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Preparation of Structurally Modified Plumbagin Silver Nanoparticle for its Prospective Binding to Trastuzumab

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ABSTRACT

Combination therapy plays a major part in reducing side effects in treatment of breast cancer. For treating HER2-positive breast cancer, HER2-targeted therapy is common. A natural compound, Plumbagin, gives activity against the HER2 receptor that can be employed in breast cancer therapy. A conjugation of plumbagin with Trastuzumab increases its targeting specificity to the HER2 receptor. However, the low stability of plumbagin necessitates an encapsulation in silver nanoparticles (AgNPs) to widen its stability and therapeutic efficacy. This study using Nano-precipitation technique, the silver nanoparticles were synthesized in varying concentrations of polyvinylpyrrolidone (PVP), silver nitrate, and sodium borohydride. The parameters of formulations are particle size, polydispersity index (PDI), and zeta potential, were optimized through statistical analysis by QBD design. Based on these parameters, a best formulation was selected. The successful loading of Plumbagin into the nanoparticles attained 85% entrapment efficiency. The release kinetics of the drug within 24 hours was approximately 89%. Nanoparticles were structurally modified with carbonyl groups to facilitate the conjugation with Trastuzumab, also this promotes attachment of the monoclonal antibody through its amine group. Scanning electron microscopy (SEM), Transmission electron microscopy (TEM), and Fourier-transform infrared (FTIR) spectroscopy tools recorded the confirmation of surface conjugation. This approach ensures a obligation strategy for the target delivery and also stability of plumbagin.

Keywords: *Antibody conjugation, Plumbagin, Trastuzumab*

1. INTRODUCTION

The most common cancer types seen in female are the breast cancer. The development of breast cancer involves several type of gene that need to be activated or inactive for promoting the malignancy. Understanding the key molecule pathway for tumor growth and survival will facilitated the development of the targeted therapies [1]. The use of chemotherapy and antibody therapy as adjuvant used to treat of early stage breast cancer. Antibody therapy targeting the HER2 pathway was shown to improve progression and survival in metastasis disease condition [2]. Plumbagin PLB (2-methyl-5-hydroxy-1, 4 naphthoquinone is a chemical that is isolated from the root of plant belong to plumbaginacea family. Plumbagin can be used for the treatment of breast cancer which inhibits proliferation of cancer cell by inducing apoptosis. Since plumbagin have low stability its stability can increase by transforming to nanoparticle. Plumbagin is also have low shelf life if it is converted to silvernanoparticle it promise a slow release of drug with more stability. Plumbagin silver nanoparticle alone can be used in chemotherapy meanwhile non target tissue are also exposed [3].

Trastuzumab is a monoclonal antibody having more affinity towards HER2 receptor. This is considering as the major option in standard therapy of metastasis breast cancer. Combining trastuzumab with chemotherapy will promote the apoptosis action [4]. When conjugates with PLB silver nanoparticle to trastuzumab will target to cancer cell with less expose to normal tissue. Trastuzumab have no capability for direct combination with nanoparticle. It requires modification in its surface for controlled conjugation. Since the trastuzumab have presence of amine group successful conjugation requires presence of carboxyl group. Introduction of carboxyl group will promote the conjugation process [5].

2. MATERIALS AND METHODS

Reagents required:

Plumbagin and Trastuzumab (sigma Aldrich), silver nitrate,PVP and 11-Mercaptoundecanoic acid (otto chemic pvt limited). Milli-Q water from India Mart. N- Hydroxy succinimide (NHS) from Hemant Bioscience Hydrabad. 2-NMorpholino ethane sulphonic acid(MES), 1-ethyl-3-(3-dimethylaminopropyl carbodiimide (EDC) from India Mart.

Preparation technique of Silver Nanoparticles:

The silver nanoparticle was prepared using chemical reduction method. A synthesis mixture was prepared by dissolving $AgNO_3$ 0.1 g and PVP 0.2 g in 50 ml of Milli-Q water. The mixture was stirred in room temperature at 1000 RPM until mixture fully dissolved. A fresh solution of 0.05 M $NaBH_4$ solution was prepared by dissolving required amount of $NaBH_4$ in cold Milli-Q water. $NaBH_4$ solution was added drop wise to PVP/ $AgNO_3$ solution with continuous stirring over 10 minutes. Reaction was permit to proceed for an about 30 minutes to assure complete reduction of silver ions to silver nanoparticle. Prepared silver nanoparticle is collected and lyophilized for storage [6, 7].

Optimization Variables:

To achieve optimal characterisation of nanoparticle, the concentrations of $NaBH_4$, PVP and AgNO were changed systematically. The selection of these variables was based on their critical roles in synthesis of nanoparticle [8]. The upper and lower limit was selected appropriately was mentioned in Table 1:

Table 1: selection of range for variables

Name	Goal	Lower Limit	Upper Limit
A:PVP	is in range	50	150
B:Sodium borohydrate	is in range	20	60
C:Silver nitrate	is in range	50	150

PVP acts as stabilizing agent which prevent the agglomeration of nanoparticles and provide steric stabilization of nanoparticle. The concentration of PVP was adjusted between 0.1-0.3 g to study its effect on stability and particle size. NaBH is used as a reducing agent which converts the silver ions to silver nanoparticles. The concentration of NaBH was varied from 0.01-0.1 M to control the rate of reduction process and influences the size and polydispersity of the nanoparticles. AgNO is used as precursor for silver nanoparticles. Its concentration varied from 0.05-0.2 g to help its impact on the nucleation and growth of nanoparticles [9, 10].

Optimization Study Using QbD:

Quality by Design (QbD) approach was used to optimize the formulation of nanoparticle. Box-Behnken design was used to assess impact of three different key variables (PVP, $NaBH_4$, and $AgNO_3$ concentrations) as the critical quality attributes (CQAs) of the nanoparticles, such as, zeta potential particle size and polydispersity index (PDI) [10].

Plumbagin loaded to silver loading:

Silver nanoparticle was synthesized through chemical reduction method where the synthesis solution prepared by dissolving silver nitrate and PVP in distilled water. Sodium borohydride solution was added drop wise to the mixture for about 10 minute by continueous stirring for about 1000 RPM. The prepared nanoparticle is separated and centrifuged. Plumbagin solution was added to the silver nanoparticle solution [11, 12].

