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Quality by Design (Qbd) Based Development and Evaluation of Carvedilol Loaded Polymeric Nanoparticles for Enhanced Solubility

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Abstract

By preparing a polymeric nanoparticle by nanoprecipitation using specific polymers like Chitosan and HPMC K15M and Poloxamer 407 as a surfactant, the drug solubility of Carvedilol, a BCS class-II drug with poor water solubility, can be improved through the release of drug over time. Critical quality parameters, such as drug release (%), entrapment efficiency (%), particle size (nm), and zeta potential (mV), are used to eliminate unnecessary process and formulation variables. The model drug has a sharp melting point, and the FT-IR and DSC investigations show that it does not interact with the polymers or show any additional peaks. Particle size, zeta potential, entrapment efficiency and in-vitro drug release were among the characteristics studied for the generated drug-loaded polymeric nanoparticles. According to the results of the drug release and stability investigations, the F6 formulation was the most reliable, boasting increased drug solubility and efficient drug trapping as it formed a nano-sized polymeric particle. Non-Fickian diffusion-controlled drug release with Higuchi kinetics was demonstrated by the correlation coefficient data and the release exponent numbers from Koresmeyer Peppa's. Drug entrapment efficiency and in vitro dissolution rate remained constant during the 6-month stability trial, indicating that the optimised formulation (F6) is more stable.

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

1. Introduction

Like ultrafine particles, nanoparticles (NPs) are 1–100 nanometers. Nanoparticles may or may not have size-related features different from bulk materials and tiny particles [1]. Polymeric nanoparticles are 1–1000 nm colloids. Pharmaceutically active compounds are embedded in macromolecules [2]. Due to the quantum size effect, polymeric NPs affect oxidative stress, cytotoxicity, and genotoxicity [3]. Nanoprecipitation requires two miscible solvents. An acetone or acetonitrile-dissolved polymer is in the internal phase. They evaporate easily because they are water-impermeable. This approach uses polymer interfacial deposition after organic solvent displacement from a lipophilic solution to the aqueous phase. An intermediate-polarity water-miscible solvent dissolves the polymer. The solution is added to an aqueous solution dropwise or at a regulated rate [4]. Due to the fast spontaneous diffusion of the polymer solution into the aqueous phase, nanoparticles form instantly to escape water molecules. As solvent diffuses from nano-droplets, polymer forms nanocapsules or nanospheres [5]. The organic phase is usually introduced to the aqueous phase; however, it can be reversed without affecting nanoparticle synthesis. Surfactants stabilize colloidal suspensions; however, they are not necessary for nanoparticle synthesis. Superior to emulsification solvent evaporation, the nanoparticles have a well-defined size and constrained size distribution [6, 7].

Absorbable polymeric nanoparticles that respond to internal or external stimuli improve therapeutic delivery to sick areas. Micelles, vesicles, cross-linked nanoparticles, and hybrid nanoparticles are used to make stimulus-sensitive polymeric nanoparticles [8]. Polymeric nanoparticles' chemical or physical characteristics change with single, dual, or multiple stimuli. This lets them hold payloads during circulation, target sick areas, and release them after cell internalization [9].

Carvedilol is RS-I-(9H-carbazol-4-yloxy)-3-[[2-(2-methoxyphenoxy) ethyl] amino]. The sympathetic nervous system signals via adrenergic receptors and propan-2-ol to promote breast cancer. Since this discovery, cardiac-blocker drugs are considered cutting-edge anticancer treatments. Due to first-pass metabolism and limited water solubility, this medication has 25% to 35% absolute bioavailability [10].

This study developed and optimized nanoprecipitation-prepared carvedilol-loaded polymeric nanoparticles to improve medication solubility in a sustained release mechanism and reduce drug dose intervals. There are numerous ways to make nanoparticles, but nanoprecipitation is the most common since it involves the fewest steps. The following objectives were chosen to conduct pre-formulation studies: analytical method development to develop polymeric nanoparticulate systems using selected polymers to optimize formulation variables or conditions and characterize prepared formulations.

