-4. FT-IR spectra of optimized lyophilized nano formulation

Differential scanning calorimetry (DSC)

DSC studies assessed the interaction between the drug and the polymer. A thermogram of carvedilol, polymers, and poloxamer 407 was determined. The DSC curve of carvedilol showed an endothermic peak at 120.9^0C, an onset temperature of 113.2^0C, and an end set temperature of 125.5^0C, corresponding to its melting point (**Figures-5 to 7**) [14].

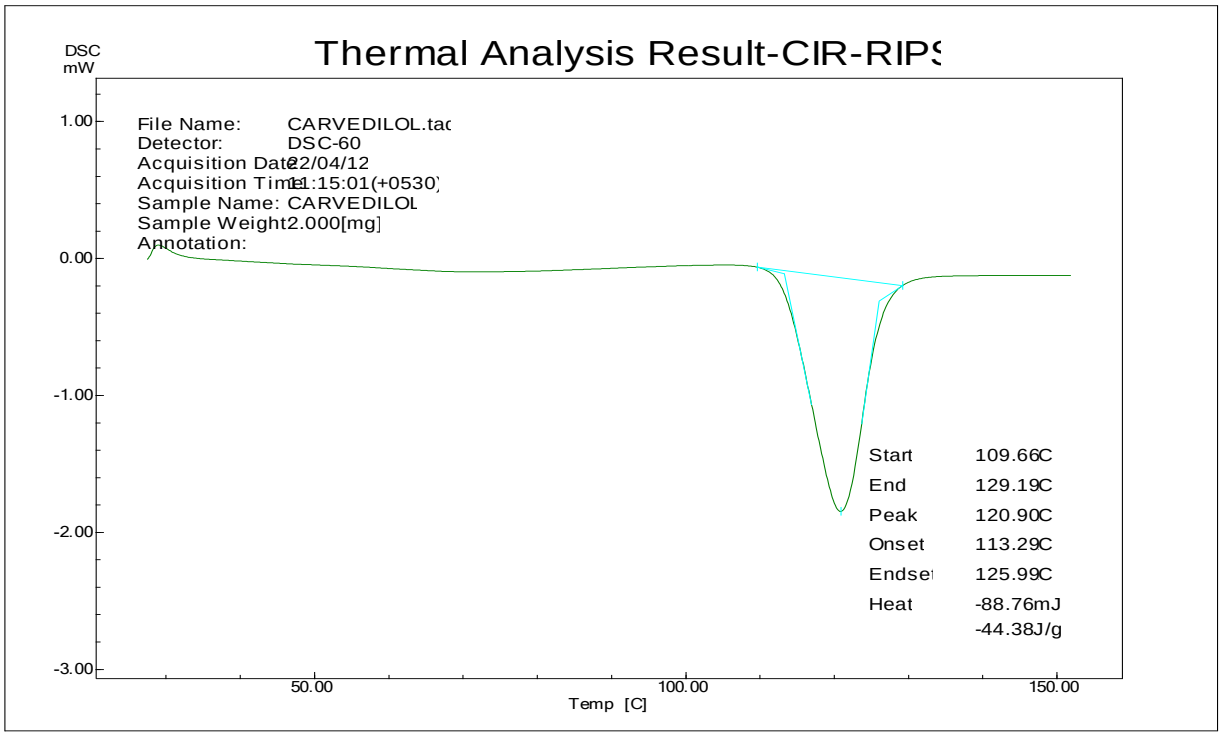

Figure-5. DSC thermogram of carvedilol

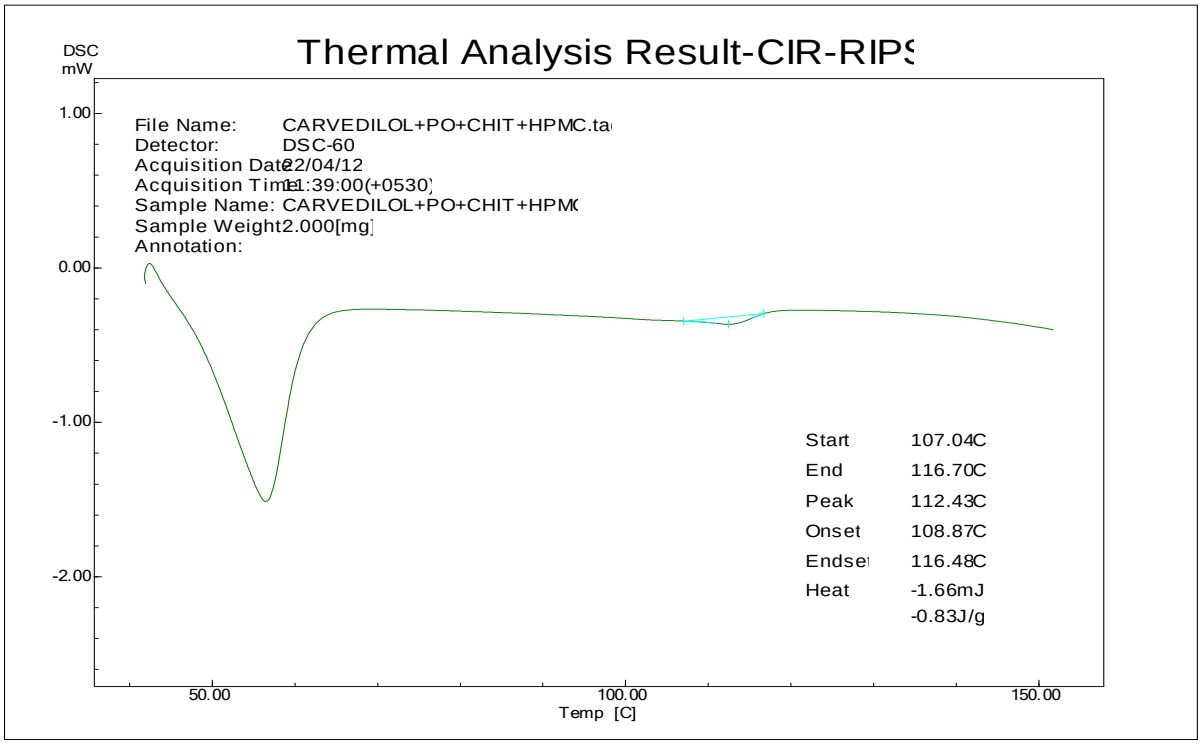

Figure-6. DSC thermogram of physical mixture of carvedilol + poloxamer 407 + HPMC K15M + chitosan

