Solubility Enhancement of Carvedilol Polymeric Nanoparticles by Nanoprecipitation Technique

Mallika Tamminana[1]*, and B.V.V. Ravi Kumar[2]

[1]Ph.D. Research Scholar, Pharmacy, Biju Patnaik University of Technology, Rourkela, Roland Institute of Pharmaceutical Sciences, Berhampur-760010, Odisha, India

[2]Roland Institute of Pharmaceutical Sciences, Berhampur-760010, Odisha, India

***Corresponding Author:**

Ms. Mallika Tamminana

Ph.D. Research Scholar, Pharmacy,

Biju Patnaik University of Technology, Rourkela,

Roland Institute of Pharmaceutical Sciences, Berhampur-760010, Odisha, India

Abstract:

Carvedilol is an anti-cancer drug which is used in treatment of breast cancer. But it has poor water solubility property. The research work is based on improvement of solubility by formulation and development of polymeric nanoparticles by nanoprecipitation technique. HPMC K 15M is used as polymer and Poloxamer 407 is used as surfactant. After pre formulation study the drug interaction study is performed by FTIR and DSC analysis. After preparation various evaluation methods are adopted as per the specification. The in vitro drug release study and stability study shows F6 formulation produce good entrapment capacity. The result of various kinetic studies is also acceptable. So we concluded that HPMCK15M is the appropriate polymer and Poloxamer 407 is the appropriate surfactant and nanoprecipitation is the best technique to enhance the stability of and able to enhance the anti-cancer activity.

Key word: Carvedilol, Nanoprecipitation technique, HPMC K15 M, Poloxamer 407.

Introduction

Particles that are anyplace between one and one hundred nano meters in size are known as nanoparticles (NPs). Unlike bulk materials and small particles, nanoparticles may or may not exhibit size-related characteristics [1]. The size range of polymeric nanoparticles is from 1 to 1000 nm, and they are colloidal particles. Included in this category are macromolecules that have pharmaceutically active compounds embedded or adsorbed onto them [2]. The oxidative stress,

cytotoxicity, and geno toxicity are affected by polymeric NPs because of the quantum size effect [3]. It takes two solvents that are not incompatible to perform nanoprecipitation. Since they are insoluble in water, they are quickly depleted by evaporation. Placing the organic solvent out of a lipophilic solution and into an aqueous phase causes polymers to deposit at surfaces; this is the fundamental idea behind this method. The polymer can be dissolved using a water-miscible solvent with an intermediate polarity. The solution is added to an aqueous solution while stirring using a controlled addition rate or a drop wise approach [4]. Nanoparticles are formed when the polymer solution quickly diffuses into the water phase by spontaneous diffusion.. Nanocapsules or nanospheres of polymer precipitate out of nano droplets when solvent diffuses from them [5]. Nanoparticle production is unaffected by switching the order of the phases,. To stabilize colloidal suspensions, surfactants are commonly used, yet they are not required for nanoparticle production. [6, 7].

Through the absorption of polymeric nanoparticles which shows both internal and external stimuli, it is possible to construct stimulus-sensitive polymeric nanoparticles from a variety of nanostructures, including vesicles, hybrid nanoparticles, cross -linked nanoparticles, and micelles [8]. Polymeric nanoparticles may change their physical or chemical characteristics in reaction to one, two, or even more stimuli. They are able to target the affected area, keep their payloads in circulation[9]

RS-I-(9H-carbazol-4-yloxy)-3-[[2-(2-methoxyphenoxy) ethyl] amino] is the chemical formula for carvedilol. The SANS contributes to the progression of breast cancer via adrenergic receptor and propan-2-ol signaling pathways. This revelation has opened the door to cardiac-blocker drugs as potential novel anticancer treatments. This medication has an absolute bioavailability of around 25% to 35% due to its poor water solubility and strong first-pass metabolism [10].

The main objective of this study was to develop and improve carvedilol-loaded polymeric nanoparticles synthesised by nanoprecipitation in order to increase the medication's solubility in a sustained-release mechanism, thereby reducing drug dosing intervals. There are several ways to generate nanoparticles, but the most common is nanoprecipitation since it is easy and requires few steps. Optimisation of formulation variables or conditions, development of analytical methods, creation of polymeric nanoparticle systems using selected polymers, and characterization of the final nanoparticulate formulations are all crucial steps in reaching this objective.

Materials and methods:

Materials:

In order to fund this research, Reddy's Laboratories of Hyderabad, India, provided free samples of the drug Carvedilol as well as other polymers (chitosan, HPMC K 15M, Poloxamer 407). Chemical supplier acetone was Sigma Aldrich of India. The research has relied on just the highest grade vital elements throughout.

Methods:

Preformulation studies

Solubility Analysis

Add 1 milliliter of ethanol to serve as a co-solvent, and bring the total volume to 10 millilitres. Then, combine 10 milligrams of the drug with 9 millilitres of 0.1 N hydrochloric acid. Water, acetonitrile, phosphate buffers 6.8 and 7.4, 0.1 N HCl, methanol, ethanol, DMSO, PEG 200 and 400, and n-octanol were among the solvents investigated in the drug's quantitative solubility tests. Starting with 5 ml of each solvent, a little amount of the drug was added until it was completely dissolved. Finally, it was shaken for about three hours. Then, it was possible to determine which solvents dissolved the medicine. Taking 2 ml of each solvent and adding the medication till it reached its saturation point, the mixture was shaken for 3 hours to see whether it was completely soluble. We then used a UV spectrophotometer to examine the filtrates from each solvent. Tabulated and shown in Figure 1 are the results of the solubility tests conducted on pure pharmaceuticals in various solvents.[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