2. Materials And Methods

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

Preformulating 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

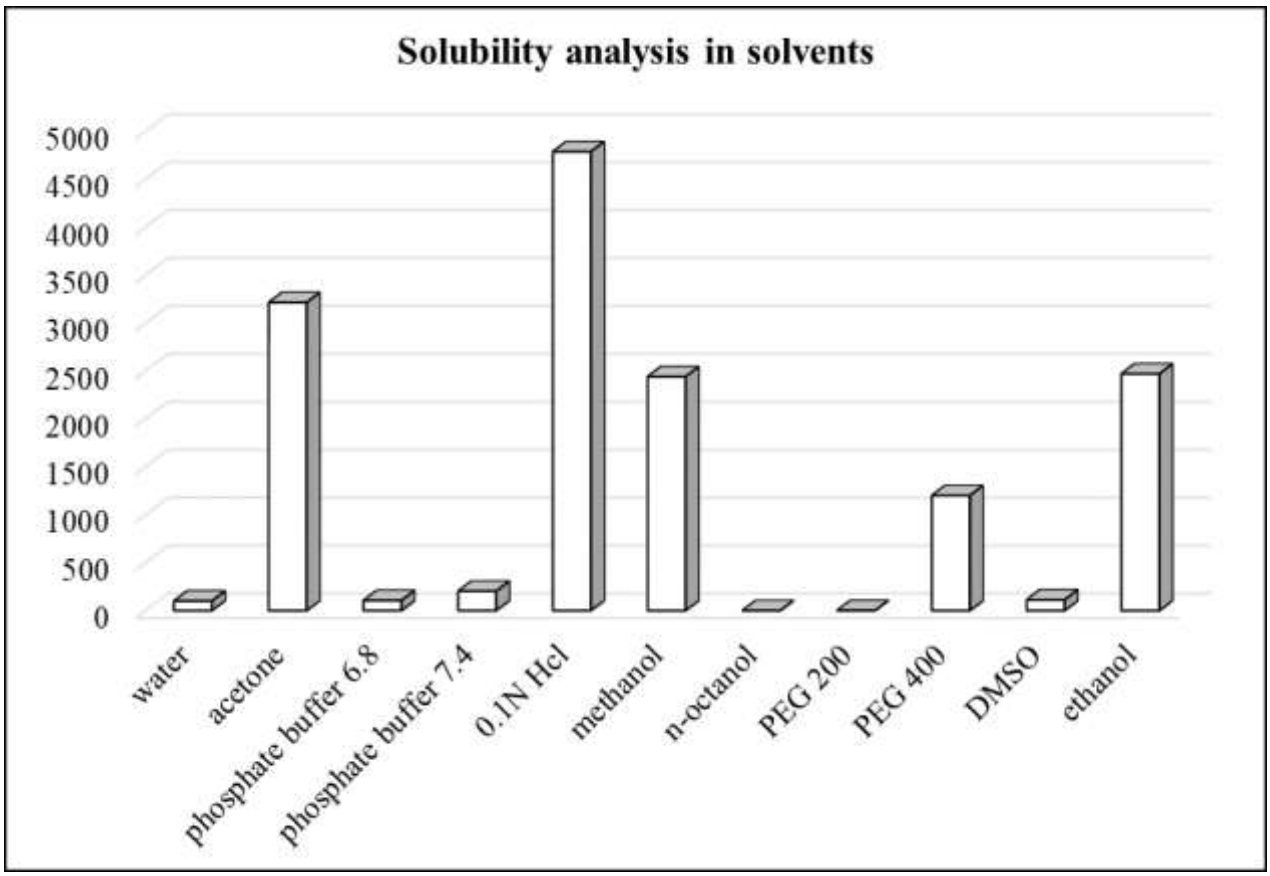

Figure 1. Bar graph showing solubility of pure drug in different solvents

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

Nanoparticles were made via solvent evaporation and nanoprecipitation. Step one: drug ratios 1:1, 1:2, 1:3. 20 mg drug: 20mg polymer, 40 mg drug: 80 mg polymer, 60 mg drug:180 mg of chitosan and HPMC K15M were dissolved in 5ml glacial acetic acid and acetone. The polymeric and drug solutions were combined using a magnetic stirrer after the pure drug carvedilol was dissolved separately in 10ml of acetone. From this, 20ml of the drug-polymer solution and 5ml of the solution were added to distilled water containing 1%, 1.5%, and 2% poloxamer 407 to make 20 ml. We used a rotary flask evaporator to evaporate the acetone under reduced pressure to 10ml. Slowly add the medicine to the polymer solution to dissolve it. The medication and polymer were gently added using a #27-gauge needle-size

syringe at 1 ml/min. The adding procedure should take 1hr at 1000 rpm. To remove acetone, the 20ml final volume was evaporated in a rotating flask. We adjusted the volume to 10ml. The nano suspension was freeze-dried for 36 h to produce polymeric nanoparticle powder.

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

Run	Factor 1	Factor 2	Response 1	Response 2	Response 3	Response 4
	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].