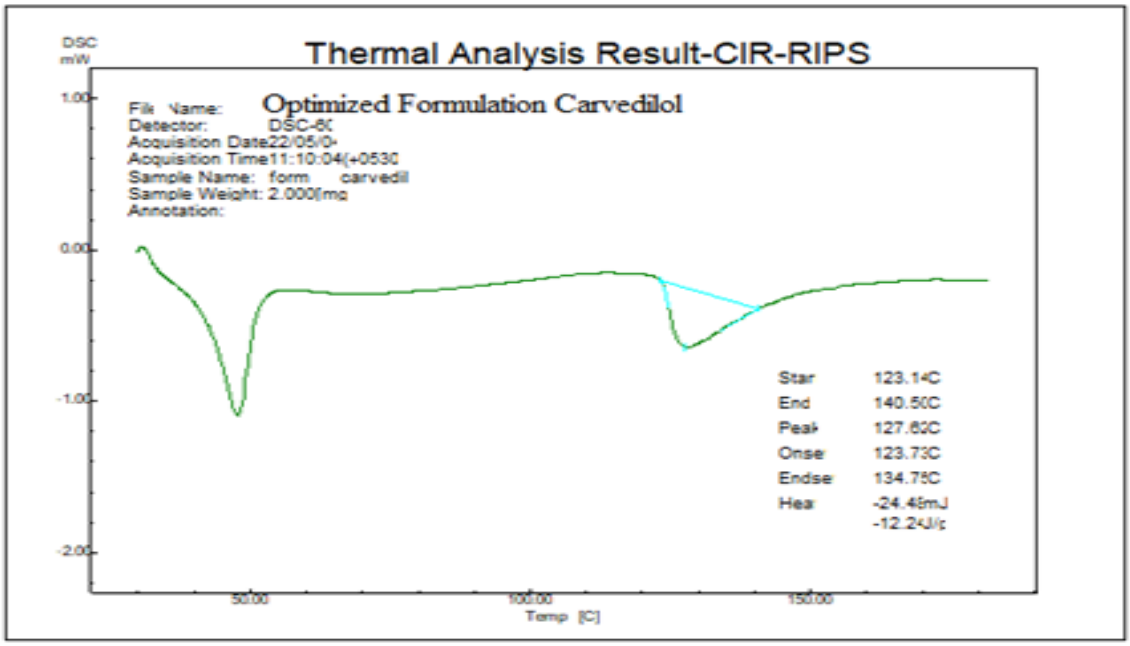

Figure-7. DSC thermogram of optimized lyophilized nano formulation

Entrapment efficiency

Exactly 2 ml of the sample was taken from the respective formulations in a centrifuge tube. First, the centrifuge tube was taken, and centrifugation was performed at 8000 rpm for about 25 minutes. After 25 minutes, the centrifuge tube was carefully removed and observed for the formation of a supernatant layer above the sample. Next, the supernatant layer of the liquid, i.e., about 1 ml, was carefully transferred into a test tube, and the volume was made up to 10 ml (Ethanol and 0.1 N HCl). Then the sample solution was analyzed under UV at λ_{max} 282 nm [15].

Statistical analysis and optimization of variables using experimental design

Statistical analysis

Design-Expert® (Version 12), Stat-Ease Inc., Minneapolis, MN, advanced statistical software of USA, was employed for formulation optimization and the estimation of its critical method parameters (CMAs). Microsoft performed the data evaluations excels 2007 (Microsoft, USA).

Optimization of process variables

To explore the influence of formulation and preparative variables of the nanoprecipitation technique on the formation of nanoparticles and their size, the polymer type and concentration, selection of organic solvent, stabilizers and their concentration, and the ratio of solvent (S) to nonsolvent (N.S.), etc. were studied to control and optimize the process. The drug and polymer ratio were varied to see the effect on drug release, entrapment efficiency, particle size, and zeta potential. To know the impact of stabilizer concentration and the temperature used during the formulation of nanoparticles and similarly, the ratio/proportion of the solvent and nonsolvent was varied to see the effect on nanoprecipitation of carvedilol nanoparticles and the stirring speed on the formation of nanoparticles: While following the nanoprecipitation method the stirring speed was various to observe the impact on the appearance of nanoparticles [16].

Particle size distribution and zeta potential determination

The droplet size and size distribution analysis were performed on optimized formulation by using Malvern Zetasizer (Nano ZS-90 U.K). The statistical distribution of droplet size of optimized formulation is shown in the **Figure-8**.

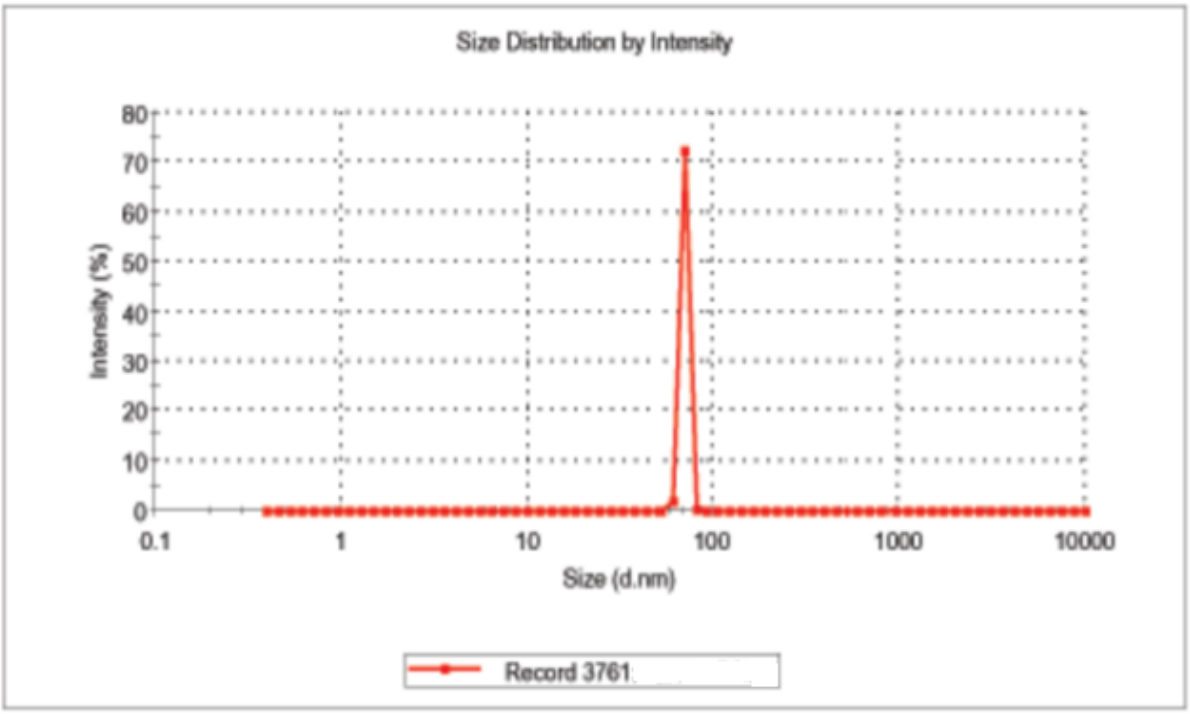

Figure-8. Droplet size distribution of formulation

***In-vitro* diffusion study**

Initially, 150ml of 0.1 N HCl was taken in respective beakers. Then, about 5ml of the sample (F1-F9) was taken from the individual formulations, loaded with the dialysis membrane bag, and carefully tied with the help of thread. Dip the membrane bag into the beakers containing 0.1 N HCl for the first 2h and then change the dissolution medium with phosphate buffer solution with pH 6.8. Adjust the dialysis membrane properly inside the solution and on the magnetic stirrer at the stirring speed of 100 rpm. At 0 h, pipette 2ml of the sample and transfer it to the centrifuge tube, then add 2ml of 0.1 N HCl into the beaker to maintain the sink condition. A similar process was repeated at 2h, 4h, 6h, 8h, 12 h,18h, and 24 h. This process was repeated for at least 24 h, and the 2ml of the samples collected was further divided, where 1ml of the sample was taken out and added with 1ml of ethyl acetate. The above solution was vortexed for about 15 minutes in a cyclomixture and kept aside for about 15 minutes. The formation of the supernatant clear liquid layer was carefully removed into a test tube and subjected to drying in a water bath. After

completely drying this test tube, it was further analyzed under UV by adding the respective solvent into the tube (**Table-3**).