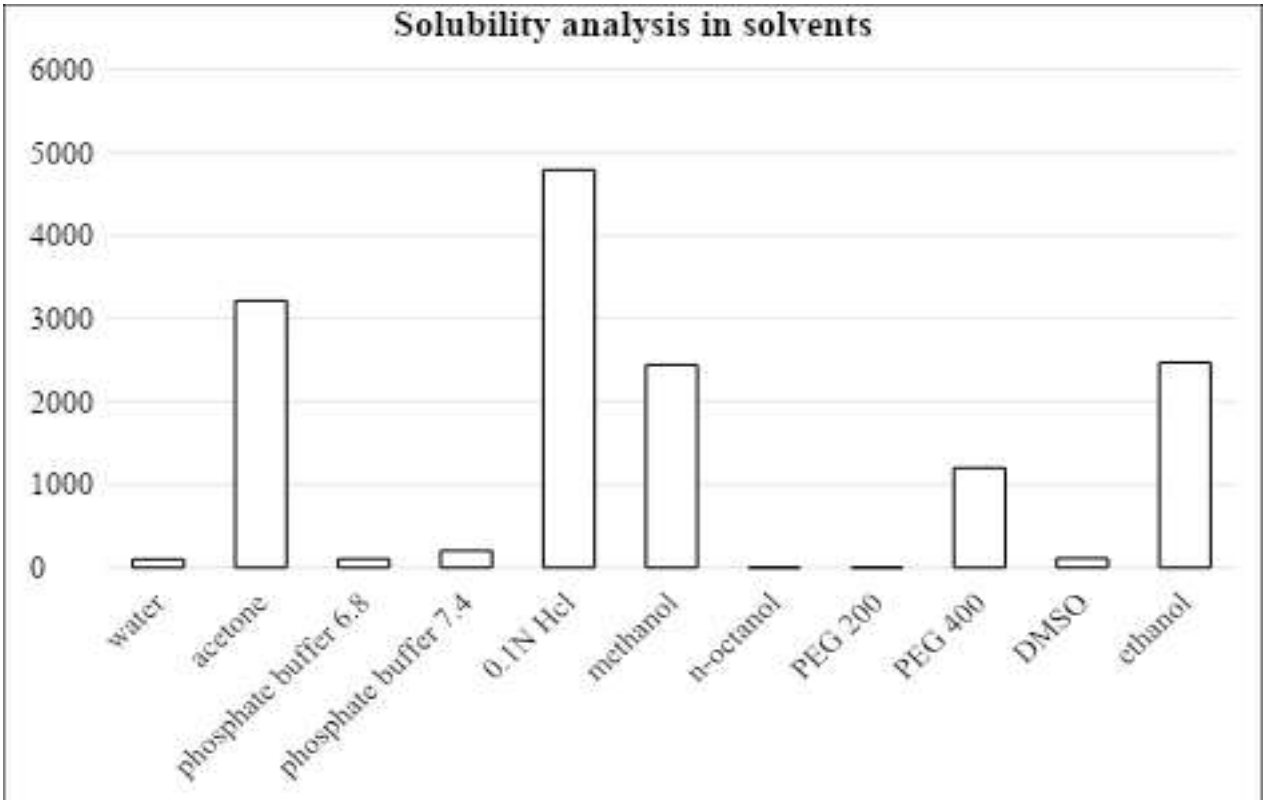

Figure 1. Bar graph showing solubility of pure drug in different solvents

Determination of λ_{max} of carvedilol

An ethanol-and-0.1N HCl combination was used to dissolve 100 mg of carvedilol in 90 ml to make the stock solution. Hence, 1000 µg/liter. After that, 1 milliliter was collected and made into a solution containing 100 microgram's per litre. Concentrations of 10, 20, 40, 60, 80, and 100 µg/ml were achieved by using these to make further dilutions. In the end, the UV technique was used to analyse it at λmax 282nm.

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

A more broad definition of QTPP would be the collection of predicted medication qualities that are necessary to demonstrate the product's safety and efficacy and to detect any possible product CQAs. The QTPP was determined in compliance with all applicable scientific and regulatory standards, as demonstrated in Table-2. In order to regulate the progress of products and methods, QTPPs are in charge, and this allows for Critical Quality Assessments. Critical process parameters (CPPs) and important material characteristics (CMAs) are two resources that are utilised during the synthesis of nanomaterials.[12].

Optimization by response surface methodology

The optimisation was carried out using Design Expert 12.1.1, which is a product of Minneapolis, MN-based State-Ease Inc. Drug release (%), entrapment efficiency (%), particle size (nm), and zeta potential (mV) were among the assessed responses. The effects of the drug-to-polymer ratio (A) and stabilizer concentration (B) on these variables were also studied independently. The two-component, three-level optimisation scheme is shown in Table 2. There were thirteen separate runs outlined in the model, and the results of each run were recorded. Ultimately, the mixture that yielded the best results was selected for further investigation.

Method of Preparation of Polymeric nanoparticles

Solvent evaporation and nanoprecipitation were used to create nanoparticles. The first thing to do is to measure out 1:3, 1:2, or 1:1 medication to water ratios. (20 milligrams of medication): 20 milligram of polymer, 40 milligrams of medication: (80 mg polymer, 60 mg medication)5 millilitres of glacial acetic acid and acetone were used to dissolve 180 milligrams of chitosan and HPMC K15M. A magnetic stirrer was used to combine the polymeric and drug solutions after 10 millilitres of acetone had been used to dissolve the pure prescription carvedilol. The drug-polymer solution and 5 ml of the solution were combined with 20 ml of distilled water containing 1%, 1.5%, or 2% poloxamer 407 to make 20 ml. A rotating flask evaporator running at decreased pressure was used to evaporate 10 ml of acetone. Slowly adding the medicine to the polymer mixture will cause it to dissolve. The medication and polymer were carefully added at a rate of 1 ml/min using a #27-gauge needle-size syringe. You should be able to complete the addition procedure in one hour at 1000 rpm. Up until 20 ml of acetone remained, the rest was

evaporated in a rotating flask. A capacity of 10 millilitres was specified. The nano fluid was freeze-dried for 36 hours to produce powdered polymeric nanoparticles.

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)

The solid substance (drug) should be ground into a fine powder using dry potassium bromide. Make sure the dosage is enough to cover the disc's area in terms of the substance's weight. Use a specialized die to place a little amount of the mixture and push it down under intense suction. Then, place the finished disc in an appropriate mount. Potassium bromide undergoes 45 IR scans in 1.5 minutes. After gathering blank spectra of the ambient air, we were able to acquire the sample spectrum. Scannable samples included both polymer-only and medication-containing formulations. (**Figures-2 to 4**) [13].