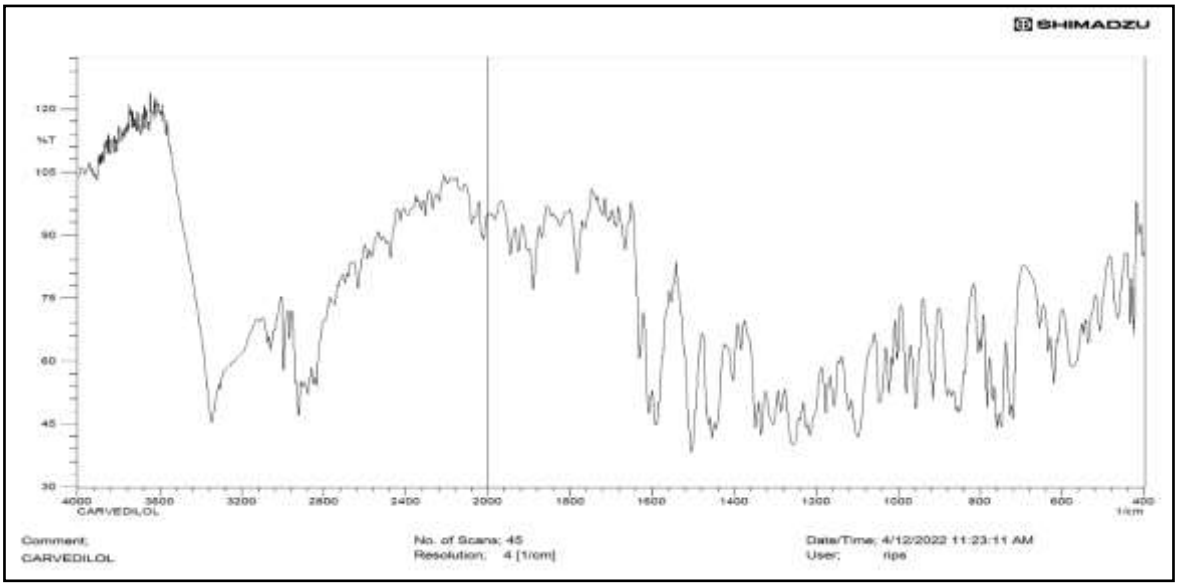

Figure-2. FT-IR spectra of Carvedilol

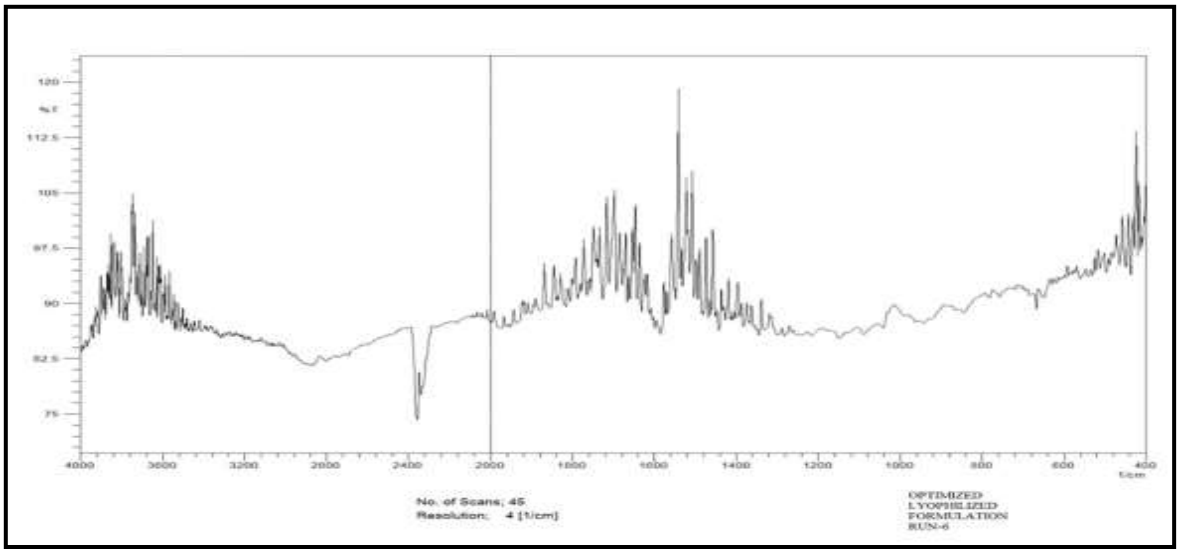

Figure-3. FT-IR spectra of physical mixture of carvedilol + poloxamer 407 + HPMC K15M + chitosan