Entrapment Efficiency:

After preparing the nanoparticles was centrifuged, and the amount of free Plumbagin in supernatant solution was quantified using UV-visible spectrophotometry at the λmax of 420 nm [13]. The initial amount of drug added to the solution and the entrapped amount drug present in the supernatant solution were calculated using entrapment efficiency (EE %) using the following formula:

EE(%)= [(Total amount of drug present- Free drug present in supernatant liquid)/Total Drug] × 100

In Vitro Drug Release Study of loaded nanoparticle:

The *in vitro* release of Plumbagin from the nanoparticle can calculated using Franz diffusion apparatus. Phosphate-buffered saline solution (PBS, pH 7.4) was used as medium which simulate the physiological conditions. The nanoparticle suspension was placed in the donor chamber separate receptor chamber using dialysis membrane (MWCO ~12,000 Da). Samples from the receptor chamber were withdrawn at regular interval (replaced with fresh PBS), and total amount of drug released was calculated using UV-Vis spectrophotometry at 420 nm [13].

Conjugation with HER-2 Antibody (Trastuzumab):

Preparation of Trastuzumab Solution:

Reconstitute the trastuzumab solution in PBS to achieve concentration of 1 mg/mL. Filter solution through 0.22 µm membrane filter to ensure sterility and remove any particulate contaminants present in solution [16].

Nanoparticle-Antibody Conjugation:

Add the activated silver nanoparticles to the prepared trastuzumab solution in a ratio of 1:10 (nanoparticles to antibody). For example, if 1 mg of nanoparticles uses 10 mg of trastuzumab has to be added. Incubate the mixture at room temperature for about2-4 hours with gentle continuous stirring to allow conjugation with silver nanoparticle. Alternatively, the mixture can be incubated at 4°C overnight to enhance conjugation efficiency and preserve antibody activity [17].

Purification of Conjugated Nanoparticles:

Centrifuge the conjugated nanoparticles at 10,000 rpm for about 10 minutes to ensure the pellet formation of nanoparticles. Carefully decant the supernatant liquid and re suspend the pellet in PBS. Repeat the centrifugation and resuspension steps thrice to remove any unbound trastuzumab and any residual activation agents present. Perform dialysis against PBS using dialysis membrane having an appropriate molecular weight of 50 kDa to further purify the conjugated nanoparticle. Ensure the sterility of final suspension by filtering through a 0.22 µm membrane filter [17].

Confirmation of Conjugation:

Scanning Electron Microscopy (SEM):

Prepare samples for SEM by drop-casting nanoparticle suspensions into silicon wafers by drying them at room temperature. Sputter-coat the samples with gold or carbon thin layer to prevent charging. Capture SEM images to visualize the morphology of the unconjugated and conjugated nanoparticles and confirm the presence of uniform coating of trastuzumab on the nanoparticles [18].

Dynamic Light Scattering (DLS):

Hydrodynamic diameter and polydispersity index (PDI) of the unconjugated and conjugated nanoparticle prepared with DLS analyser. An increase in particle size and PDI after conjugation indicates the successful attachment of trastuzumab to the nanoparticles [19].

Zeta Potential Analysis:

Measure the zeta potential of the unconjugated and conjugated nanoparticles to assess changes in surface charge. The zeta potential of trastuzumab-conjugated nanoparticles should differ from that of the unconjugated nanoparticle; indicate presence of antibody on the surface. Compare the resultant value with that of non-conjugate one

Fourier-Transform Infrared (FTIR) Spectroscopy:

Perform FTIR spectra of unconjugated silver nanoparticle, Trastuzumab, and the conjugated nanoparticles. Note the characteristic peaks of trastuzumab, such as amide I (1650 cm^{-1}) and amide II (1550 cm^{-1}) bands, in the spectrum of conjugated nanoparticles. characterastic peak of carboxylic acid can see in the surface modification step [20].

3. RESULTS

The optimization study involved 17 preparations with varying concentrations of PVP, sodium borohydride, and silver nitrate. After applying lower and upper limit .The design will show relative concentration for PVP, Sodium borohydride and silver nitrate.

The value for each formulation's particle size, PDI, and zeta potential is measured using dynamic light scattering (DLS) and zeta potential analyzers. The results were analysed using stat-ease design expert software was used to identify optimal formulation needed for preparation. The values obtained from QBD analysis is mentioned in Table 2

Table 2 : optimization of variables.

		Factor-1	Fator 2	Factor3	Response 1	Response 2	Response 3
Standard	Sample Run	A(PVP)	B(Sodium borohydride)	C(Silver nitrate)	particle size	PDI	Zeta Potential
		mg	Mg	Mg	Nm		mV

15	1	100	40	100	21.42	0.662	-19.2
7	2	50	40	150	61.38	0.489	-22.2
4	3	150	60	100	21.71	0.622	-21.4
9	4	100	20	50	23.24	0.634	-20.1
6	5	150	40	50	73.62	0.512	-11.57
3	6	50	60	100	74.3	0.531	-20.91
16	7	100	40	100	21.42	0.662	-19.2
8	8	150	40	150	60.82	0.501	-11.1
14	9	100	40	100	21.42	0.662	-19.2
1	10	50	20	100	98.42	0.621	-10.2
5	11	50	40	50	150	0.439	-15.62
12	12	100	60	150	57.49	0.511	-11.78
2	13	150	20	100	57.44	0.56	-15.24
11	14	100	20	150	31.64	0.529	-18.4
13	15	100	40	100	21.42	0.662	-16.49
10	16	100	60	50	20.62	0.531	-20.51
17	17	100	40	100	21.42	0.662	-19.2

ANOVA used for quadratic model:

Response 1 for particle size:

Table 3: ANOVA study for particle size.