Table-3. *In-vitro* drug release data of F1-F13 formulations

Cumulative percentage drug release													
Time (h)	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**	**F10**	**F11**	**F12**	**F13**
0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	6.393	7.231	4.837	5.872	4.215	7.282	8.321	5.736	5.786	4.877	8.432	8.321	6.812
2	15.299	16.923	22.341	29.221	12.394	19.272	19.787	11.229	28.897	10.327	18.392	17.483	13.786
4	38.383	48.821	32.342	42.237	23.382	34.222	43.221	29.383	43.786	20.339	32.344	39.384	21.638
6	53.889	67.892	39.839	62.289	43.382	53.728	64.282	39.839	61.978	42.337	54.223	61.387	34.739
8	76.939	79.291	71.383	69.767	54.392	74.222	76.222	71.383	69.767	64.288	79.339	79.892	61.230
12	88.332	87.455	83.383	75.828	65.992	87.238	88.373	83.383	76.986	69.221	89.493	90.340	76.870
24	97.389	96.908	97.282	80.281	75.393	96.586	99.123	98.383	83.998	76.822	99.760	99.780	99.768

Results and Discussion

The UV spectroscopy determined the standard curve for the pure drug and the initial solubility analysis shown in **Table-1**. The linearity range was determined up to 80 µg/mL in 0.1 N HCl. Hence, it obeyed Beer Lambert's law in this concentration range. Pure drug characterizations for compatibility and melting point were carried out with different polymers and excipients with the help of FT-IR and DSC. The DSC studies revealed no interaction between the medication and

the polymer. Carvedilol-loaded polymeric nanoparticles were created successfully by the nanoprecipitation method. Initially, the technique was performed at 500 rpm stirring speed and 70^0 C temperature, where the formulations of batches 1, 2, and 3 showed a hazy appearance. Hence the rate was decreased to 1200 rpm and 37^0 C temperature; by doing so, the clear formulations of carvedilol nanoparticles were obtained at different concentrations.

Particle size analysis

The size and dispersion of nanoparticles play a crucial role in their adhesion and interaction with cells. The particle size was minimum at 263.8, i.e. (-1, 0) in Run 6, while the maximum particle size in 1329.35 nm, i.e., at (1, 1) Run 11.

Zeta potential

Zeta potential is a scientific notion for electrokinetic potential in colloidal systems and is one of the essential properties playing a significant role in nanomedicine. Zeta potential can affect the physical and pharmacokinetic aspects of nanosystems in the body or may affect nanoparticle phagocytosis in the bloodstream. The zeta potential was maximum at -5.85mV at Run 11, i.e., (1,1), while the zeta potential was minimum at 24.2mV at Run 6.

Entrapment efficiency

In this work, the influence of various process parameters The EE is the proportion of the drug-loaded into polymeric matrices. The percentage of the entrapped drug was minimum at 35.448, i.e. (1,1) in run 11. The percentage of entrapped drugs was maximum in Run 6, i.e., 86.225%—i.e. (-1, 0). The zeta potential carried out for the respective formulations is significantly less, which may affect the stability of the formulation.

Differential scanning calorimetry

The differential scanning calorimetry was performed to determine the peak temperature, onset and end set temperature, and heat energy for the pure drug carvedilol, polymers stabilizer poloxamer 407, as shown in Figure-3. DSC study revealed no interaction between the drug and polymers used.

Optimization of process variables

It optimized the various preparative variables and the conditions; Drug: Polymer ratio (mg) and stabilizer concentration (gm %) were used to develop polymeric nanoparticles for carvedilol by

nanoprecipitation technique as shown in Table-2. The polymer was dissolved in the selected nontoxic solvent, PEG 400, to form the diffusing phase with the help of a vortex mixer. The specified amount of drug was accurately weighed, added to the polymeric solution, vortexed for a few minutes, and allowed to stand for a few minutes to obtain an air bubble-free and transparent solution. The aqueous dispersing phase containing the stabilizer constituted the nonsolvent in which the polymer and the drug were insoluble. Accurately measured 1 ml volume of the diffusing phase was added to the 19 ml of the dispersing phase (nonsolvent) with the help of a syringe which was properly positioned the needle to immerse directly into the aqueous medium under moderate (1200 rpm) magnetic stirring at 35^0C, resulted in the formation of nanoparticles. The polymer precipitated as soon as the polymer-containing solvent diffused into the dispersing medium, resulting in immediate drug entrapment. The so-called Marangoni effect governed the rapid nanoparticle formation due to interfacial turbulences at the solvent interface and nonsolvent and results from complex and cumulated phenomena such as flow diffusion and surface tension variations.

Response surface analysis of 2D and 3D plots

Effect of the factor on CQA (% of drug release)

The **Figures-9** and **10** counter (2D) and response (3D) plots responses elucidate the impact of observed responses % drug release upon the stabilizer concentration and drug: polymer ratio. The stabilizer concentration gradually increased (Low level coded value -1, 0 was embedded in the model), and there is a significant variation in % drug release characteristic. However, when the level changes from low to high (0, to +1), i.e., results in a significant increase in drug: polymer concentration, there is a prevalence of dark green colour region replicating the considerable influence upon % drug release of polymeric nanoparticles.