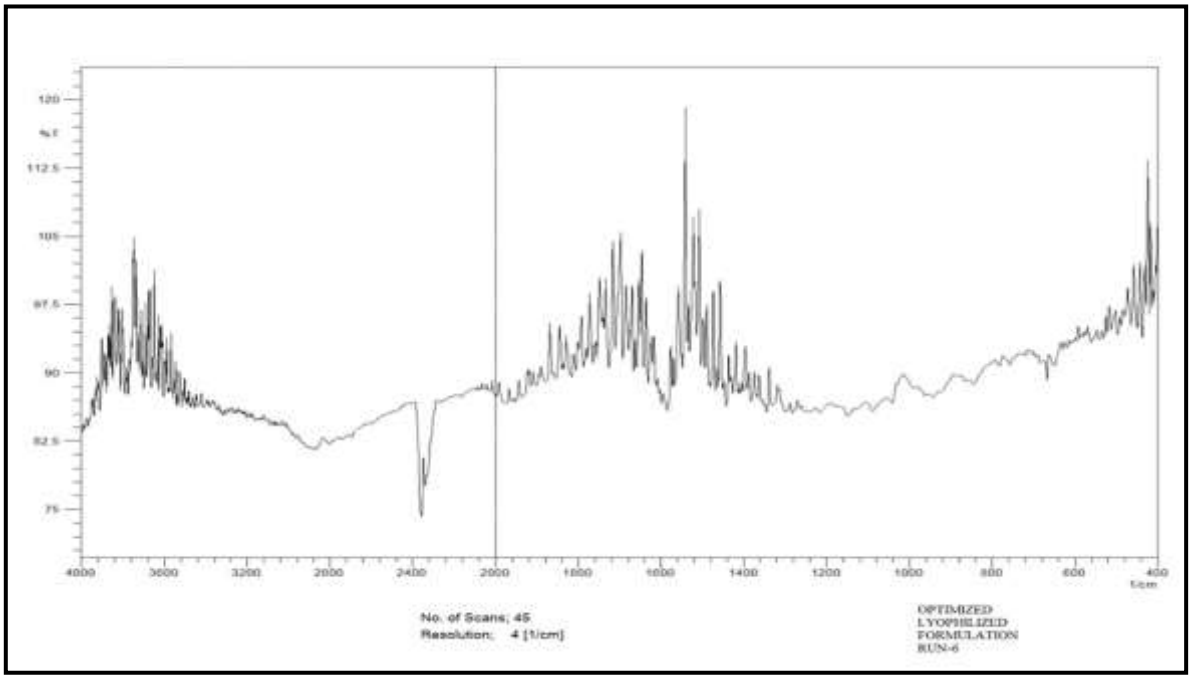

Figure-4. FT-IR spectra of optimized lyophilized nanoformulation

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^{0}C, an onset temperature of 113.2^{0}C, and an end set temperature of 125.5^{0}C, corresponding to its melting point (**Figures-5 to 7**) [14].

