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Formulation and Optimization of Ansamycin-Loaded Polymeric Nanoparticles Using Response Surface Methodology for Bacterial Meningitis

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Abstract
Response surface methodology utilizing the central composite rotatable design version was made use of to optimize formulation of ansamycin-loaded polymeric nanoparticles. The central composite rotatable design including three-factored factorial designs with 3 levels was utilized in this research study. The drug encapsulation efficiency, particle size and zeta potential of the nanoparticles were examined relative to 3 independent variables consisting of polymer concentration (X1), surfactant concentration (X2) and also proportion of organic to aqueous phase volume (X3). The outcome revealed that the ideal formula can be gotten from this response surface methodology. The ideal solution for the nanoparticles was made up of polymer concentration (X1) of 5% w/v, surfactant concentration (X2) of 1% w/v and also proportion of aqueous to organic phase volume (X3) of 10:1 v/v. Ansamycin nanoparticles under the optimized conditions generated the encapsulation efficiency of 89%, mean particle size of 121 nm and zeta potential value of − 25 mV. SEM of the optimized polymeric nanoparticle showed spherical particles. The in vitro experiments verified that ansamycin in the polymeric nanoparticles released progressively over the duration of 36 h. This research study revealed that the response surface methodology central composite rotatable design can successfully be gotten the modelling of ansamycin polymeric nanoparticles.

Keywords Ansamycin · Polymeric nanoparticles · Response surface methodology · Central composite rotatable design

1 Introduction

Bacterial meningitis is a life-threatening health condition that requires prompt interest as well as treatment. The extremely most usual factors of bacterial meningitis are really Nesseriaeningitidis, *Streptococcus pneumoniae* and also Haemophilus flu. Greater than 1.2 million circumstances of bacterial meningitis are estimated to occur internationally annually. Frequency and case fatality rates for bacterial meningitis vary by place, country, microbe and also age. Without having treatment, the case fatality rate has the ability to be as high as 70%, as well as one in 5 survivors of bacterial meningitis can be left irreparable sequelae including hearing loss, neurologic problems or loss of an arm or leg [1].

Most of the small molecule medicines do not go across the blood-brain barrier (BBB). Exceedingly 7000 medicines in the extensive medical chemistry data source, simply 5% of medications deal with the central nervous system (CNS), and also these CNS active medications deal with just anxiety, schizophrenia, and also sleeplessness. The typical molecular mass of the CNS active medicine is 357 Da. In one more research study, just 12% of medicines were active in the CNS, yet just 1% of all medications were active in the CNS for the conditions besides affective conditions [2, 3].

Nanoparticles have in fact been thoroughly made use of for brain-targeted distribution. The aspects for this presumption are over all linked to the chance of nanoparticles multifunctionalization, coupled to their ability to carry medicines, contained BBB-impermeant medications. Polymeric nanoparticles are items comprised of a polymer matrix consisting of fragments with a minimum of one measurement in the nanometre range [4–6]. The presence of such nanofillers can significantly customize the regional characteristics as well as likewise morphology of the polymer chains along with the macroscopic response of the polymer, including its mechanical, thermal, along with rheological properties, with which it can, passes through the blood-brain barrier to supply the incorporated medication [7–9]. If moderately produced,

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polymeric nanoparticles can have a high result on a range of crucial medicine delivery applications [10, 11]. Or else, the similar would absolutely experience a selection of issues associating with the certain components of the formulation which would definitely seek that taken care of a lot less significantly [12–14].

Ansamycin is a broad spectrum antibiotic that is being used as prophylaxis against bacterial meningitis. Although ansamycin has a good bioavailability of 85% when administered orally, portion of drug reaches the brain is just 4% which is not sufficient enough for its therapeutic potential. Therefore, the development of the new formulation of ansamycin that enables quick availability to the targeted area brain is in in great need.

In the advancement of ansamycin-loaded polymeric surface methodology nanoparticles, a vital concern was to develop an optimized pharmaceutical formulation with optimum medicine encapsulation efficiency (EE) and also ideal mean particle size with minimum tests. For this objective, a computer optimization technique based upon a response surface methodology (RSM) was made use of. Response surface methodology is a collection of mathematical as well as analytical methods based upon the fit of a polynomial formula to the experimental data, which need to explain the actions of a data set with the purpose of making analytical previsions. It can be well used when an action or a set of responses of interest is affected by a number of variables.