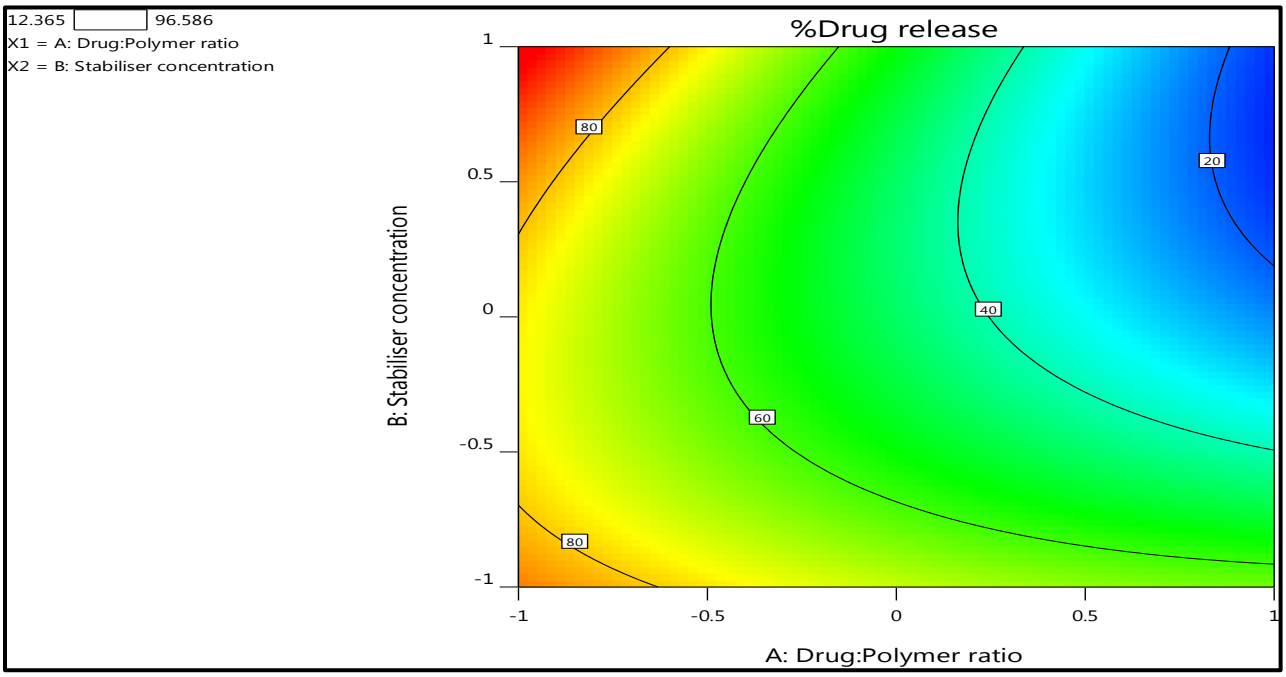

Figure-9. 2D graph showing effect of X1 and X2 on percentage of drug release

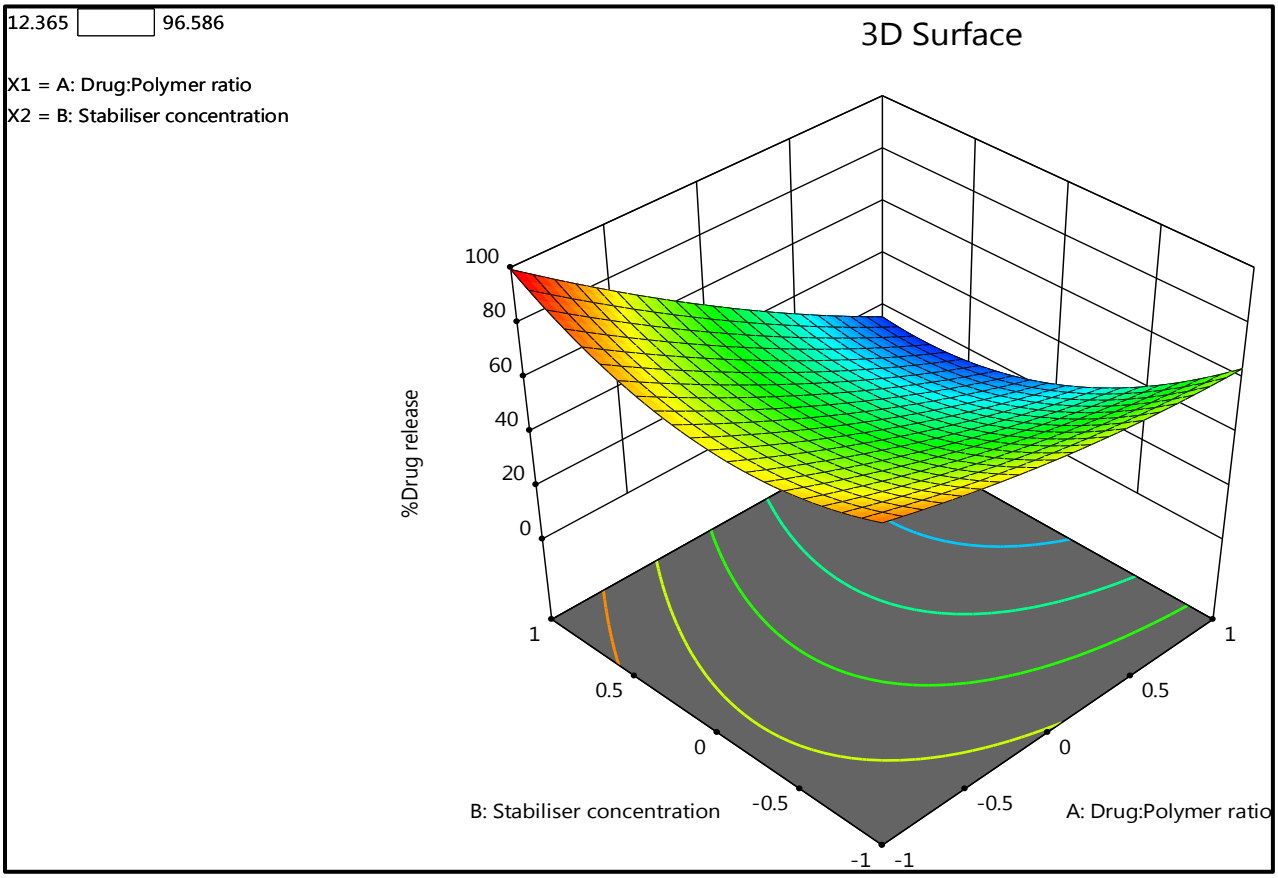

Figure-9. 3D graph showing effect of X1 and X2 on percentage of drug release

Effect of the factor on CQA (% of drug entrapment efficiency)

Figures-10 and **11** counter (2D) and response (3D) plots depict that improvement in the drug: polymer concentration upsurges the level of size aggregation, which retards the release behaviour. This, in turn, enhances an optimum % entrapment efficiency specified in the prevalence of the red region. For example, in the case of polymeric nanoparticles of carvedilol, prepared with HPMC K15M and chitosan polymers, it was perceived that an abundant, desirable sustain release profile of 24 h was achieved with a much lesser proportion of HPMC K15M in comparison to other polymers.