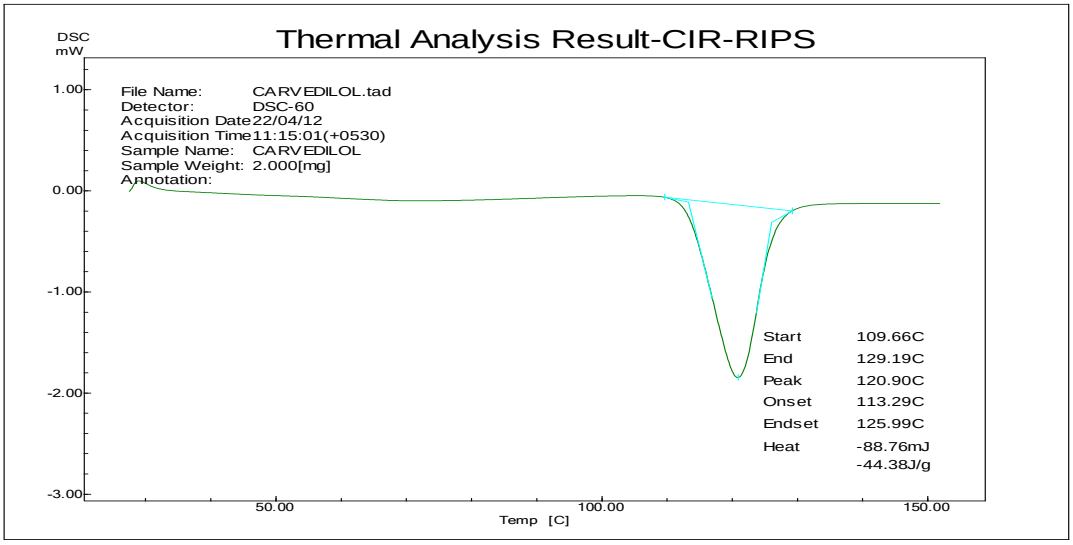

Figure-5. DSC thermogram of Carvedilol

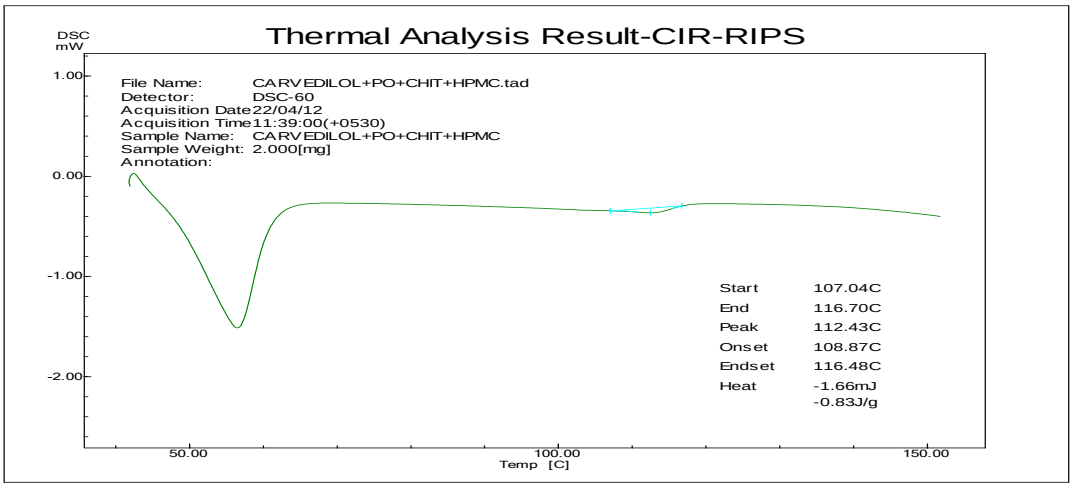

Figure-6. DSC thermogram of physical mixture of carvedilol + poloxamer 407 + HPMC K15M + chitosan

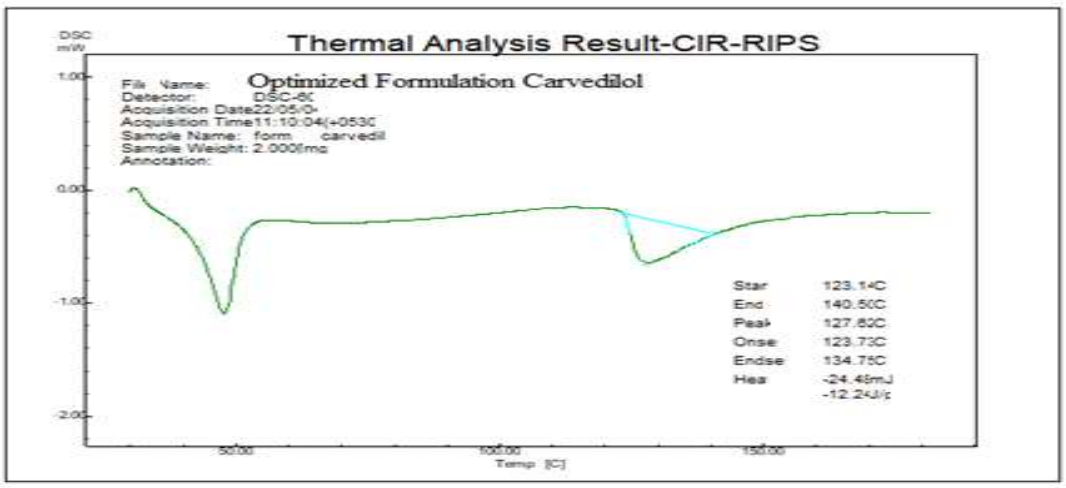

Figure-7. DSC thermogram of optimized lyophilized nano formulation

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 non-solvent (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 non-solvent 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 Zeta sizer (Nano ZS-90 U.K). The statistical distribution of droplet size of optimized formulation is shown in the **Figure-8**.