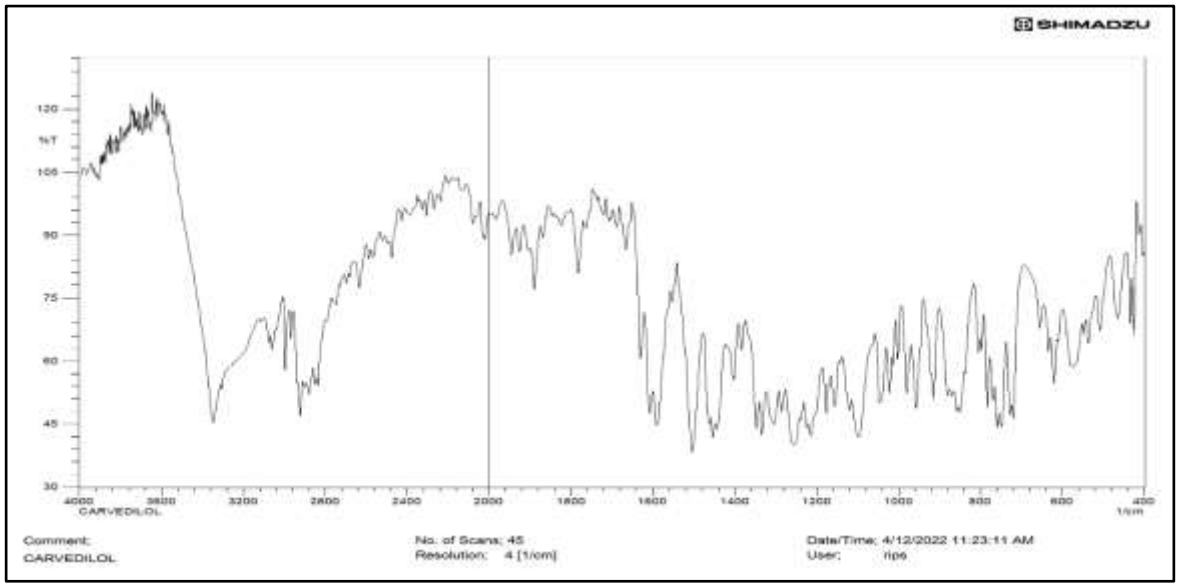

Figure-2. FT-IR spectra of Carvedilol

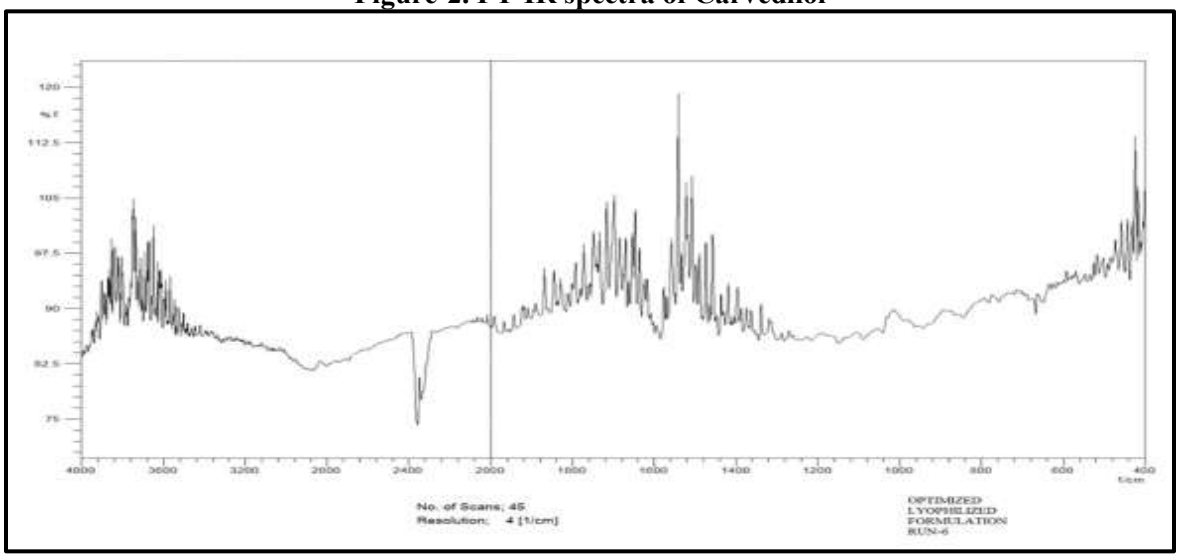

Figure-3. FT-IR spectra of physical mixture of carvedilol + poloxamer 407 + HPMC K15M + chitosan

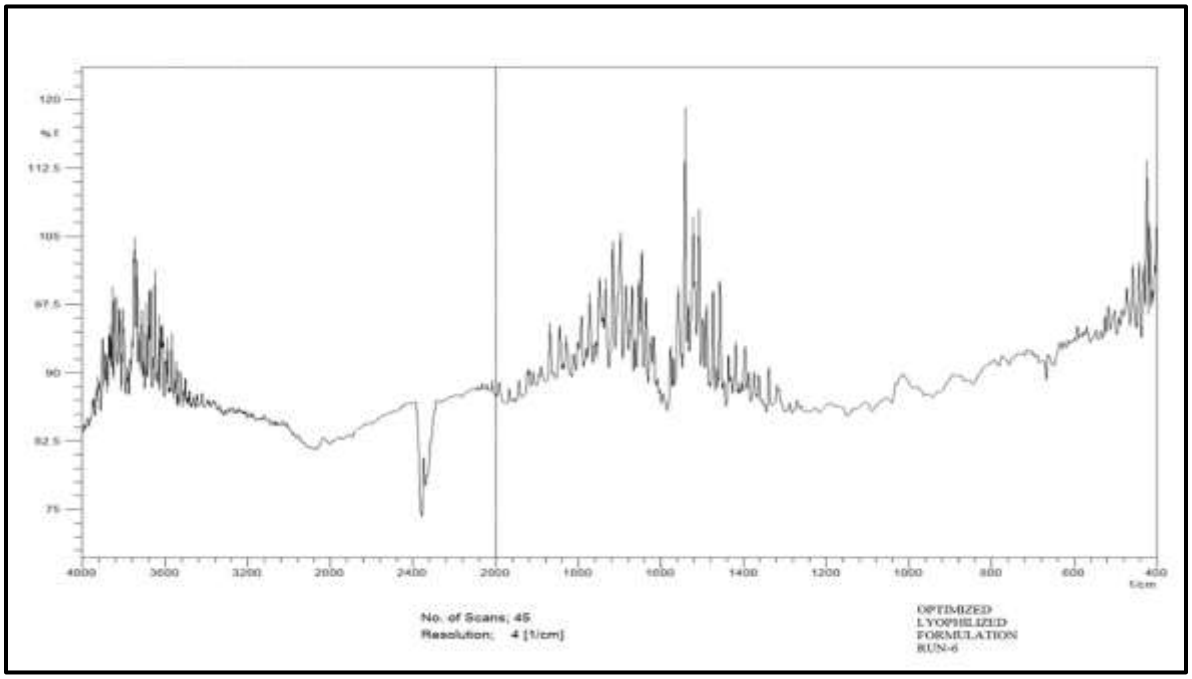

Fig:4 FT-IR Spectra of Optimized Lyophilized Nanoformulation Differential Scanning Calorimetry

Research using DSC measured how the medicine and polymer interacted. A thermogram was taken of poloxamer 407, carvedilol, and polymers. Figures 5–7 indicate that the melting point of carvedilol, at 125.50°C, is correlated with an endothermic peak on its differential scanning calorimetry (DSC) curve, which began at 113.20°C and ended at 120.90°C.[14].