Sources	**Sum of squre value**	**df value**	**Mean squre value**	**F value**	**P value**	
Model	17055.82	9	1895.09	3.70	0.0493	Significant
A-PVP	3634.21	1	3634.21	7.09	0.0323	
B-Sodium borohydrate	167.63	1	167.63	0.3271	0.5853	
C-Silver nitrate	394.10	1	394.10	0.7691	0.4096	
AB	33.70	1	33.70	0.0658	0.8050	
AC	1437.17	1	1437.17	2.80	0.1379	
BC	202.64	1	202.64	0.3954	0.5494	
A^2	9451.06	1	9451.06	18.44	0.0036	
B^2	143.11	1	143.11	0.2793	0.6135	
C^2	1312.79	1	1312.79	2.56	0.1535	
Residual	3587.10	7	512.44			
Lack of Fit	3587.10	3	1195.70			
Pure Error	0.0000	4	0.0000			
Cor Total	20642.91	16				

The F –value of model is 3.70 which show that model is found to be significant. There is only chance of 4.93% that an F-value is large to occur (Table 3).

When p-value is less than 0.05, which means term is significant, and we can keep it in the model. For example, if the terms AAA and A2A²A2 have p-values less than 0.05, they are considered important and should stay. When a term has a p-value greater than 0.10, means term is not significant and could be removed from the model. However, we can't just remove any term some terms are needed to support the model's structure, like keeping both AAA and A2A²A2 together. When there are too many insignificant terms, we can simplify the model by removing them, which can make the model easier to understand and reduce overfitting.

P-values: By analyzing a model, we can use p-values to understand which parts of the model are important. Here P value was found to less than 0.0500 which indicate model terms are significant. In this model term A, A² are significant (Fig1). Values obtained greater than 0.1000 indicate model terms are not showing significant. Model reduction can improve model. Here there are not so many significant term

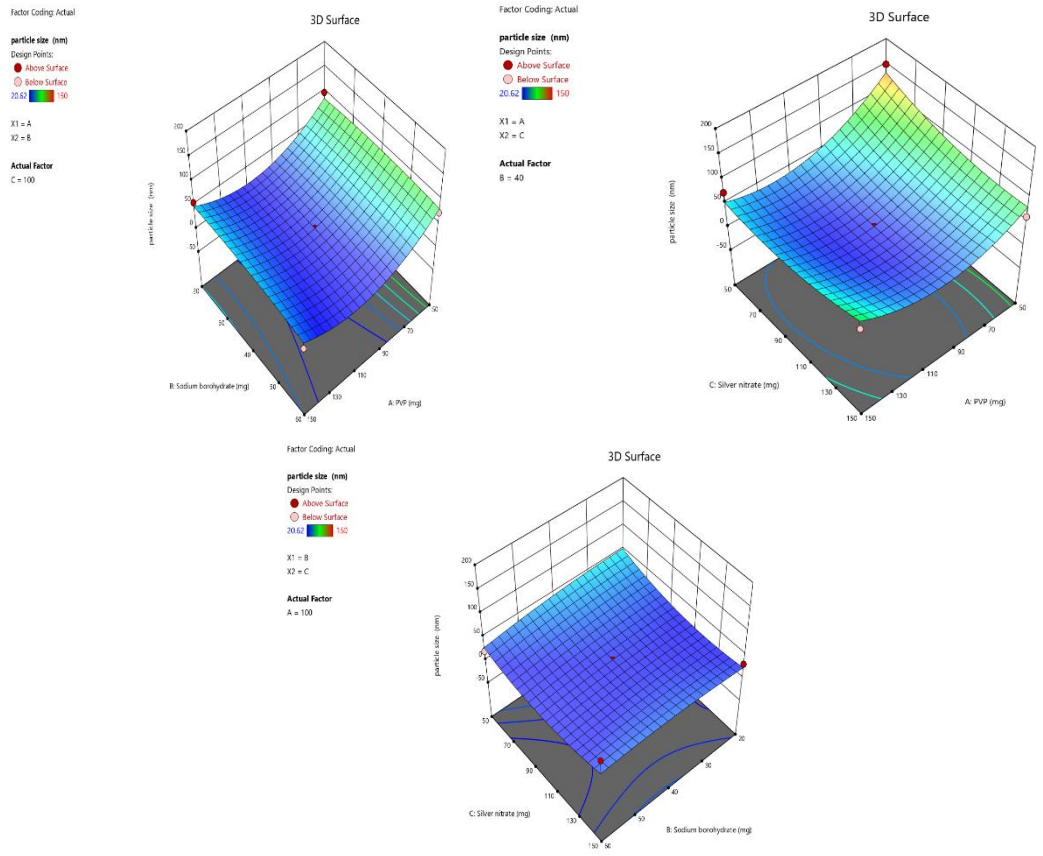


Figure 1: 3D surface response for particle size

ANOVA used for Quadratic model

Response 2: PDI

Table 4: ANOVA study analysis of PDI.

Source	Sum of square value	df value	Mean square value	F value	P value	
Model	0.0865	9	0.0096	13.95	0.0011	significant
A-PVP	0.0017	1	0.0017	2.40	0.1653	
B-Sodium borohydrate	0.0028	1	0.0028	4.03	0.0847	
C-Silver nitrate	0.0009	1	0.0009	1.34	0.2846	
AB	0.0058	1	0.0058	8.39	0.0231	

AC	0.0009	1	0.0009	1.35	0.2833	
BC	0.0018	1	0.0018	2.62	0.1494	
A^2	0.0220	1	0.0220	31.91	0.0008	
B^2	0.0002	1	0.0002	0.2388	0.6400	
C^2	0.0460	1	0.0460	66.76	< 0.0001	
Residual	0.0048	7	0.0007			
Lack of Fit	0.0048	3	0.0016			
Pure Error	0.0000	4	0.0000			
Cor Total	0.0913	16				

Here factor is coded and sum of square is partial of **Type III**. Here coded factor and the sum of square is Type 3 partial. The model F value is 13.95 which show a significant model. The F value of model is13.95 which indicates the model is significant. There is only a 0.11% chance of an F-value to be occurring (Table 4).