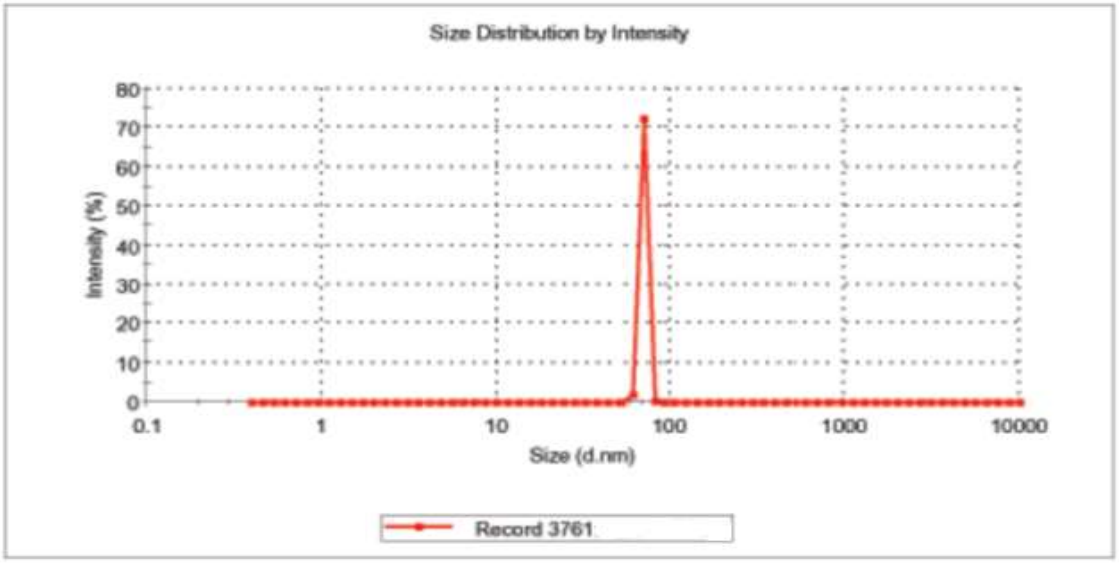

Figure-8. Droplet size distribution of formulation

***In-vitro* diffusion study**

Initially, 150 ml 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

3. 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 electro kinetic 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 optimised preparation variables and conditions; Drug: Table-2 demonstrates how nanoprecipitation created carvedilol polymeric nanoparticles with polymer ratio (mg) and stabilizer concentration (gm %). Vortex mixing the polymer in PEG 400, a benign solvent, created the diffusing phase. The drug was carefully weighed, added to the polymeric solution, vortexed, and left to stand to generate an air-free, transparent solution. Non-solvent polymer and medicament were insoluble in stabilizer-containing aqueous dispersion phase. Adding 1 ml of the diffusing phase to 19 ml of the dispersion phase (non-solvent) with a syringe positioned to insert the needle directly into the aqueous medium under moderate (1200 rpm) magnetic stirring at 35^0C formed nanoparticles. The polymer crystallized shortly after the solvent diffused into the dispersion medium, entrapping the medicine. Due to interfacial turbulences at the solvent:non-solvent interface and complex and cumulated phenomena including flow diffusion and surface tension variations, the Marangoni effect produced nanoparticles quickly

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.

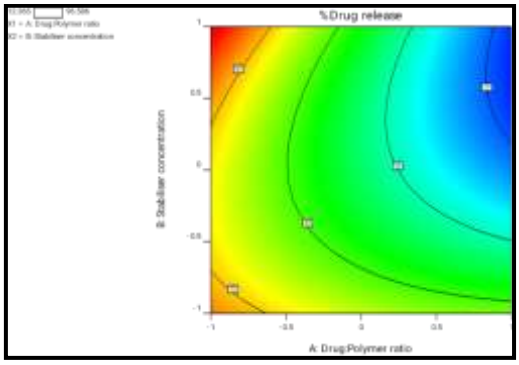

Figure-9. 2D graph showing effect of X1 and X2 on percentage of drug release

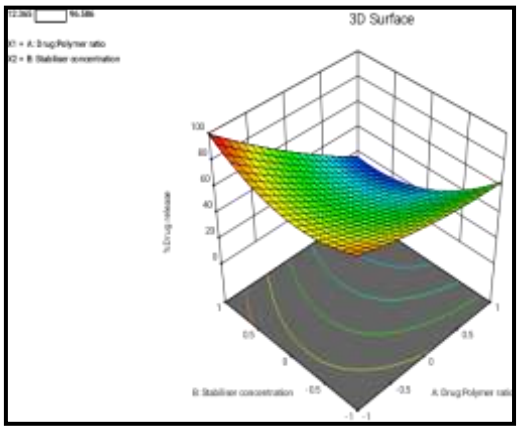

Figure-9. 3D graph showing effect of X1 and X2 on percentage of drug release

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.

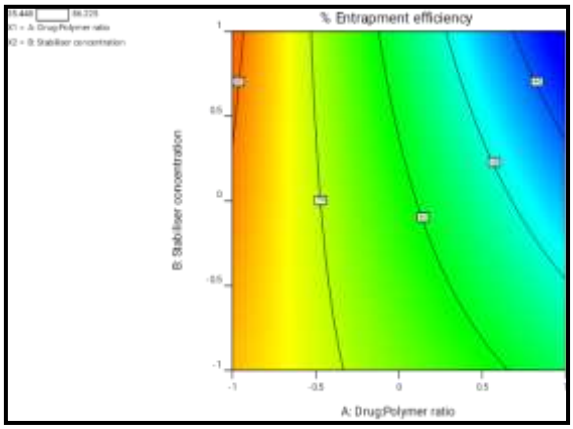

Figure-10. 2D graph showing effect of X1 and X2 on percentage of entrapment efficiency