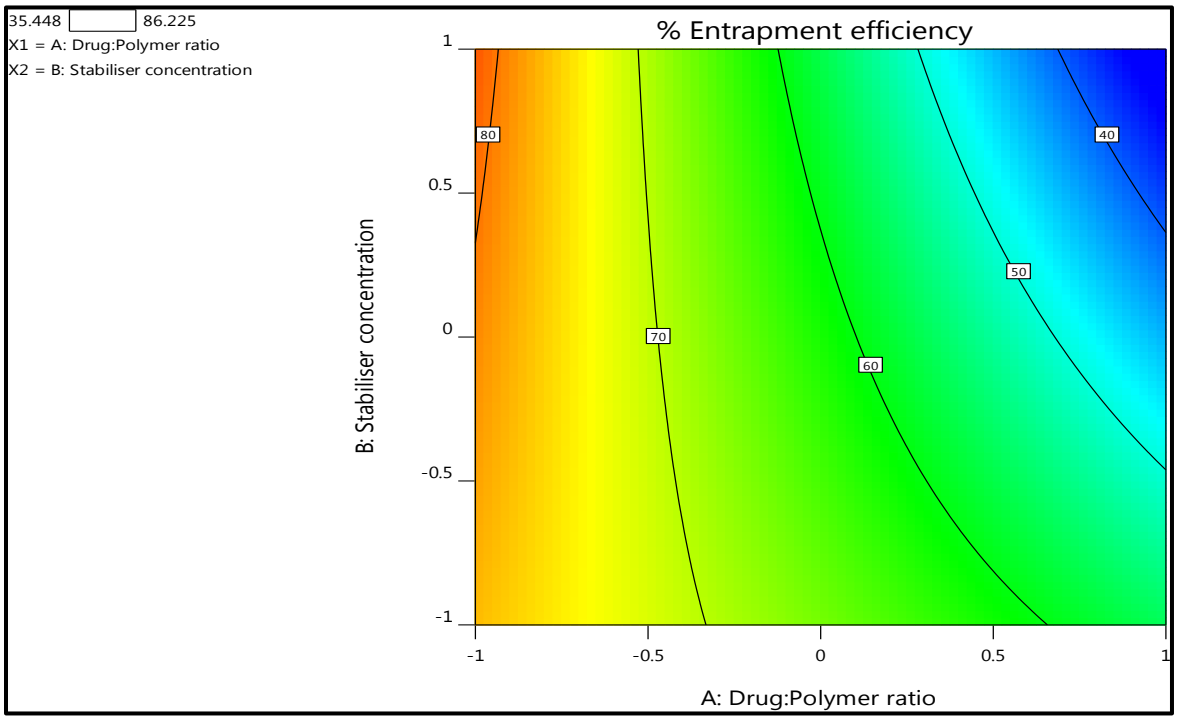

Figure-10. 2D graph showing effect of X1 and X2 on percentage of entrapment efficiency

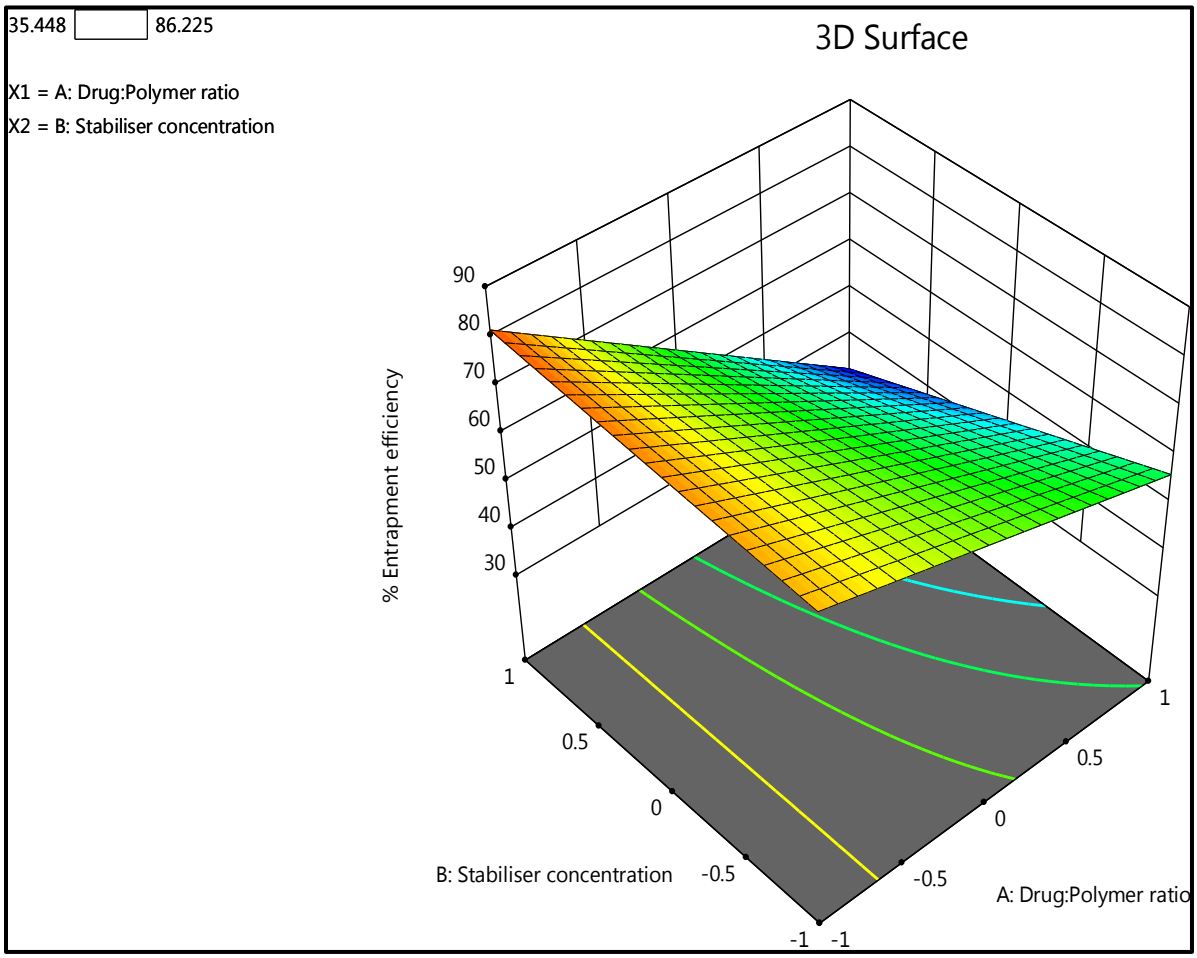

Figure-11. 3D graph showing effect of X1 and X2 on percentage of entrapment efficiency

Effect of the factors on CQA (Particle size)

Figures-12 and **13** counter (2D) and response (3D) plots detected desired particle size of the polymeric nanoparticles for HPMC K15M and chitosan-based formulation. This can be accredited to chemical bonding and high molecular weight of selected polymers. The particle size can be altered due to a significant change of high viscoelastic and swellable polymeric properties of HPMCK 15M rather than others indicated by the prevalence of green color region.