P Value is less than 0.0500 which indicate that model terms were found to be significant. This case AB, A^2, C^2 is significant model terms (Fig.2). Values greater than 0.1000 indicate the model is not including in significant. Here there is not much model that shows insignificant

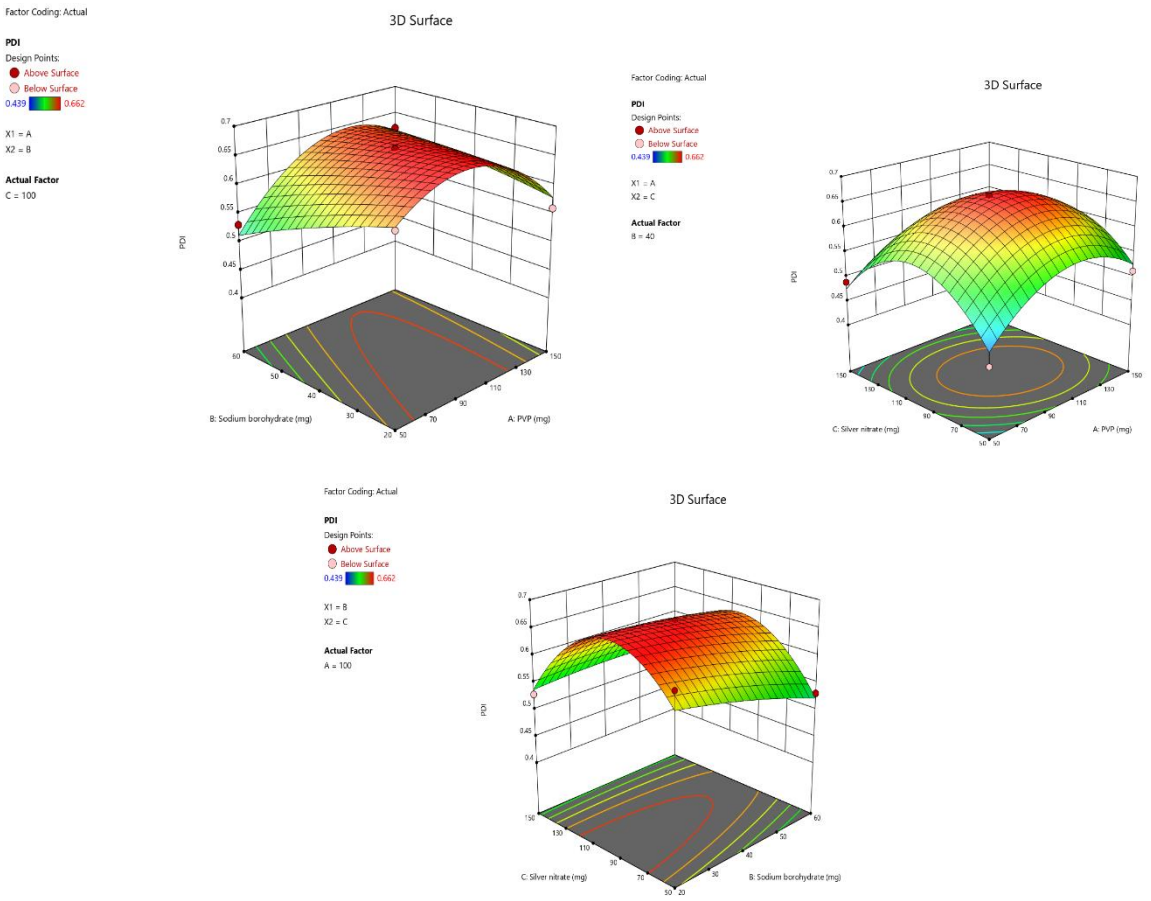


Figure 2: 3D surface response for PDI

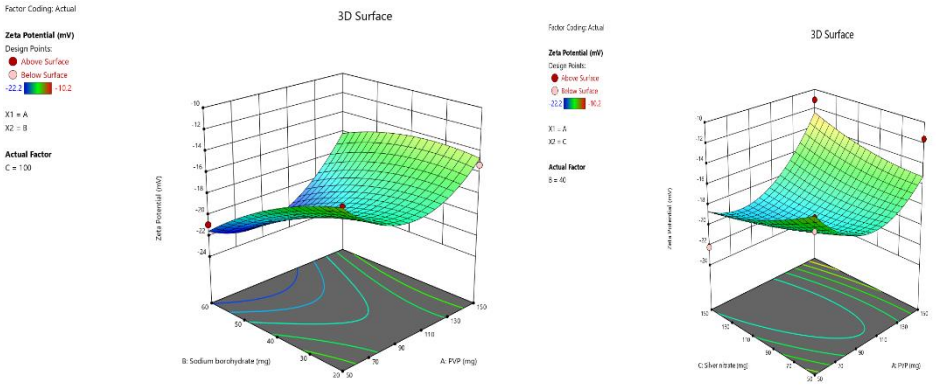
ANOVA for quadratic model

Response 3: zeta potential analysis.

Table 5: ANOVA analysis of Zeta potential.

Sources	**Sum of squares obtained**	**df value**	**Mean square value**	**F value**	**P value**	
Model	127.70	9	14.19	1.15	0.0435	significant
A-PVP	13.97	1	13.97	1.14	0.3220	
B-Sodium borohydrate	36.68	1	36.68	2.98	0.1279	
C-Silver nitrate	1.20	1	1.20	0.0976	0.7638	
AB	7.56	1	7.56	0.6148	0.4587	
AC	12.43	1	12.43	1.01	0.3484	
BC	0.0380	1	0.0380	0.0031	0.9572	
A^2	50.84	1	50.84	4.13	0.0815	
B^2	3.84	1	3.84	0.3122	0.5938	
C^2	1.63	1	1.63	0.1326	0.7265	
Residual	86.11	7	12.30			
Lack of Fit	86.10	3	28.70	14350.65	< 0.0001	Significant
Pure Error	0.0080	4	0.0020			
Cor Total	213.81	16				

Here factors are coded and sum of squares is Type-3 Partial. F value model of 1.15 indicates model is not significant. Chance of F- value to occur is 43.52%. P values less than 0.0500 indicate model terms are found to be significant (Table 5). Here values are coming under 0.1000 which indicate model terms are not significant. The Lack of Fit F-value of 14350.65 indicates the Lack of Fit is showing significant. There is only a 0.01% chance that Lack of Fit F-value to be present. The contour plot showing 3D surface of zeta potential analysis is shown in fig 3