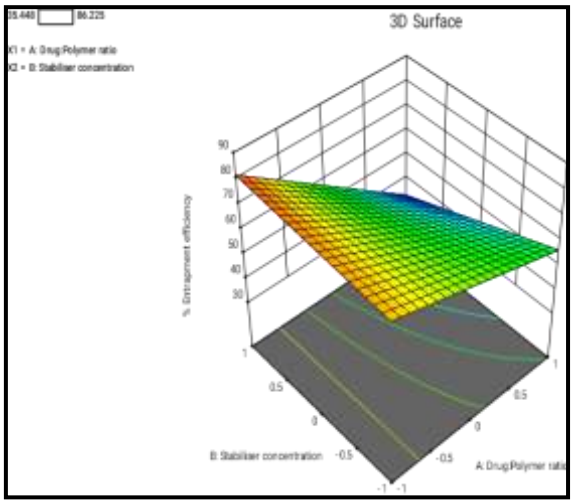

Figure-11. 3D graph showing effect of X1 and X2 on percentage of entrapment efficiency

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.

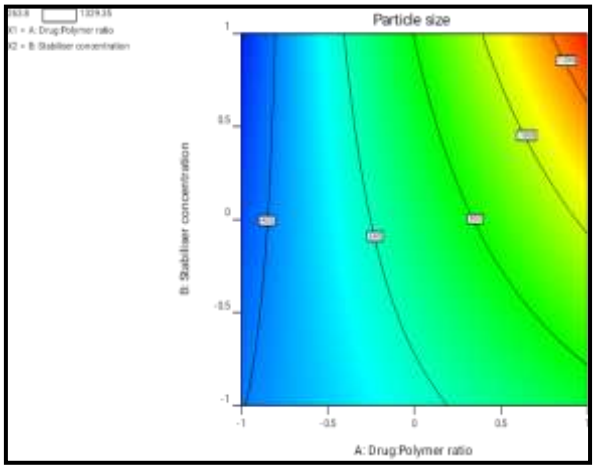

Figure-12. 2D graph showing effect of X1 and X2 on particle size

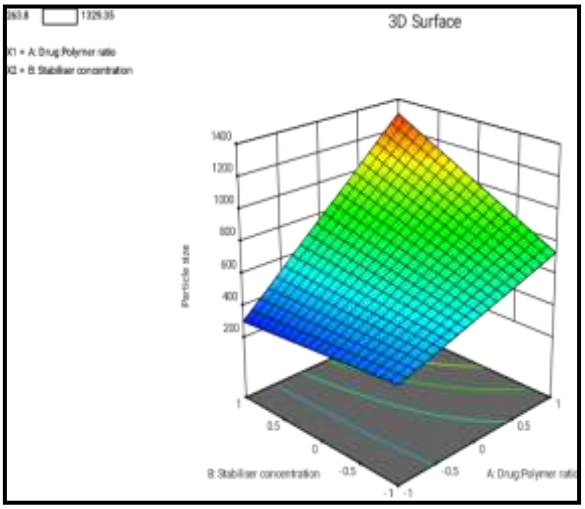

Figure-13. 3D graph showing effect of X1 and X2 on particle size

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.