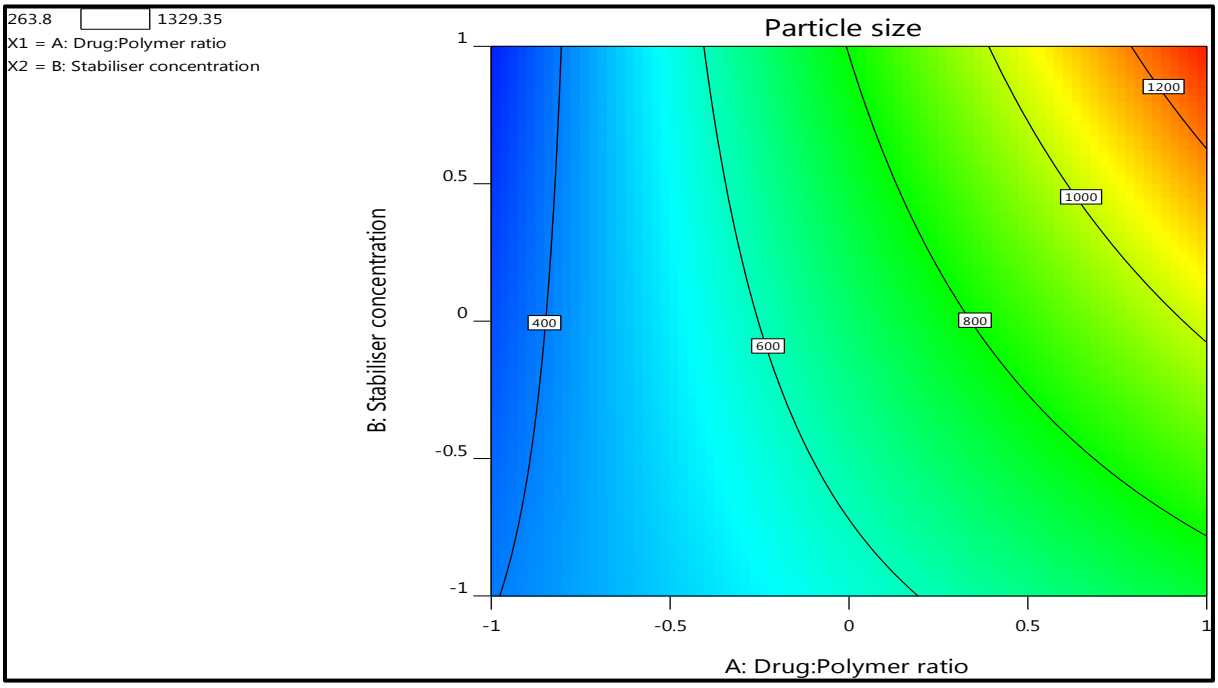

Figure-12. 2D graph showing effect of X1 and X2 on particle size

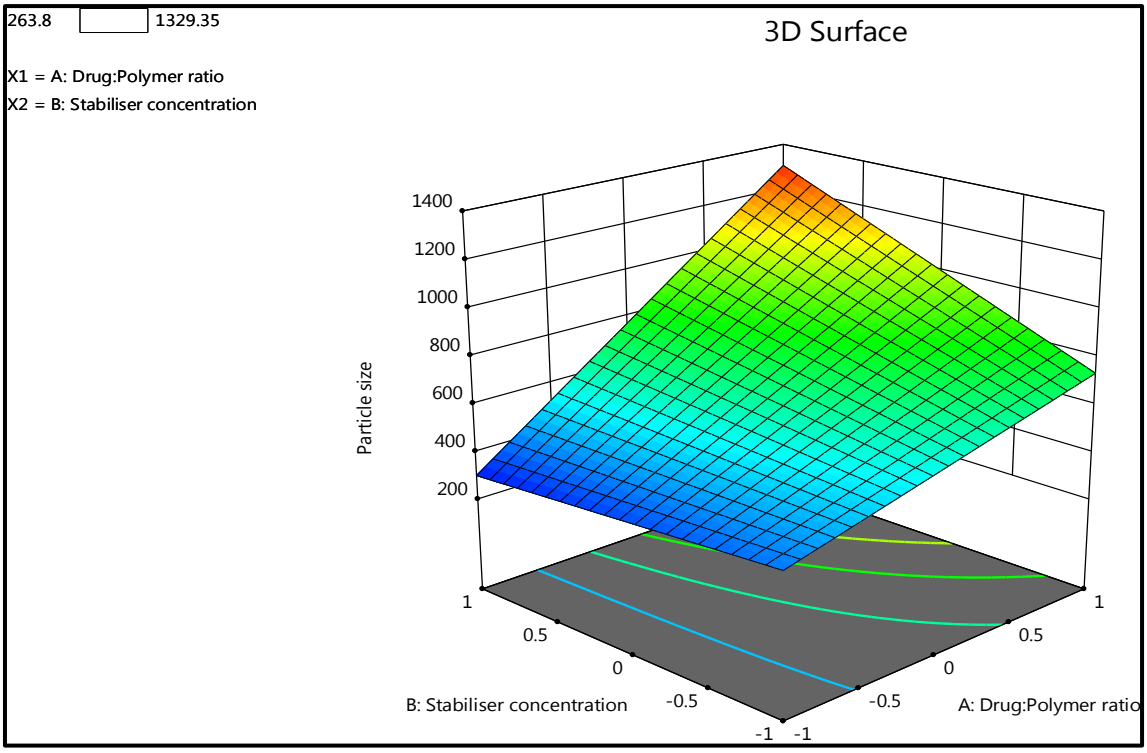

Figure-13. 3D graph showing effect of X1 and X2 on particle size

Effect of the factors on CQA (Zetapotential)

Figures-14 and **15** counter (2D) and response (3D) plots depict those higher values of zetapotential were detected for HPMC K15M, and chitosan-based polymeric nanoparticles. This can be accredited to optimum concentration of the stabilizer and selected polymers. The zetapotential can be altered due to a significant change of high concentration of drug:polymer rather than others indicated by the prevalence of green colour region.