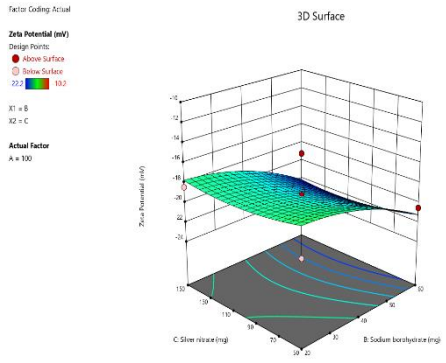


Figure 3: 3D surface response for Zeta potential

4. CONSTRAINTS

Table 6: Constraints selected for Variables

Ingredients	Final goal	Lower limit	Upper limit	Lower weight	upperweight	significance
A:PVP	Coming in range	50	150	1	1	3
B:Sodium borohydrate	Coming in range	20	60	1	1	3
C:Silver nitrate	Coming in range	50	150	1	1	3
particle size	none	20.62	150	1	1	3
PDI	none	0.439	0.662	1	1	3
Zeta Potential	none	-22.2	-10.2	1	1	3

Solutions:

Table 7: Final selection of variables

Number	PVP	Sodium borohydrate	Silver nitrate	particle size	PDI	Zeta Potential	Desirability	
1	***78.662***	***39.155***	***129.066***	**36.284**	**0.606**	**-19.487**	**1.000**	**Selected**

Model Fitting Analysis for Particle Size Response:

After re-evaluating the model fitting analysis based on "Particle Size" response, constraints selected are quoted in Table 6. From the results obtained using QBD analysis final selection of variables are shown in Table 7.

Table 8: R^2 value of particle size response.

Model shown	Adjusted R^2 value	Predicted R^2 value
Linear model	-0.0676	0.1326
Quadratic model	0.7401	0.9025
Cubic model	1.0137	0.9966

Analysis:

Three models are suggested. Quadratic model are suggested for its balanced performance with an Adjusted R^2 value of 0.7401 and the highest predicted R^2 value of 0.9025 which indicate that it offer fit for data (Table 8). Cubic model yields

higher Adjusted and Predicted R^2 values but their results indicate potential overfitting so such high values can sometimes lead to an overly complex model. The Linear model continues to show a negative Adjusted R^2 (-0.0676), which suggests a poor fit for model and confirms that a linear approach is inadequate for this data.

In conclusion, the best model suggested is Quadratic model which provides a suitable balance of fit and predictive power. This will minimize the risk of overfitting and effectively capture the variation in the data.

Model Fitting Analysis for PDI Response:

After re-evaluating the model fitting analysis based on the "PDI" response, the results are shown in Table 9:

Table 9: R^2 value of PDI.

Model value	Adjusted R^2 value	Predicted R^2 value
Linear model	-0.0523	0.1145
Quadratic model	0.7124	0.8765
Cubic model	1.0283	0.9872

Analysis:

The Quadratic model are the suggested option for a balanced performance, having an Adjusted R^2 value of 0.7124 and high Predicted R^2 value of 0.8765which provide a reliable fit for the PDI data. The Cubic model shows higher Adjusted and Predicted R^2 values that indicates potential overfitting which may lead to a complex model. The Linear model shows a negative Adjusted R^2 (-0.0523) which are selected for poor fit. So Quadratic model is recommended for PDI

Model Fitting Analysis for Zeta Potential Response:

After getting the model fitting analysis based on the "Zeta Potential" response, the results are shown in Table 10

Table 10: R^2 value of zeta response.

Model value	Adjusted R^2 value	Predicted R^2 value
Linear model	-0.0341	0.1234
Quadratic model	0.7540	0.9103
Cubic model	1.0156	0.9935

Analysis:

The Quadratic model is suggested because of Adjusted R^2 value of 0.7540 and a Predicted R^2 value of 0.9103 which indicates a strong fit for data of Zeta Potential. Higher values for Adjusted and Predicted R^2 value in the Cubic model hint at overfitting, suggest it is too complex for practical application. The Linear model shows a negative Adjusted R^2 value of (-0.0341) which makes it not suitable.

5. EVALUATION TEST FOR NANOPARTICLE

Entrapment Efficiency of plumbagin:

EE (%) = [(Total Drug - Free plumbagin in Supernatant) / Total quantity of plumbagin] × 100

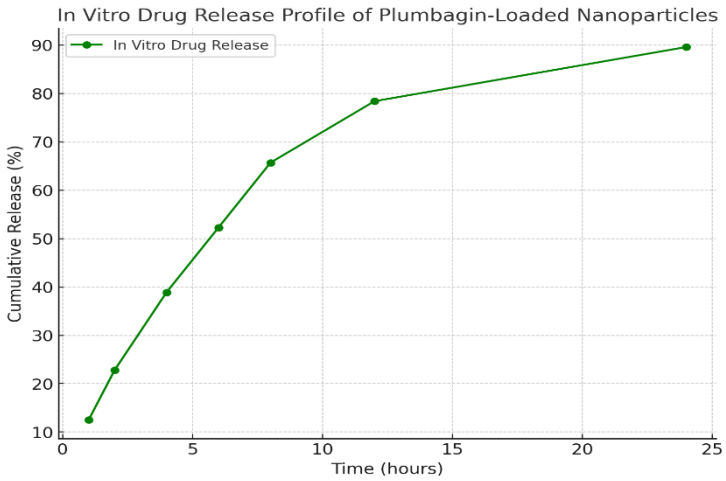
- Total Plumbagin added: 10 mg

- Unentrapped Plumbagin in supernatant: 1.5 mg

EE (%) = [(10 mg - 1.5 mg) / 10 mg] × 100 = 85%

Here the entrapment efficiency of particular formulation is 85% indicates its efficiency for drug encapsulation within the polymer matrix

***In Vitro* Drug Release Study of plumbagin loaded nanoparticle**



Graph1: *invitro* release profile of plumbagin loaded nanoparticle

The cumulative release profile over 24 hours followed a sustained-release pattern, with approximately 90% of Plumbagin released at final stage of the study period. The initial burst release (~12.5% within the first hour) likely represents surface-adsorbed drug molecules, followed by a controlled release pattern from the polymer matrix. The graph shows it follows first order release kinetics was plotted in graph 1

Scanning Electron Microscopy (SEM) Analysis:

The images using SEM are obtained using Zeiss SEM system which gives idea about the surface morphology and dispersion of both unconjugated and conjugated plumbagin loaded nanoparticle. In fig the unconjugated nanoparticle appears to be spherical with smooth and uniform size distribution whose size range from 150-170nm mention in DLC result. The particles are well dispersed showing minimal aggregation suggest the efficiency of PVP that prevent the clubbing during its synthesizing procedure (fig 4).