Goals of this research study were for that reason to make use of response surface methodology (RSM) combined with central composite rotatable design (CCRD) to develop the useful connections in between 3 processing variables of polymer concentration (X1), surfactant concentration (X2) and proportion of aqueous to organic phase volume (X3) and also 3 responses of encapsulation efficiency (EE), mean particle size and zeta potential of the nanoparticles specifically. In order to optimize ansamycin-loaded polymeric nanoparticles, mathematical model equations were acquired by computer simulation programming Design-Expert ® 11. For a much better understanding of the 3 variables for the optimum nanoparticle efficiency, the designs existed as three-dimensional (3D) response surface graphs. Additionally, morphological attributes, particle size, zeta potential value and cumulative release percent in optimized nanoparticles were evaluated.

2 Materials and Methods

2.1 Materials

Ansamycin, PLGA, Poloxamer were purchased from Sigma Aldrich, India. HPLC grade DMSO and water were purchased from Fisher Scientific, Mumbai, India. All other solvents were of HPLC grade.

2.2 Formulation of Nanoparticle

Ansamycin-loaded polymeric nanoparticles were prepared by Emulsification solvent diffusion technique. An organic phase was made dissolving properly 4 g of PLGA as well as 100 mg of ansamycin in 10 ml of DMSO as well as an aqueous phase was made dissolving 0.5 g of poloxamer dissolved in 100 ml of sterilized water. To 10 ml of aqueous phase, organic phase was included drop wise at the rate of 1 ml/ min. The nanoparticle suspension was maintained under constant mixing at 300 rpm for 3 h at 300 °C to enable total dissipation of DMSO leaving the colloidal suspension of ansamycin PLGA nanoparticle in the aqueous phase. The colloidal nanosuspension was centrifuged at 12000 rpm for 30 min at 40 °C to obtain the final nanoprecipitate consisting of pellets as encapsulated ansamycin. The pellet was cleaned with deionized water two times to eliminate the unentrapped medicine from the surface area of nanoparticle. Nanoparticle pellets was re-distributed in water.

2.3 Experimental Design

Initial experiments showed that the variables, such as polymer concentration, surfactant concentration, and also proportion of aqueous to organic phase throughout preparation work, were the major variables that influenced the particle size, zeta potential and encapsulation effectiveness of the ansamycin polymeric nanoparticles. Hence, a central composite rotatable design–response surface methodology (CCRD–RSM) was made use of to systemically examine the impact of these 3 important formulation variables on particle size, zeta potential and encapsulation efficiency of the prepared ansamycin polymeric nanoparticles. The information of the design are detailed in Table 1. For every element, the speculative range was picked on the basis of the outcomes of initial experiments and also the feasibility of preparing the ansamycin polymeric nanoparticles at the extreme values. The value range of the variables was polymer concentration (X1) of 5–20 mg/ml, surfactant concentration (X2) of 0.25–1%, and also proportion of aqueous to organic phase (X3) of 10:1. A total amount of 20 examinations were performed. All the formulations in these experiments were prepared in replicate.

Table 1 Considered variables along with their levels

Independent variables	Levels		
	− 1	0	+ 1
Polymer concentration	5	12.5	20
Surfactant concentration	0.25	0.625	1.0
Aqueous to organic phase ratio	5	10	15

2.4 Fourier Transmission Infrared Spectroscopy Analysis

The chemical integrity of the drug and also polymer can be figured out by Fourier transmission infrared spectroscopy (FTIR) evaluation (SPECTRUM RX I, Perkin Elmer, USA). About 2 mg of ansamycin nanoparticle samples was combined individually in 300–400 mg of anhydrous KBr and also ground correctly in a mortar pestle. The sample blend was pressed by using hydraulic pressure of 2000 kg/cm^2 (Jasco MP2 mini press) for 2 min. The FTIR range was gotten by scanning all samples with resolution of 2 cm^{-1} in the series of 4000–400 cm^{-1}.

2.5 Differential Scanning Calorimetry Analysis

The physical status of drug in the polymer as well as drug-polymer interaction was identified by Differential Scanning Calorimetry (DSC-60, Shimadzu, Japan). The samples ansamycin nanoparticles and also native ansamycin (2–4 mg) were secured individually in basic aluminium pans and scanned at a heating rate of 10 °C/min over a temperature range of 50–350 °C with continual nitrogen gas circulation of 65 ml/min.