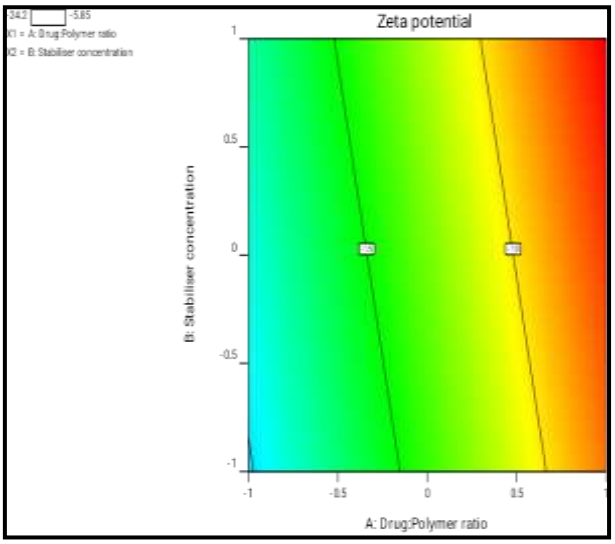

Figure-14. 2D graph showing effect of X1 and X2 on zeta potential

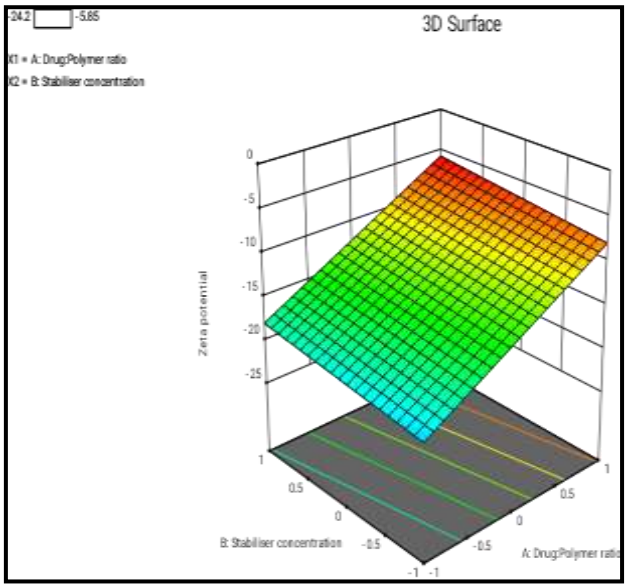

Figure-15. 3D graph showing effect of X1 and X2 on zeta potential

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

Source	Sum of Squares	Df	Mean Square	F-value	p-value	
Model	873700.00	3	291200.00	19.45	0.0003	Significant
A-Drug: Polymer ratio	679900.00	1	679900.00	45.41	< 0.0001	
B-Stabilizer concentration	84099.52	1	84099.52	5.62	0.0419	
AB	109600.00	1	109600.00	7.32	0.0242	
Residual	134800.00	9	14974.32			
Lack of Fit	123200.00	5	24636.30	8.50	0.0296	Significant
Pure Error	11587.40	4	2896.85			
Cor Total	1008000.00	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 Table-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: Polymer ratio	224.73	1	224.73	10.77	0.0083	
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-16**). 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 Peppa'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.

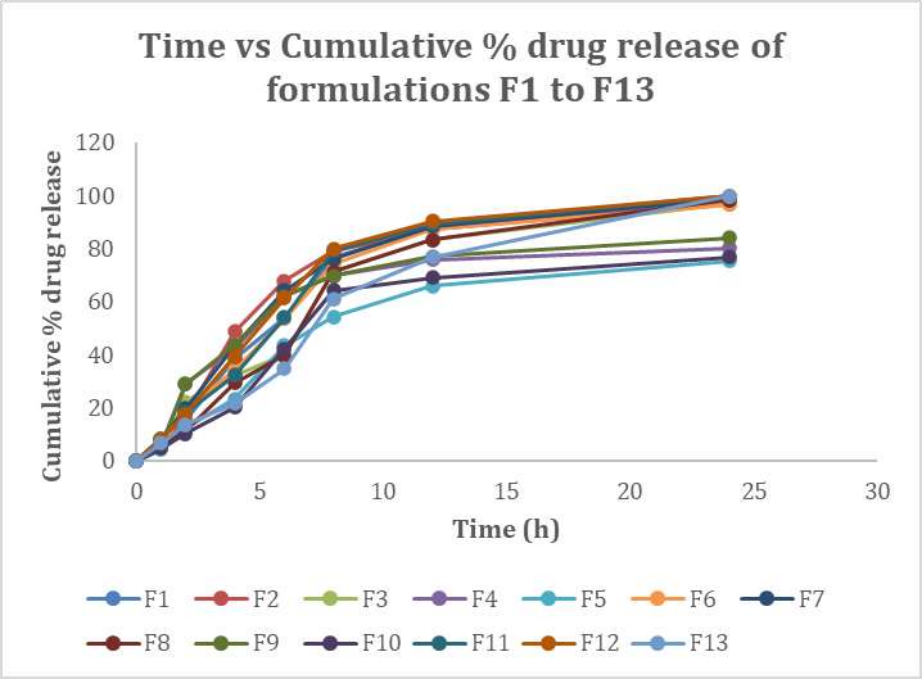

Figure-16. Drug release profile of F1-F13 formulations.

4. Conclusion

In order to create polymeric nanoparticles of carvedilol formulation, an alternative anticancer treatment, in a systematic way while optimizing the drug solubility and dissolving rate, this work used a nanoprecipitation process. After 24 hours of in vitro dissolution, the optimized polymeric nanoparticle formulation released over 90% of the medication with a particle size of 42.54 nm and an entrapment efficiency of 99.5%. Accelerated stability data showed that the optimized polymeric nanoparticles showed no significant change in storage after six months. This study's findings demonstrated that synthetic polymeric carvedilol nanoparticles released their payload efficiently in suspension and had an excellent sustained release profile when tested in vitro. More consistent and long-lasting formulations with unique qualities were developed with the help of quality-by-design. Hydrophobic drugs like carvedilol benefit greatly from NPs because of their enhanced solubility and controlled release properties.

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. Ravi Kumar: 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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