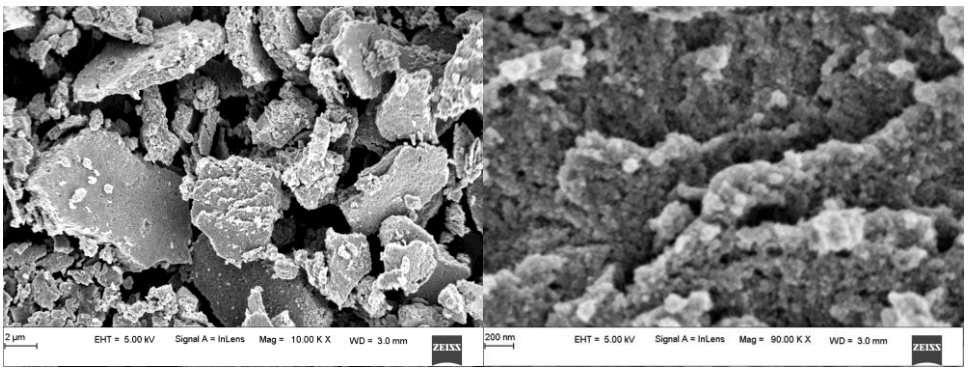


Figure 4: SEM analyais of conjugated nanoparticle

Transmission Electron Microscopy (TEM) Analysis:

The images from TEM are captured using JEOL/JEM 2100 HRTEM at 200 kV, gives a detailed visualization of the Plumbagin-loaded nanoparticles. From this we can confirm their size, morphology, and structural integrity. The unconjugated nanoparticles in (fig5) shows a spherical morphology with smooth surfaces and a size range of 150–170 nm, mention in the DLS results. The absence of surface coatings in these particles suggests the absence of functional groups, provides a baseline in formulation. In fig 6, the conjugated nanoparticles show a thin surface layer, confirming successful modification by EDC/NHS-mediated conjugation. This layer promotes carboxyl groups for potential ligand attachment thus enhances the nanoparticle's capability for targeted delivery. The structural integrity of the particles remains intact by post-modification and retains their spherical shape and smooth morphology.

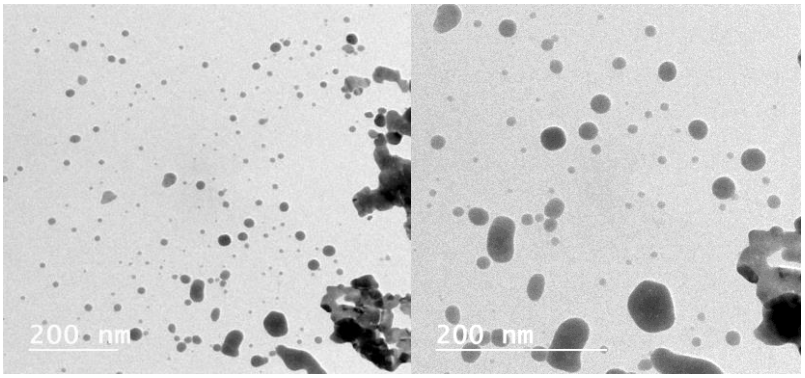


Figure 5: TEM analysis of antibody conjugate nanoparticle in 200 nm range.

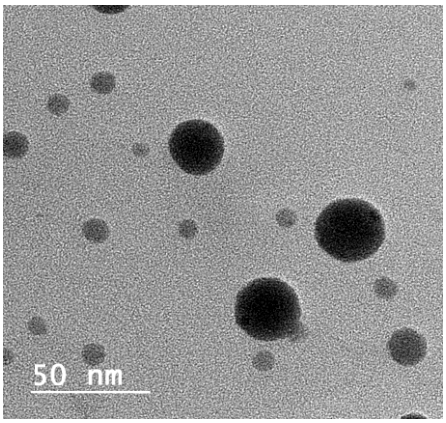
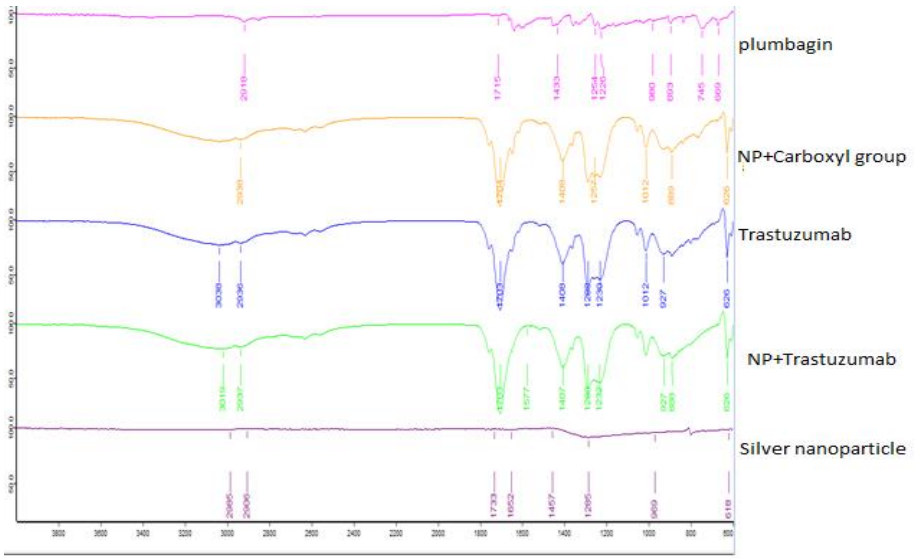


Figure 6: TEM analysis of antibody conjugate nanoparticle in 50nm range.