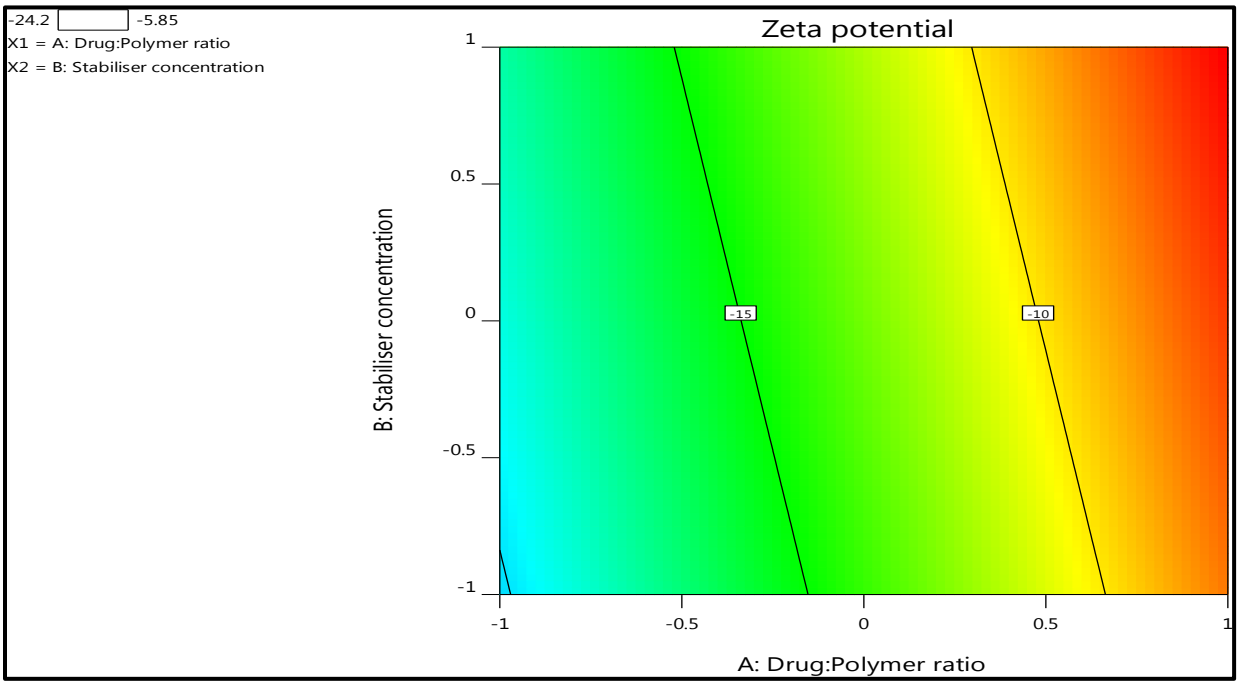

Figure-14. 2D graph showing effect of X1 and X2 on zeta potential

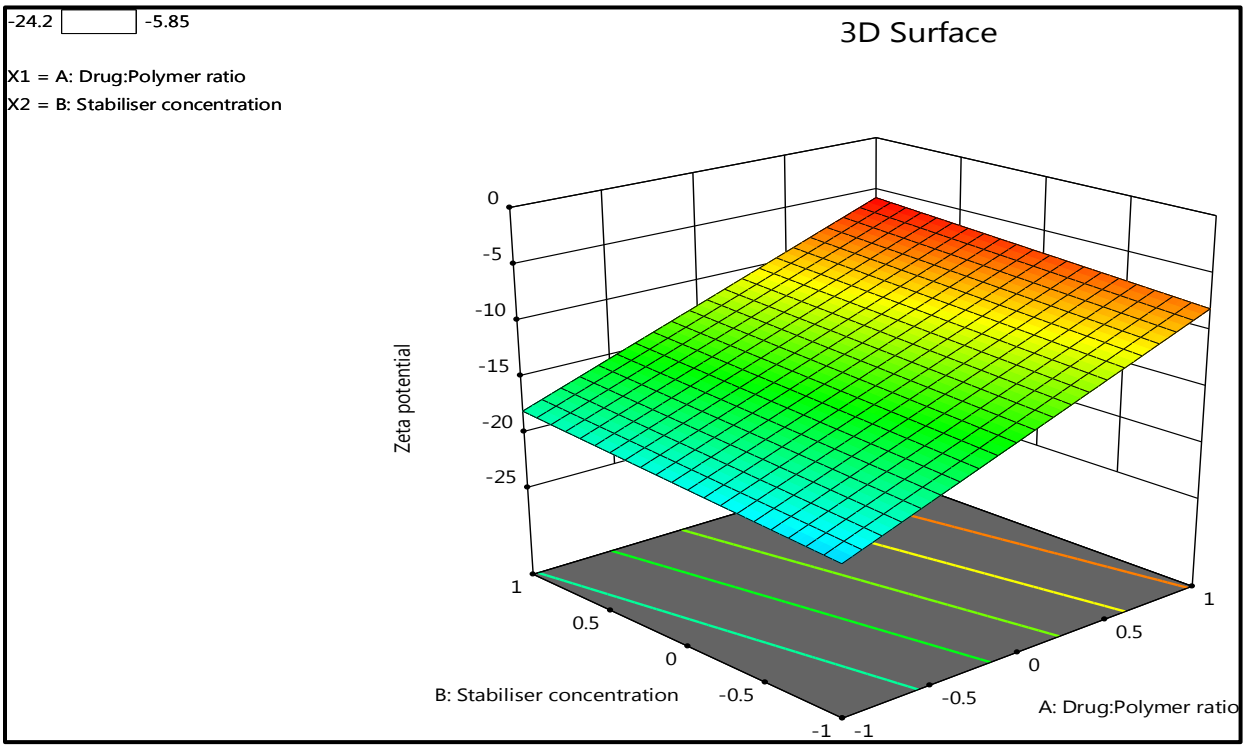

Figure-15. 3D graph showing effect of X1 and X2 on zeta potential

ANOVA for quadratic model on percentage of drug release

The model's F-value of 8.21 indicates that it is significant. An F-value this large might arise owing to noise just 0.77 percent of the time. Model terms with P-values less than 0.0500 are significant. In this scenario, A and AB are important model terms. Values larger than 0.1000 imply that the model terms are unimportant. Model reduction may improve your model if there are many inconsequential model terms (except those required to enable hierarchy). The F-value for Lack of Fit of 14.14 indicates that the Lack of Fit is substantial. A Lack of Fit F-value this significant might arise due to noise just 1.35 percent of the time. A significant lack of fit is undesirable; we want the model to fit (**Supplementary Table-1**).

Supplementary Tabl-1. ANOVA for quadratic model data on percentage of drug release

Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**P-value**	
Model	6645.10	5	1329.02	8.21	0.007	Significant

A-Drug: Polymer ratio	4133.06	1	4133.06	25.52	0.001	
B-Stabilizer concentration	484.24	1	484.24	2.99	0.127	
AB	945.16	1	945.16	5.84	0.046	
A^2	45.75	1	45.75	0.282	0.611	
B^2	739.62	1	739.62	4.57	0.069	
Residual	1133.68	7	161.95			
Lack of Fit	1035.98	3	345.33	14.14	0.013	Significant
Pure Error	97.71	4	24.43			
Cor Total	7778.78	12				

ANOVA for quadratic model on percentage of drug entrapment efficiency

The Model's F-value of 16.26 indicates that it is significant. An F-value this large might arise owing to noise only 0.06 percent of the time. Model terms with P-values less than 0.0500 are significant. A is a crucial model term in this scenario. Values larger than 0.1000 imply that the model terms are unimportant. Model reduction may improve your model if there are many inconsequential model terms (except those required to enable hierarchy). The Lack of Fit F-value of 2.86 indicates that the Lack of Fit is insignificant in comparison to the pure error. A large Lack of Fit F-value owing to noise has a 16.52 percent chance of occurring. A non-significant lack of fit is desirable because we want the model to fit (**Supplementary Table-2**).

Supplementary Tabl-2. ANOVA for quadratic model data on percentage of drug entrapment efficiency

Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**P-value**	
Model	2169.00	3	723.00	16.26	0.0006	Significant
A-Drug: Polymer ratio	1816.07	1	1816.07	40.84	0.0001	
B-Stabilizer concentration	140.66	1	140.66	3.16	0.1090	

AB	212.27	1	212.27	4.77	0.0567	
Residual	400.26	9	44.47			
Lack of Fit	312.79	5	62.56	2.86	0.1652	Significant
Pure Error	87.46	4	21.87			
Cor Total	2569.26	12				

ANOVA for quadratic model on particle size (nm)

The Model's F-value of 19.45 indicates that it is significant. An F-value this large might arise owing to noise only 0.03 % of the time. Model terms with P-values less than 0.0500 are significant. A, B, and AB are important model terms in this situation. Values larger than 0.1000 imply that the model terms are unimportant. Model reduction may improve your model if there are many inconsequential model terms (except those required to enable hierarchy). The F-value for Lack of Fit of 8.50 indicates that the Lack of Fit is substantial. A Lack of Fit F-value this significant might arise due to noise just 2.96 percent of the time. We want the model to fit, so a significant lack of fit is negative (**Supplementary Table-3**).

Supplementary Tabl-3. ANOVA for quadratic model data on particle size (nm)

Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**	
Model	8.737E+05	3	2.912E+05	19.45	0.0003	Significant
A-Drug: Polymer ratio	6.799E+05	1	6.799E+05	45.41	< 0.0001	
B-Stabilizer concentration	84099.52	1	84099.52	5.62	0.0419	

AB	1.096E+05	1	1.096E+05	7.32	0.0242	
Residual	1.348E+05	9	14974.32			
Lack of Fit	1.232E+05	5	24636.30	8.50	0.0296	Significant
Pure Error	11587.40	4	2896.85			
Cor Total	1.008E+06	12				

ANOVA for quadratic model on zeta potential

The Model's F-value of 5.57 indicates that it is significant. An F-value this large might arise due to noise just 2.37 percent of the time. Model terms with P-values less than 0.0500 are significant. A is a crucial model term in this scenario. Values larger than 0.1000 imply that the model terms are unimportant. Model reduction may improve your model if there are many inconsequential model terms (except those required to enable hierarchy). The F-value for Lack of Fit of 8.91 indicates that the Lack of Fit is substantial. A Lack of Fit F-value this significant might arise due to noise just 2.64 % of the time. We want the model to fit, so a significant lack of fit is negative (**Supplementary Table-4**).