2.6 Scanning Electron Microscopy

Surface morphological evaluation of ansamycin-loaded polymeric nanoparticles was carried out by scanning electron microscope (EVO, ZEISS Germany) for which lyophilized powder samples were spread on the carbon adhesive sample holder and coated with gold, used for scanning electron microscope.

2.7 Particle Size Analysis

Particle size evaluation was done by dynamic light scattering (DLS) with a Malvern Zetasizer 3000 HSA (Malvern Instruments, UK). DLS generates the mean size as well as the polydispersity index (PI) which is a procedure of the width of the size distribution. The mean size and PI values were gotten at an angle of 90° in 10-mm diameter cells at 25 °C. Prior to the measurements, all samples were diluted with double distilled water to create an ideal scattering intensity.

2.8 Zeta Potential

The zeta potential, mirroring the electrical charge on the particle surface as well as suggesting the physical stability of colloidal systems, was gauged by establishing the electrophoretic movement utilizing the Malvern Zetasizer 3000 HSA (Malvern Instruments, UK). The sample was gauged in double distilled water as well as adapted to a conductivity of 50 IS/cm with sodium chloride solution (0.9% w/v). The pH remained in the range of 5.5–7.5 and also the used field strength was 20 V/cm.

2.9 Chromatographic Conditions

Chromatographic separation was performed at ambient temperature on a reversed phase Phenomenex C-8 Luna (250 × 4.6 mm, 5 μm) column using a mobile phase consisting of methanol:water (75:25) at flow rate 1 ml/min. The detector wavelength was set at 240 nm as determined by Perkin Elmer Lambda 25 UV/VIS spectrometer.

2.10 Determination of Drug Encapsulation Efficiency

When it comes to the determination of drug encapsulation efficiency of drug-loaded nanoparticles, initially, the nanoparticles were precipitated by adding 0.1 M hydrochloric acid to change the pH of nanoparticle suspension to 1.20, and afterwards, the supernatant (S1) and also solid deposit were gathered after centrifugation (MIKR022, HEETTICH, Germany) at 80,000×*g* for 50 min. The solid deposit was re-dispersed in 10 ml of 0.3% sodium dodecyl sulfate (SDS) solution, after that centrifuge-separated as well as the supernatant (S2) was gathered. The drug encapsulation efficiency percentage of drug-loaded nanoparticles was after that determined from formula (1):

$$\mathrm{EE} = \frac{\mathrm{WT}-\mathrm{WS1}-\mathrm{S2}}{\mathrm{WT}} \times 100\%$$

where EE is entrapment efficiency, WT is the total amount of charged drug. WS_1 is the amount of drug in the supernatant after the first centrifugation and WS_2 is the amount of drug in the supernatant after the second centrifugation.

2.11 The In Vitro Drug Release Study

The in vitro ansamycin release pattern from nanoparticle formulation was checked out utilizing dialysis bag technique in PBS medium with a pH of 7.4. The samples (0.5 ml of nanoparticle) were transferred into a dialysis bag. The bag was after that positioned in 50 ml PBS. The release study was carried out for 72 h at 37 °C with gentle shaking at 100 rpm. At predetermined time points, 2-ml aliquots were taken out from the beaker and also changed with the very same quantity of fresh PBS. At 8 h, 24 h and also 48 h, all buffered solutions outside the dialysis bag were changed with fresh PBS. The ansamycin concentration in the dispersing medium was spectrophotometrically determined at 275 nm. To mimic in vivo condition, in another set of experiments, the combination of ansamycin nanoparticle and also human plasma (1:1 v/v) was

positioned in a dialysis bag. The drug release profile was reviewed by the very same procedure.

2.12 Data Analysis

The relationships between responses and formulation variables of all model formulations were dealt with by Design-Expert ® software application. Statistical analysis consisting of step-by-step linear regression and also response surface analysis was performed. The significant terms ($p < 0.05$) were picked for final equations. Appropriate models containing 3 elements consist of linear, quadratic and special cubic models. The very best suitable mathematical design was chosen based upon the contrasts of a number of statistical specifications consisting of the coefficient of variant (c.v.), the multiple correlation coefficient (R^2) and adjusted multiple correlation coefficient (adjusted R^2) verified by Design-Expert software program [15]. Significance of difference was evaluated making use of Student's *t* test and also one-way ANOVA at the probability level of 0.05.