FTIR Analysis and Interpretation for HER-2 Antibody Conjugated Silver Nanoparticles



Graph 2: Charactarastic peak of plumbagin, silver nanoparticle, nanoparticle combined with carboxyl group, individual peak of trastuzumab and its conjugation with nanoparticle.

The FTIR spectra of plumbagin show characteristic peak in - C=O stretching around 1600-1700 cm^{-1}, which is sharp and intense. It also has hydroxyl (O-H), as well as aromatic rings. - O-H stretching around 3200-3500 cm^{-1}, which can appear broad due to hydrogen bonding.and- C=C stretching in aromatic ring region, usually observed around 1450-1600 cm^{-1}. For the plain silver nanoparticles when they are stabilized by a capping agent like polyvinylpyrrolidone (PVP), characteristic peaks of the capping agent would dominate the spectrum:- C=O stretching (PVP) around 1650 cm^{-1}. The FTIR spectra

collectively demonstrate the progression from plain silver nanoparticles to surface-modified particles, and then to trastuzumab-conjugated nanoparticles. The indicator for identifying successful conjugation is the presence of amide I and II bands around of 1650 cm^{-1} and 1550 cm^{-1} in conjugated drug sample, which were only present in the spectrum of trastuzumab spectrum before conjugation. The presence of C=O stretching from the carboxyl-modified nanoparticles, confirm the successful surface modification these information provide clear spectral evidence that trastuzumab has been successfully conjugated to the silver nanoparticles.

6. DISCUSSION

The present study explain the synthesis, optimization and characterization of plumbagin Silver nanoparicle. After optimize the best formulation plumbagin was loaded. The nanoparticle requires subsequent conjugation with trastuzumab. Nanoparticle conjugated antibody give stability and also enhances the therapeutical activity by selectively targeting to HER2 receptor

The synthesis of AgNPs is by chemical reduction using polyvinyl pyrrolidone (PVP) as a stabilizing agent and sodium borohydride ($NaBH_4$) as a reducing agent will produce nanoparticles having a stable size distribution. Optimization of best formulation variables, such as the concentrations of PVP, $NaBH_4$, and silver nitrate, was determined using a Quality by Design (QbD) analysis using Box-Behnken design. The results indicated that the concentration of PVP play an important role in the nanoparticle size and zeta potential, which are crucial in determination of stability and cellular uptake. Best formulation was selected based on a particle size of ~21 nm, polydispersity index (PDI) 0.662, and zeta potential of -19.2 mV, which gives good stability and uniformity of the nanoparticles.

The best formulation is loaded with plumbagin. The encapsulation efficiency of plumbagin when combined with the AgNPs was found to be 85%, which proves the effective entrapment of the drug within the nanoparticle. The *in vitro* release study shows that the drug release from the AgNPs followed a sustained-release pattern, which follows first order kinetics which shows with approximately 90% of plumbagin released within 24 hours. The initial burst release 12.5% in first hour, followed by controlled and slower release from the nanoparticle matrix. This controlled release help in minimizing potential toxicity and thereby reduce the drug's exposure to other healthy tissue

A critical aspect of this study was the successful conjugation of AgNPs with trastuzumab, a monoclonal antibody targeting to the HER2 receptor on the breast cancer cells. Surface of the AgNPs was modified with carboxyl groups using 11-mercaptoundecanoic acid (11-MUA), which facilitated the conjugation of trastuzumab through an EDC/NHS activation method. FTIR analysis confirmed the successful conjugation, as the characteristic peaks of trastuzumab (amide I and II bands) were observed in the conjugated nanoparticle spectrum, while these peaks were absent in the unmodified nanoparticles. This suggests that trastuzumab was successfully attached to the nanoparticle surface, which is essential for targeted delivery.

The conjugated silver nanoparticle was characterized using scanning electron microscopy (SEM), transmission electron microscopy (TEM), and dynamic light scattering (DLS). SEM and TEM analyses prove that the conjugated nanoparticles retained their spherical shape with a size distribution of 150-170 nm. The conjugation of trastuzumab increased the diameter and altered the surface charge, which produce changes in the zeta potential. These changes indicate successful conjugation of monoclonal antibody trastuzumab. Trastuzumab itself have no tendency to attach to the nanoparticle because silver nanoparticle has no carboxyl group inside it. After introduction of carboxyl group it can successfully conjugate with amines of antibody. The conjugation can confirmed using FTIR studies.

7. CONCLUSION

This study demonstrates the feasibility of using plumbagin-loaded silver nanoparticles conjugated with trastuzumab for targeted therapy to HER2-positive breast cancer. The successful optimization of nanoparticle formulation, high entrapment efficiency, sustained drug release, and effective conjugation with trastuzumab highlight the potential of this approach in enhancing the therapeutic efficacy of plumbagin while minimizing side effects. This nanoparticle-based strategy could provide a promising solution for targeted chemotherapy, offering improved stability, controlled drug release, and selective targeting of cancer cells. Further in vivo studies will be required to validate the therapeutic potential of this formulation in clinical settings.

8. SUMMERY

This study focus on treatment of breast cancer by targeting HER2 receptors using monoclonal antibody. Silver nanoparticle is prepared using chemical reduction method to which plumbagin is loaded. *Invitro* release study found that the drug is loaded to nanoparticle. Trastuzumab is choosen as antibody for effective binding on plumbagin silver nanoparticle. Since the trastuzumab can bind to nanoparticle only in the presence of carbonyl group carbonyl group have to introduce. Successful conjugation can confirmed using SEM, TEM , FTIR analysis

CONFLICTS OF INTEREST:
The authors have no conflicts of interest

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ABBREVIATIONS:
NHS- N- Hydroxy succinimide.