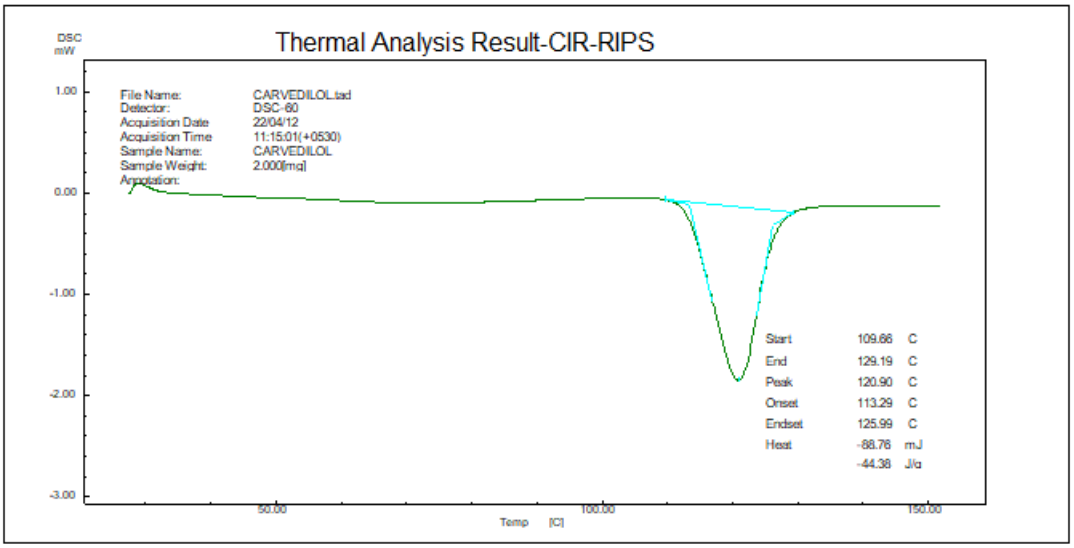

Figure-5. DSC thermogram of Carvedilol

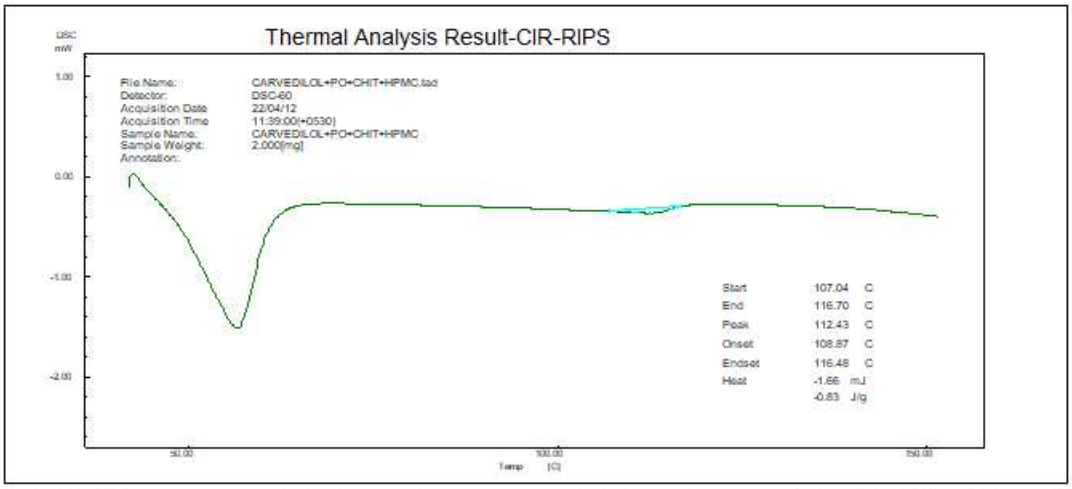

Figure-6. DSC thermogram of physical mixture of carvedilol + poloxamer 407 + HPMC K15M + chitosan

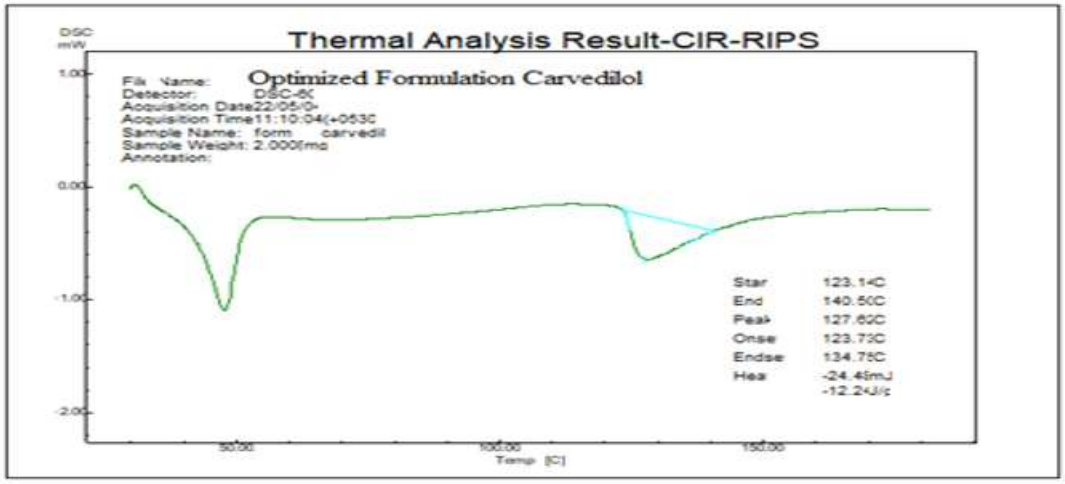

Figure-7. DSC thermogram of optimized lyophilized nano formulation

Entrapment Efficiency:

An additional 2 ml of each formulation was added to a centrifuge tube. We spun the sample for around 25 minutes at 8000 rpm after we got the tube. At the end of the 25-minute period, we carefully removed the tube from the centrifuge and observed if a layer of supernatant had formed above the sample. After that, 1 millilitre of the liquid's supernatant was carefully transferred to a test tube, and then 10 millilitres of solution containing ethanol and 0.1 N hydrochloric acid was added. Afterwards, the sample solution was examined using ultraviolet light at a wavelength of 282 nm. [15].

Statistical analysis and optimization of variables using experimental design

Statistical analysis: The formulation optimisation and estimate of its key method parameters (CMAs) were carried out using Design-Expert® (Version 12), a sophisticated statistical program developed by Stat-Ease Inc. of Minneapolis, MN, USA. Microsoft Excel 2007 (Microsoft, USA) was used to evaluate the data.

Optimization of Process Variables:

A number of formulation and preparative factors of the nanoprecipitation technology were investigated in order to regulate and improve the process. Among these factors were the polymer's type and concentration, the chosen organic solvent, the stabilizer's content, and the solvent-to-non-solvent ratio. Modifying the drug-to-polymer ratio allowed us to study the effects on drug release, entrapment efficiency, particle size, and zeta potential. We conducted this experiment to see how various stabilizer concentrations, temperatures, and solvent/non-solvent ratios affected the nanoprecipitation of carvedilol nanoparticles. We also wanted to know how varied stirring rates affected nanoparticle production. To observe the impact of varying the stirring speed on the nanoparticles' visual appearance, the nanoprecipitation method was used.[16].

Particle Size Distribution and Zeta Potential Determination

Size distribution and droplet size examinations were performed on the improved formulation using a Malvern Zeta sizer (Nano ZS-90 U.K.). Figure-8 displays the distribution of the optimal formulation droplet size.