Supplementary Tabl-4. ANOVA for quadratic model data on zeta potential

Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**	
Model	232.32	2	116.16	5.57	0.0237	Significant
A-Drug:	224.73	1	224.73	10.77	0.0083	

Polymer ratio						
B-Stabilizer concentration	7.59	1	7.59	0.3639	0.5598	
Residual	208.65	10	20.87			
Lack of Fit	194.13	6	32.35	8.91	0.0264	Significant
Pure Error	14.52	4	3.63			
Cor Total	440.97	12				

In vitro drug release

A diffusion study was performed to obtain the drug release in different hours after administration of the drug formulation shown in **Table-3**. The percentage of drug release was seen as a maximum of 96.58 % in the case of run 6 (**Figure-~~17~~**). In vitro, kinetic diffusion study showed that drug release followed first-order kinetics. The primary drug release mechanism was diffusion-controlled due to the higher correlation coefficient for the Higuchi equation. Korsmeyer Pappa's release exponent was more than 0.5 and less than 1 for all formulations implying that the drug was released via a non-Fickian diffusion mechanism.

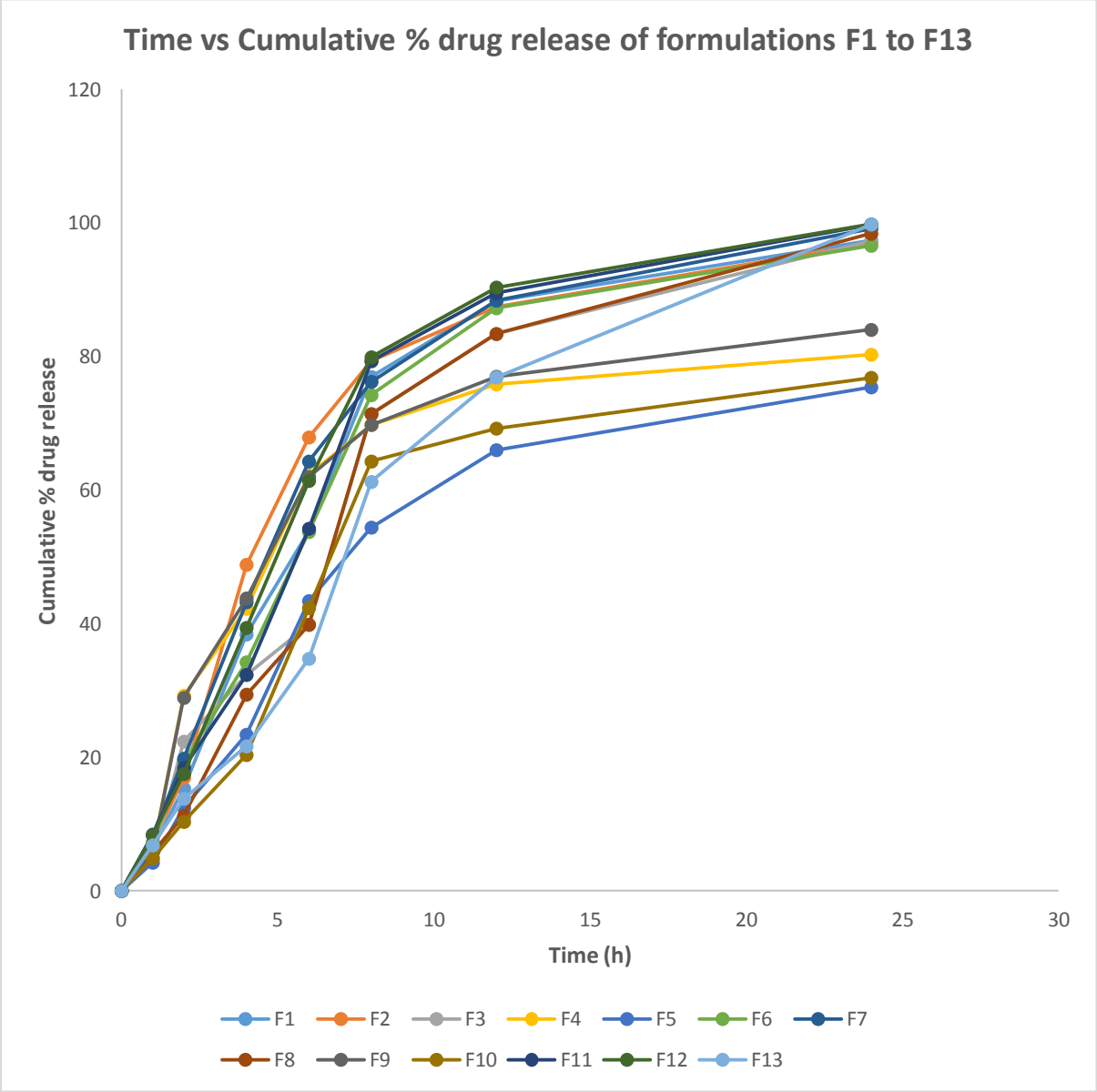

Figure-~~17~~. Drug release profile of F1-F13 formulations

Conclusions

~~This study used a nanoprecipitation technique to optimize the drug solubility and dissolution rate to systematically create polymeric nanoparticles of carvedilol formulation, an antihypertensive medication. The optimized polymeric nanoparticle formulation had an optimal in vitro drug release of more than 90% after 24h, a particle size of 42.54 nm, and 99.5% entrapment efficiency. The accelerated stability data showed no significant changes in storage after six~~

~~months for the optimized polymeric nanoparticles. The current study found that the formulated polymeric carvedilol nanoparticles exhibited a good sustained release profile with effective in-vitro release in the suspension form. The QbD-assisted development helped fabricate more sustainable formulations with distinct characters expected to be more stable and steadier. Hence, NPs were discovered to be an effective medication delivery mechanism in encapsulating hydrophobic drugs like carvedilol for improved solubility with sustained release behavior.~~

Author's statement

The manuscript includes contributions from all the authors. The authors have all approved the final version of the manuscript, but the author declares no conflicts of notice in preparing the manuscript.

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Authors' contributions

Mallika Tamminana: Major contributor towards writing and language editing or substantively the final form of the document. B.V.V. Ravikumar: Conceptualization, methodology, software applications, formal analysis, Investigation, Writing-original draft, visualization collected the literature related to the title and interpreted in the form of tables and images, and he made the acquisition and drafting of the manuscript and also checked grammatical and typographical errors, etc. as per the journal guidelines. All authors have read and approved the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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List of abbreviations

BCS: Biopharmaceutical classification system

CQAs: Critical quality characteristics

DSC: Differential scanning calorimeter

DMSO: Dimethyl sulfoxide

EE.: Entrapment efficiency

FT-IR: Fourier transform infrared

HCl: Hydrochloric acid

HPMC: Hydroxypropyl methylcellulose

NPs: Nanoparticles

PNs: Polymeric nanoparticles

PDI: Polydispersity index

PEG: Polyethylene glycol

QbD: Quality by design

QTPP: Quality target product profile

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