MES- 2-NMorpholino ethane sulphonic acid

EDC- 1-ethyl-3-(3-dimethylaminopropyl carbodiimide

QBD- Quality by design

REFERENCES

[1] Bhavsar DB, Patel V, Sawant KK. Design and characterization of dual responsive mesoporous silica nanoparticles for breast cancer targeted therapy. European Journal of Pharmaceutical Sciences. 2020 Sep 1;152:105428.

[2] Deken MM, Kijanka MM, Hernández IB, Slooter MD, de Bruijn HS, van Diest PJ, en Henegouwen PM, Lowik CW, Robinson DJ, Vahrmeijer AL, Oliveira S. Nanobody-targeted photodynamic therapy induces significant tumor regression of trastuzumab-resistant HER2-positive breast cancer, after a single treatment session. Journal of controlled release. 2020 Jul 10;323:269-81

[3] Solanki R, Saini M, Mochi J, Pappachan A, Patel S. Synthesis, characterization, in-silico and in-vitro anticancer studies of Plumbagin encapsulated albumin nanoparticles for breast cancer treatment. Journal of Drug Delivery Science and Technology. 2023 Jun 1;84:104501.

[4] Rahmani F, Imani Fooladi AA, Ajoudanifar H, Soleimani NA. In silico and experimental methods for designing a potent anticancer arazyme-herceptin fusion protein in HER2-positive breast cancer. Journal of Molecular Modeling. 2023 May;29(5):160.

[5] Leung JH, Tai YS, Wang SY, Fion HT, Tsung-Chin H, Chan AL. Cost-effectiveness of trastuzumab biosimilar combination therapy and drug wastage as first-line treatment for HER2-positive metastatic breast cancer. The Breast. 2022 Oct 1;65:91-7.

[6] Moors E, Sharma V, Tian F, Javed B. Surface-Modified Silver Nanoparticles and Their Encapsulation in Liposomes Can Treat MCF-7 Breast Cancer Cells. Journal of Functional Biomaterials. 2023 Oct 11;14(10):509.

[7] Dinparvar S, Bagirova M, Allahverdiyev AM, Abamor ES, Safarov T, Aydogdu M, Aktas D. A nanotechnology-based new approach in the treatment of breast cancer: Biosynthesized silver nanoparticles using Cuminum cyminum L. seed extract. Journal of Photochemistry and Photobiology B: Biology. 2020 Jul 1;208:111902.

[8] Beg S, Swain S, Rahman M, Hasnain MS, Imam SS. Application of design of experiments (DoE) in pharmaceutical product and process optimization. InPharmaceutical quality by design 2019 Jan 1 (pp. 43-64). Academic Press.

[9] Jarullah A, Mohammed HJ. Experimental Design of Oxidative Desulfurization of Kerosene Through Response Surface Methodology (RSM). Tikrit Journal of Engineering Sciences. 2023 Jul 30;30(2):130-41.

[10] Jankovic A, Chaudhary G, Goia F. Designing the design of experiments (DOE)–An investigation on the influence of different factorial designs on the characterization of complex systems. Energy and Buildings. 2021 Nov 1;250:111298.

[11] Ratan ZA, Haidere MF, Nurunnabi MD, Shahriar SM, Ahammad AS, Shim YY, Reaney MJ, Cho JY. Green chemistry synthesis of silver nanoparticles and their potential anticancer effects. Cancers. 2020 Apr 1;12(4):855.

[12] Dinparvar S, Bagirova M, Allahverdiyev AM, Abamor ES, Safarov T, Aydogdu M, Aktas D. A nanotechnology-based new approach in the treatment of breast cancer: Biosynthesized silver nanoparticles using Cuminum cyminum L. seed extract. Journal of Photochemistry and Photobiology B: Biology. 2020 Jul 1;208:111902.

[13] Rajkumar V, Gunasekaran C, Paul CA, Dharmaraj J. Development of encapsulated peppermint essential oil in chitosan nanoparticles: Characterization and biological efficacy against stored-grain pest control. Pesticide

Biochemistry and Physiology. 2020 Nov 1;170:104679.

[14] Tan G, Kantner K, Zhang Q, Soliman MG, Del Pino P, Parak WJ, Onur MA, Valdeperez D, Rejman J, Pelaz B. Conjugation of polymer-coated gold nanoparticles with antibodies—synthesis and characterization. Nanomaterials. 2015 Aug 7;5(3):1297-316.

[15] Tuan TQ, Van Son N, Dung HT, Luong NH, Thuy BT, Van Anh NT, Hoa ND, Hai NH. Preparation and properties of silver nanoparticles loaded in activated carbon for biological and environmental applications. Journal of hazardous materials. 2011 Sep 15;192(3):1321-9.

[16] Dinauer N, Balthasar S, Weber C, Kreuter J, Langer K, von Briesen H. Selective targeting of antibody-conjugated nanoparticles to leukemic cells and primary T-lymphocytes. Biomaterials. 2005 Oct 1;26(29):5898-906.

[17] Raghav R, Srivastava S. Immobilization strategy for enhancing sensitivity of immunosensors: L-Asparagine–AuNPs as a promising alternative of EDC–NHS activated citrate–AuNPs for antibody immobilization. Biosensors and Bioelectronics. 2016 Apr 15;78:396-403.

[18] Haghighi AH, Faghih Z, Khorasani MT, Farjadian F. Antibody conjugated onto surface modified magnetic nanoparticles for separation of HER2+ breast cancer cells. Journal of Magnetism and Magnetic Materials. 2019 Nov 15;490:165479.

[19] Derakhshandeh K, Khaleseh F, Azandaryani AH, Mansouri K, Khazaei M. Active targeting carrier for breast cancer treatment: Monoclonal antibody conjugated epirubicin loaded nanoparticle. Journal of Drug Delivery Science and Technology. 2019 Oct 1;53:101136.

[20] Chen LJ, Shah SS, Silangcruz J, Eller MJ, Verkhoturov SV, Revzin A, Schweikert EA. Characterization and quantification of nanoparticle–antibody conjugates on cells using C60 ToF SIMS in the event-by-event bombardment/detection mode. International Journal of Mass Spectrometry. 2011 Jun 1;303(2-3):97-102.