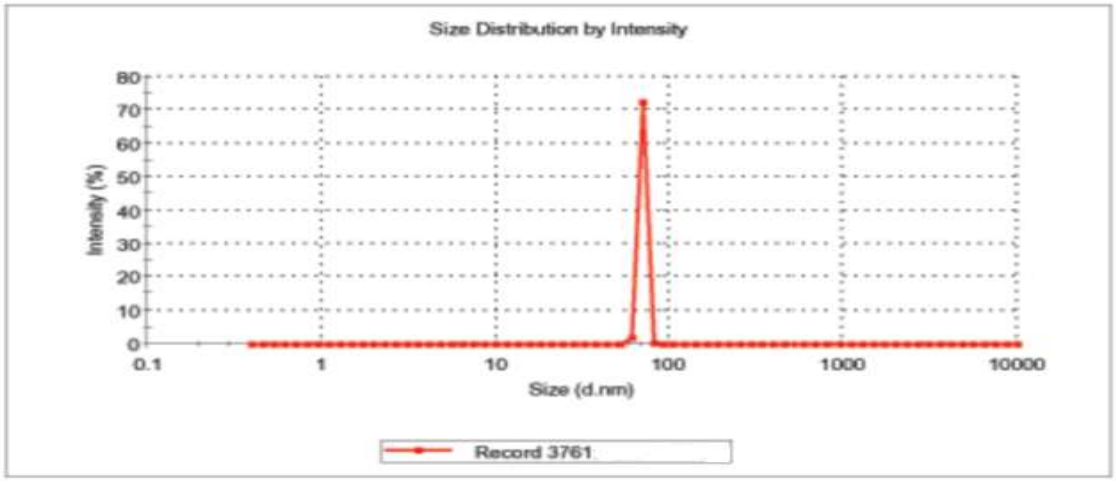

Figure-8. Droplet size distribution of formulation

***In-vitro* Diffusion Study:**

The initial stage involved adding 150 ml of 0.1 N HCl to each beaker. Then, via thread, about 5 millilitres of each formulation sample (F1–F9) was delivered to the dialysis membrane bag. In the first two hours, submerge the membrane bag in beakers containing 0.1 N HCl before moving on to a 6.8 pH phosphate buffer solution. When the dialysis membrane is at the correct position within the solution, spin it at 100 rpm using the magnetic stirrer. Start by pipetting 2 millilitres of the sample into the centrifuge tube at 0 hours. To keep the sink condition going, add 2 millilitres of 0.1 N HCl to the beaker. At 2,4,6,8,12, 18, and 24 hours, the same procedure was performed. At the end of the first 24 hours, after dividing the 2 millilitres of sample, 1 millilitre of sample was combined with 1 millilitre of ethyl acetate. Prior to setting aside for another fifteen minutes, the aforementioned solution was vortexed in a cyclomixture. The liquid layer of supernatant was carefully transferred to a test tube and allowed to dry in a water bath once it had formed. Test tubes were prepared for ultraviolet (UV) analysis by adding the correct solvent after complete drying (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 first solubility analysis and standard curve for the pure medication were obtained using UV spectroscopy, as shown in Table-1. In 0.1 N HCl, the linearity range was found to be up to 80 µg/ml. Therefore, within this concentration range, it followed Beer Lambert's rule. Using FT-IR and DSC, we characterized pure drugs for melting point and compatibility with various excipients and polymers. There was no evidence of drug-polymer interaction in the DSC investigations. We successfully generated carvedilol-loaded polymeric nanoparticles using the nanoprecipitation approach. The first three batches' formulations seemed hazy when the procedure was performed at 700 C with 500 rpm stirring speed. We were able to obtain carvedilol nanoparticle compositions with variable concentrations by lowering the speed to 1200 rpm and the temperature to 370 C.

Particle size analysis: The size and dispersion of nanoparticles have a significant impact on their adhesion and interaction with cells. The particle size peaked in Run 11 at 1329.35 nm, or (1, 1), and peaked in Run 6 at 263.8 nm, or (-1, 0).

Zeta Potential: The electro kinetic potential in colloidal systems is known technically as the zeta potential, and it is a crucial feature in nanomedicine. In addition to the physical and pharmacokinetic characteristics of nanosystems within the body, the zeta potential can affect the phagocytosis of nanoparticles in the bloodstream. The maximum zeta potential was -5.85 mV at Run 11, or (1,1), while the lowest was 24.2 mV at Run 6.

Entrapment Efficiency : This study examines how different process factors affect the amount of medication placed into polymeric matrices is known as the EE. In run 11, the lowest percentage of the entrapped drug was 35.448, or (1,1). In Run 6, specifically at (-1, 0), the percentage of entrapped pharmaceuticals reached its highest of 86.225%. There may be an effect on the formulation's stability due to the much lower zeta potential measurements taken for the individual formulations.

Differential Scanning Calorimetry: Figure 3 displays the data for the experiment, the pure drug carvedilol, and the polymers stabilizer poloxamer 407's peak temperature, start and end set

temperature, and heat energy. The experiment was carried out using differential scanning calorimetry. The medication and polymers did not interact, according to the DSC analysis.

Optimization of Process Variables:

The following factors and circumstances were optimised during preparation: Drug: The polymer ratio (in milligrams) and stabilizer concentration (in grams per millilitre) used to make carvedilol polymeric nanoparticles are shown in Table 2 as a result of nanoprecipitation. A non-toxic solvent called PEG 400 was used to form the diffusing phase by vortex mixing the polymer. An air-free, clear solution was produced by meticulously weighing the medicine, adding it to the polymeric mixture, vortexing it, and then letting it stand. In the water-based dispersion phase that included the stabilizer, the non-solvent polymer and medicine did not dissolve. One millilitre of the diffusing phase and nineteen millilitres of the dispersion phase (non-solvent) were combined to form the nanoparticles. The needle was then put straight into the aqueous medium using a syringe, and at 350C, moderate magnetic stirring (1200 rpm) was applied. The polymer crystallized, encasing the drug, shortly after the solvent had permeated the dispersion media. Surface tension variations, flow diffusion, and interfacial turbulences at the solvent : non-solvent interface were all identified as contributing elements to the Marangoni effect's quick nanoparticle formation.

Response surface analysis of 2D and 3D plots

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

The effect of the observed percentage of drug release on the stabilizer concentration and drug:polymer ratio is shown by the response (3D) plots and the counter (2D) plots in Figures 9 and 10. There is a notable fluctuation in the % medication release characteristic, and the stabilizer concentration rose with time (with a low level coded value of -1, 0 contained in the model). However, when the level climbs dramatically from low to high (from 0 to +1), that is, when the drug:polymer concentration increases greatly, a dark green colour zone mimics the big influence on the percentage drug release of polymeric nanoparticles.