3 Results and Discussions

The nanoparticles were prepared by Emulsification solvent diffusion method. PLGA was utilized as polymer to develop a drug-polymer complex which was beneficial for drug stabilizing as well as enhancement in its encapsulation efficiency. Poly-L-lysine (PLL)-based dendrons were made use of to condense pRedN-1 DNA (7.5 kbp), a fluorescent protein vector [16]. When pRedN-1 DNA was encapsulated in the kind of dendriplexes right into PLGA nanoparticles through a $w_1/o/w_2$ emulsification technique, DNA encapsulation efficiency amounted to 15.6%, greater than that observed with the uncomplexed DNA (9.9%). Poloxamer was made use of as surfactant to develop a stable emulsion. In the technique of solvent diffusion method, a too much quantity of water is included right into the emulsion; in order to assist in the quenching of the dispersed organic solvent right into the aqueous phase and also this was done to saturate water in the organic solvent. These have actually been continuously pursued 3 times, for reproducibility and also for uniformity.

3.1 Optimization of Formulas

The central composite rotatable design–response surface methodology (CCRD–RSM) makes up an alternate strategy since it offers an opportunity of examining a high variety of variables at different levels with just a restricted variety of experiments [17]. The variables in Table 1 were picked considering our initial experiments. Table 2 revealed the speculative outcomes concerning the evaluated variables on drug

Table 2 Central composite rotatable design generated by Design-Expert 11® software along with the obtained response

Std	Run	Factor 1 A:Polymer concentration (mg/ml)	Factor 2 B:Surfactant concentration (%)	Factor 3 C:Aqueous to organic phase ratio	Response 1 Particle size (Nm)	Response 2 Zeta potential (mV)	Response 3 Encapsulation efficiency (%)
18	1	12.5	0.625	10	210	−30	68
7	2	5	1	15	172	−31	78
8	3	20	1	15	176	−14	80
14	4	12.5	0.625	18.409	153	−24	94
15	5	12.5	0.625	10	210	−30	68
13	6	12.5	0.625	1.59104	161	−35	82
19	7	12.5	0.625	10	210	−30	68
11	8	12.5	−0.00567231	10	186	−28	79
6	9	20	0.25	15	159	−27	69
10	10	25.1134	0.625	10	179	−33	86
20	11	12.5	0.625	10	210	−30	68
16	12	12.5	0.625	10	210	−30	68
1	13	5	0.25	5	183	9	84
3	14	5	1	5	177	16	78
12	15	12.5	1.25567	10	166	10	91
17	16	12.5	0.625	10	210	−30	68
5	17	5	0.25	15	191	11	88
4	18	20	1	5	165	31	79
2	19	20	0.25	5	170	28	69
9	20	−0.113446	0.625	10	189	29	81