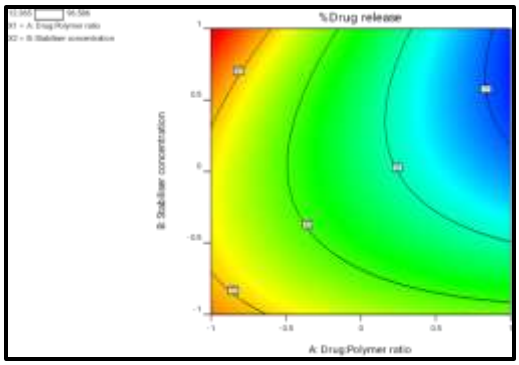

Figure-9. 2D graph showing effect of X1 and X2 on percentage of drug release

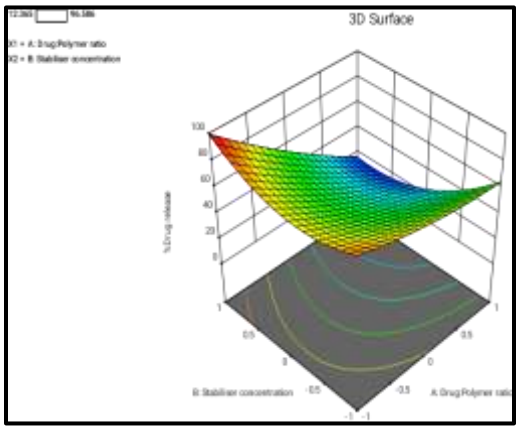

Fig:10: 3D graph showing effect of X1 and X2 on percentage of Drug Release

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

Figures 11 and 12, which show counter (2D) and response (3D) plots, respectively, show that the release behaviour slows down as the drug:polymer concentration increases due to an increase in the amount of size aggregation. Consequently, the red region's predominance specifies an ideal % entrapment efficiency, which is enhanced by this. Take carvedilol polymeric nanoparticles made using HPMC K15M and chitosan polymers as an example. Contrary to other polymers, a much lower fraction of HPMC K15M was able to produce an abundant and desirable sustain release profile of 24 hours.

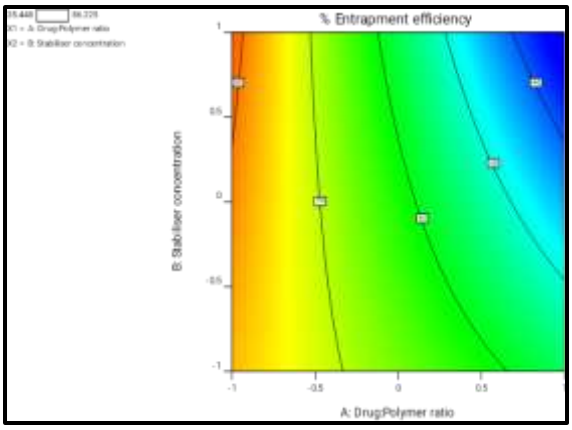

Figure-11. 2D graph showing effect of X1 and X2 on percentage of entrapment efficiency

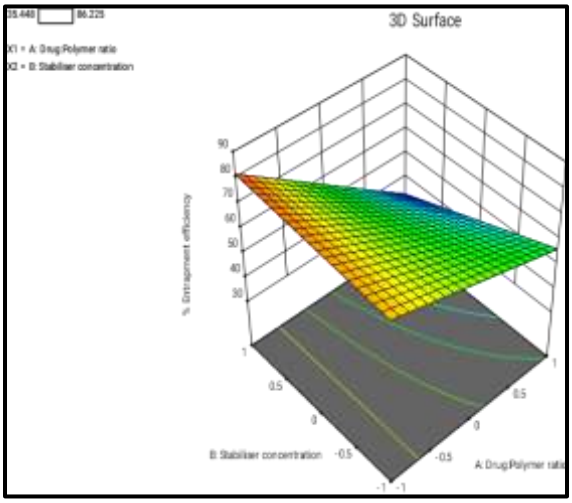

Figure-12. 3D graph showing effect of X1 and X2 on percentage of entrapment efficiency

Effect of Factors on CQA (Particle Size)

Figures-13 and 14, which are counter (2D) and response (3D) plots, respectively, showed the desired particle size of the polymeric nanoparticles for the HPMC K15M and chitosan-based formulation. This is because some polymers have a high molecular weight and chemical bonding is strong. The presence of green colour zone indicates that HPMCK 15M has a considerable shift in its high visco-elastic and swellable polymeric capabilities, which may affect the particle size.

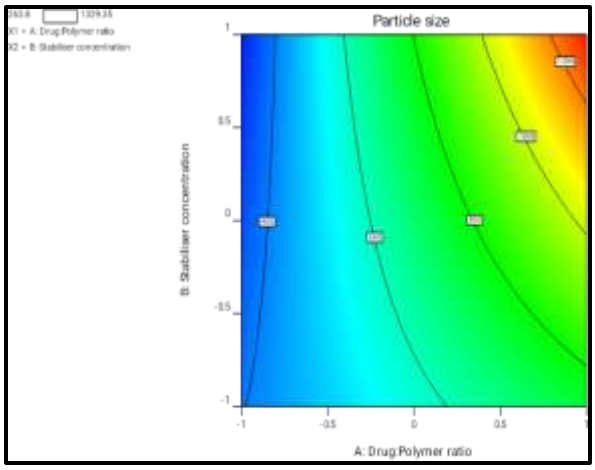

Figure-13. 2D graph showing effect of X1 and X2 on particle size

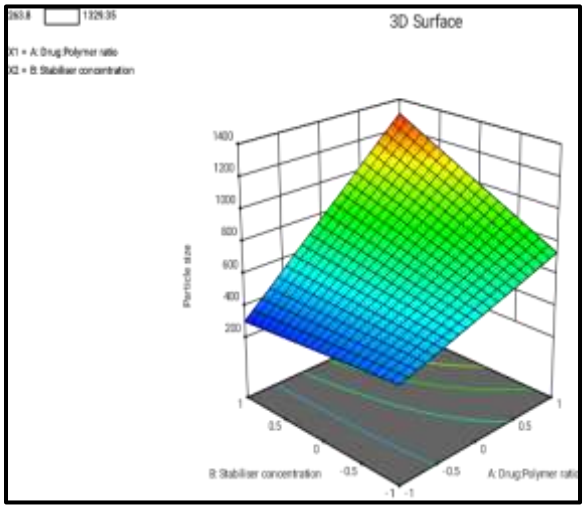

Figure-14. 2D graph showing effect of X1 and X2 on particle size

Effect of Factors on CQA (Zeta potential)

Greater zeta potential values were observed in HPMC K15M and chitosan-based polymeric nanoparticles, as shown in Figures 15 and 16 which display two-dimensional counter plots and three-dimensional response plots, respectively. The reason for this is the perfect combination of stabilizer and chosen polymers. An abnormally high drug-polymer concentration, as opposed to lower concentrations, might cause a shift in the zeta potential, as seen by the predominance of the green colour zone.

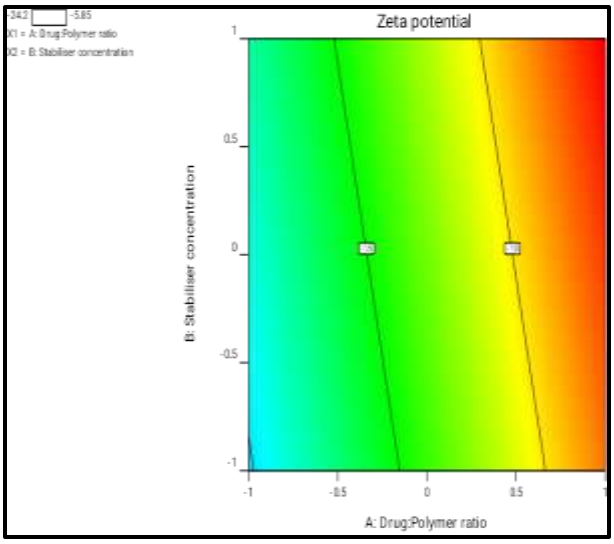

Figure-15. 2D graph showing effect of X1 and X2 on zeta potential

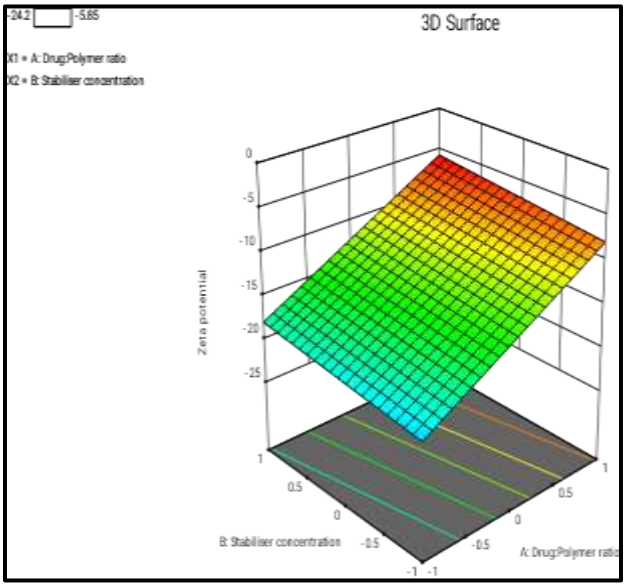

Figure-15. 2D graph showing effect of X1 and X2 on zeta potential

ANOVA for quadratic model on percentage of drug release

A model that is proven to be statistically significant with an F-value of 8.21. Just 0.77 percent of the time may this extremely high F-value be due to chance. Have P-values ≤ 0.0500 for significant model terms. In this case, the model variables A and AB are important. Any value over 0.1000 reduces the relevance of the model terms. Reducing the number of unnecessary words in any model (apart from those required to maintain hierarchy) may be beneficial. The F-value is 14.14, which indicates a significant Lack of Fit. Noise might account for such a high

Lack of Fit F-value just 1.35 percent of the time. Instead of a significant lack of fit, we would prefer a model 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 was determined to be 16.26, which is very significant. A noise-induced F-value this high is possible in 0.06% of cases. Have P-values ≤ 0.0500 for significant model terms. Here, is an important part of the model. Any value over 0.1000 reduces the relevance of the model terms. Reducing the number of unnecessary words in any model (apart from those required to maintain hierarchy) may be beneficial. Lack of Fit does not have a statistically significant impact when contrasted with pure mistake (F-value = 2.86). A notable Lack of Fit F-value is likely to be caused by noise with a probability of 16.52%. If the fit is not statistically significant, it would be fantastic as we would prefer a fitted model (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)

A high F-value of 19.45 indicates a significant model. An F-value this large might be the result of noise just 0.03% of the time. If the P-value of a model term is smaller than 0.0500, it is deemed significant. In this context, the model terms A, B, and AB are important. Values greater than 0.1000 indicate that the model terms are not significant. Improving your model could be as simple as using model reduction to get rid of any superfluous terms (beyond those required to enable hierarchy). The F-value of 8.50 indicates a substantial Lack of Fit. This big of a Lack of Fit F-value could be due to noise just 2.96 percent of the time. A big mismatch is undesirable since it is desirable for the data to be well-fitted by the model (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

A very significant model, as seen by its F-value of 5.57. Just 2.37 percent of the time might noise cause an F-value this significant. If the P-value of a model term is smaller than 0.0500, it is deemed significant. The model word "A" holds significant weight in this instance. Values greater than 0.1000 indicate that the model terms are not significant. Improving your model could be as simple as using model reduction to get rid of any superfluous terms (beyond those required to enable hierarchy). The Lack of Fit is deemed significant with an F-value of 8.91. Such a large Lack of Fit F-value can only occur 2.64 percent of the time due to noise. A big mismatch is undesirable since it is desirable for the data to be well-fitted by the model (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:**

The drug release at different hours after the pharmaceutical formulation was administered is shown in Table 3, which is the outcome of a diffusion investigation. In run 6, the maximum percentage of drug release was 96.58%, as shown in Figure 17. An research of kinetic diffusion in vitro revealed that the drug release occurred according to first-order kinetics. Drug release regulated by diffusion was the main mechanism, according to the Higuchi equation's higher correlation coefficient. Since Korsmeyer Peppa's release exponent was more than 0.5 and less than 1 for all formulations, it seems that the drug was released by a method other than Fickian diffusion.

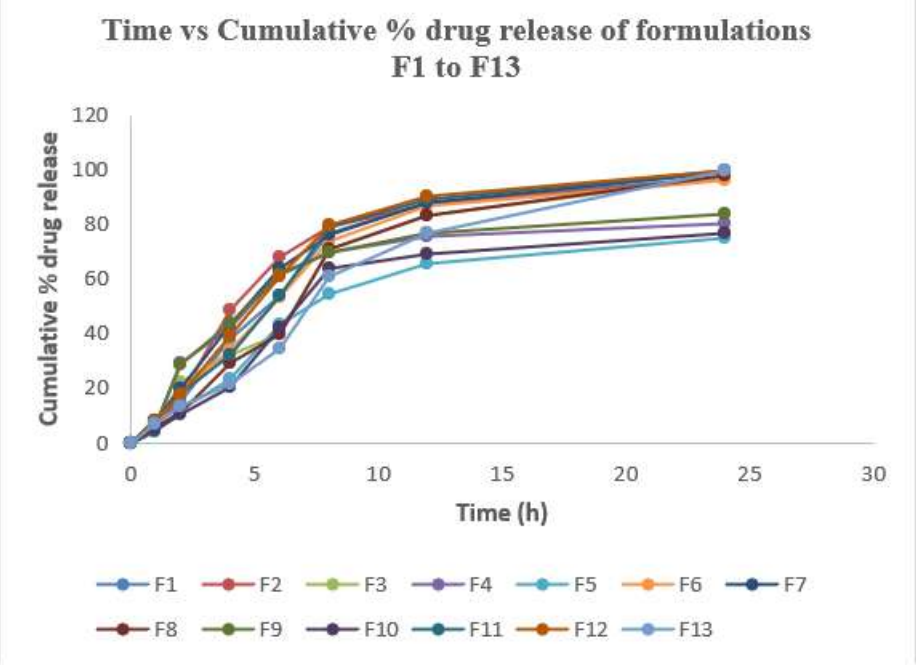

Fig: 17: Percentage of Drug Release of Formulations F1-F13

Conclusions:

An alternative anti-cancer treatment, Carvedilol, was manufactured into polymeric nanoparticles in this work by methodically optimizing the drug's solubility and dissolving rate using a nanoprecipitation approach. The optimised polymeric nanoparticle formulation had a 99.5% entrapment rate and a particle size of 42.54 nm. After 24 hours of in vitro dissolution, more than 90% of the medication was liberated. Based on the accelerated stability results, the optimised polymeric nanoparticles showed no noticeable change in storage after six months. Using a

sustained release profile in vitro, the results demonstrated that the synthetic polymeric carvedilol nanoparticles efficiently released their payload when suspended. The use of quality-by-design allowed for the development of more stable and long-lasting formulations, each with its own unique set of benefits. The regulated release and improved solubility of hydrophobic medicines, such as carvedilol, make NPs an ideal delivery vehicle.

References

1. Jeevanandam J, Barhoum A, Chan YS, Dufresne A, Danquah MK. Review on nanoparticles and nanostructured materials: history, sources, toxicity and regulations. Beilstein J Nanotechnol. 2018; 9:1050-1074.

2. Crucho CIC, Barros MT. Polymeric nanoparticles: A study on the preparation variables and characterization methods. Mater Sci Eng C Mater Biol Appl. 2017; 80:771-784. doi: 10.1016/j.msec.2017.06.004. Epub 2017.

3. Prabha AS, Dorothy R, Jancirani S, Rajendran S, Singh G, Kumaran SS. Chapter-7: Recent advances in the study of toxicity of polymer-based nanomaterials, Editor(s): Susai Rajendran, Anita Mukherjee, Tuan Anh Nguyen, Chandraiah Godugu, Ritesh K. Shukla, In Micro and Nano Technologies, Nanotoxicity, Elsevier, 2020, Page: 143-165.

4. Muddana Eswara Bhanoji Rao, Suryakanta Swain, and Sitty Manohar Babu. Chapter-8, Polymeric nanoparticles as carrier for controlled release. Pharmaceutical Drug Delivery Systems and Vehicles. Woodhead Publishing India Medicine Pvt. Ltd., an Elsevier imprint, India, 2015, ISBN: 9789385059001, 216-244.

5. Muddana Eswara Bhanoji Rao, Suryakanta Swain*, Chinam Niranjan Patra, Shakti Prasad Mund. Formulation design, optimization, and characterization of eprosartan mesylate nanoparticles. Nanoscience & Nanotechnology-Asia. 2018; 8(1): 130-143.

6. Zielinska A, Carreiro F, Oliveira AM, Neves A, Pires B, Venkatesh DN, Durazzo A, Lucarini M, Eder P, Silva AM, Santini A, Souto EB. Polymeric Nanoparticles: Production, Characterization, Toxicology and Ecotoxicology. Molecules. 2020;25(16):3731.

7. Sameer S. Sheaikh & Shrikrishna K. Harkal & Rahul P. Gaikwad & Rahul W. Gawali & Dinesh P. Deshmukh, 2018. "Formulation and Evaluation of Polymeric Nanoparticles of Rifampicin for Anti-Tubercular Therapy, Int J Healthcare Medical Sci. Academic Research Publishing Group. 2018; 4(6), 117-122.

8. Kesarla R, Tank T, Vora PA, Shah T, Parmar S, Omri A. Preparation and evaluation of nanoparticles loaded ophthalmic in situ gel. Drug Deliv. 2016;23(7):2363-2370. doi: 10.3109/10717544.2014.987333.

9. Li Y, Gao GH, Lee DS. Stimulus-sensitive polymeric nanoparticles and their applications as drug and gene carriers. Adv Healthc Mater. 2013;2(3):388-417.

10. El-Say KM, Hosny KM. Optimization of carvedilol solid lipid nanoparticles: An approach to control the release and enhance the oral bioavailability on rabbits. PLoS One. 2018;13(8): e0203405. doi: 10.1371/journal.pone.0203405.

11. Singh B, Khurana L, Bandyopadhyay S, Kapil R, Katare OO. Development of optimized self-nano-emulsifying drug delivery systems (SNEDDS) of carvedilol with enhanced bioavailability potential. Drug Deliv. 2011;18(8):599-612. doi: 10.3109/10717544.2011.604686. Epub 2011.

12. Arun JK, Vodeti R, Shrivastava B, Bakshi V. Integrated Quality by Design Approach for Developing Nanolipidic Drug Delivery Systems of Olmesartan Medoxomil with Enhanced Antihypertensive Action. Adv Pharm Bull. 2020;10(3):379-388. doi: 10.34172/apb.2020.046. Epub 2020.

13. Mishra A, Imam SS, Aqil M, Ahad A, Sultana Y, Ameeduzzafar, Ali A. Carvedilol nano lipid carriers: formulation, characterization and in-vivo evaluation. Drug Deliv. 2016;23(4):1486-94. doi: 10.3109/10717544.2016.1165314. Epub 2016.

14. Ravikumar AA, Kulkarni PK, Osmani RAM, Hani U, Ghazwani M, Fatease AA, Alamri AH, Gowda DV. Carvedilol Precipitation Inhibition by the Incorporation of Polymeric Precipitation Inhibitors Using a Stable Amorphous Solid Dispersion Approach: Formulation, Characterization, and In Vitro In Vivo Evaluation. Polymers (Basel). 2022;14(22):4977. doi: 10.3390/polym14224977.

15. Selselehjonban S, Garjani A, Osouli-Bostanabad K, Tanhaei A, Emami S, Adibkia K, Barzegar-Jalali M. Physicochemical and pharmacological evaluation of carvedilol-eudragit® RS100 electrosprayed nanostructures. Iran J Basic Med Sci. 2019;22(5):547-556. doi: 10.22038/ijbms.2019.34246.8139.

16. Xia D, Quan P, Piao H, Piao H, Sun S, Yin Y, Cui F. Preparation of stable nitrendipine nanosuspensions using the precipitation-ultrasonication method for enhancement of dissolution and oral bioavailability. Eur. J. Pharm. Sci. 2010; 40, 325-334