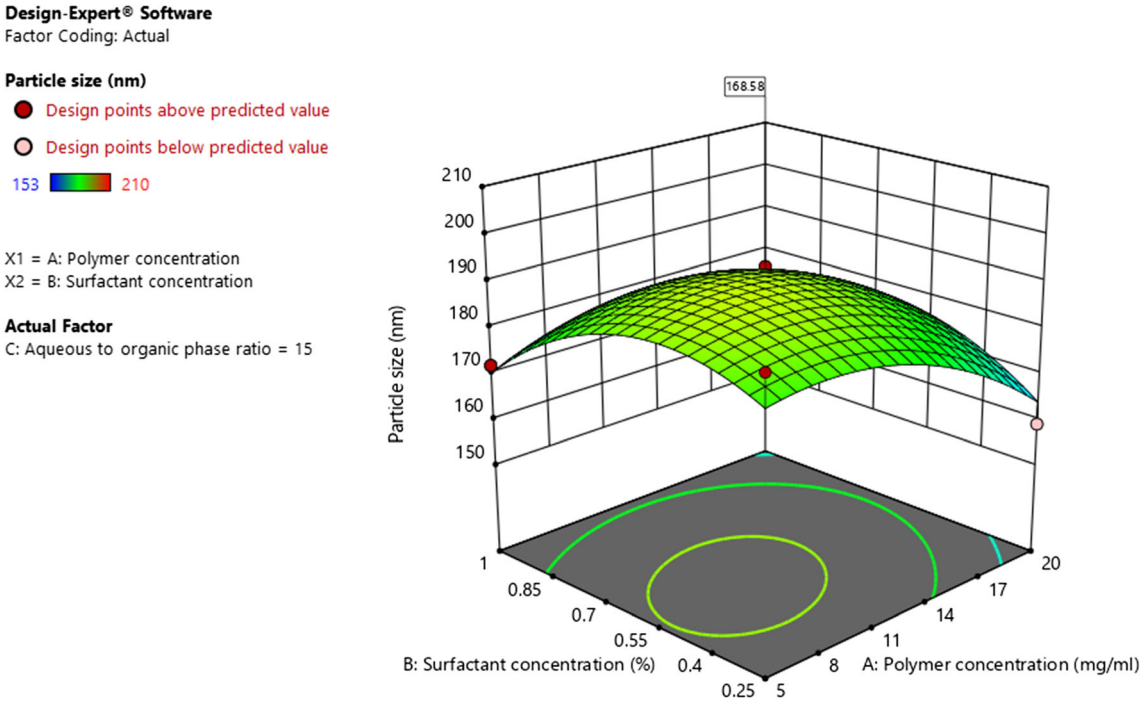

Fig. 1 Three-dimensional (3D) response surface plots showing the effect of the variable on response. The effect of polymer concentration and surfactant on particle size

encapsulation efficiency, mean particle size and also zeta potential. The 3 dependent values varied from 68 to 94% by weight, 153 to 210 nm and − 35 to 31 mV. A mathematical relationship between factors and also parameters was produced by response surface regression evaluation making use of Design-Expert ® 11 software application. The three-dimensional (3D) response surface graphs for the most statistical significant variables on the evaluated parameters are displayed in Figs. 1, 2 and 3. The response surface diagram revealed that the mean particle size and encapsulation

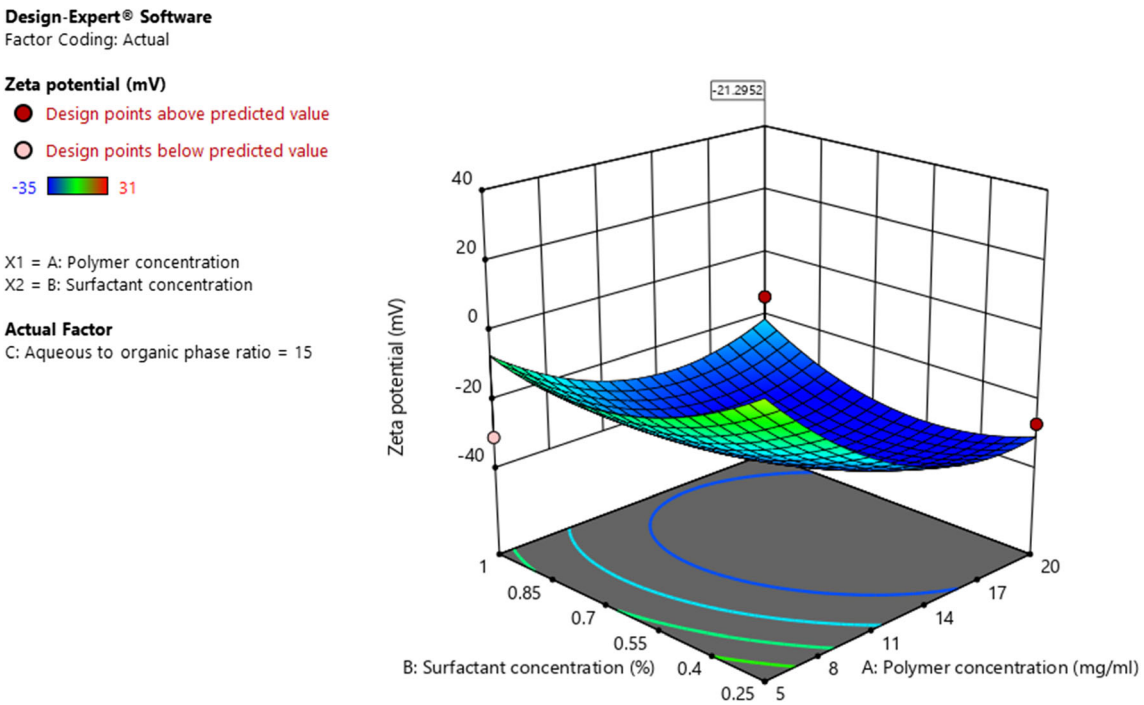

Fig. 2 Three-dimensional (3D) response surface plots showing the effect of the variable on response. The effect of polymer concentration and surfactant concentration on zeta potential

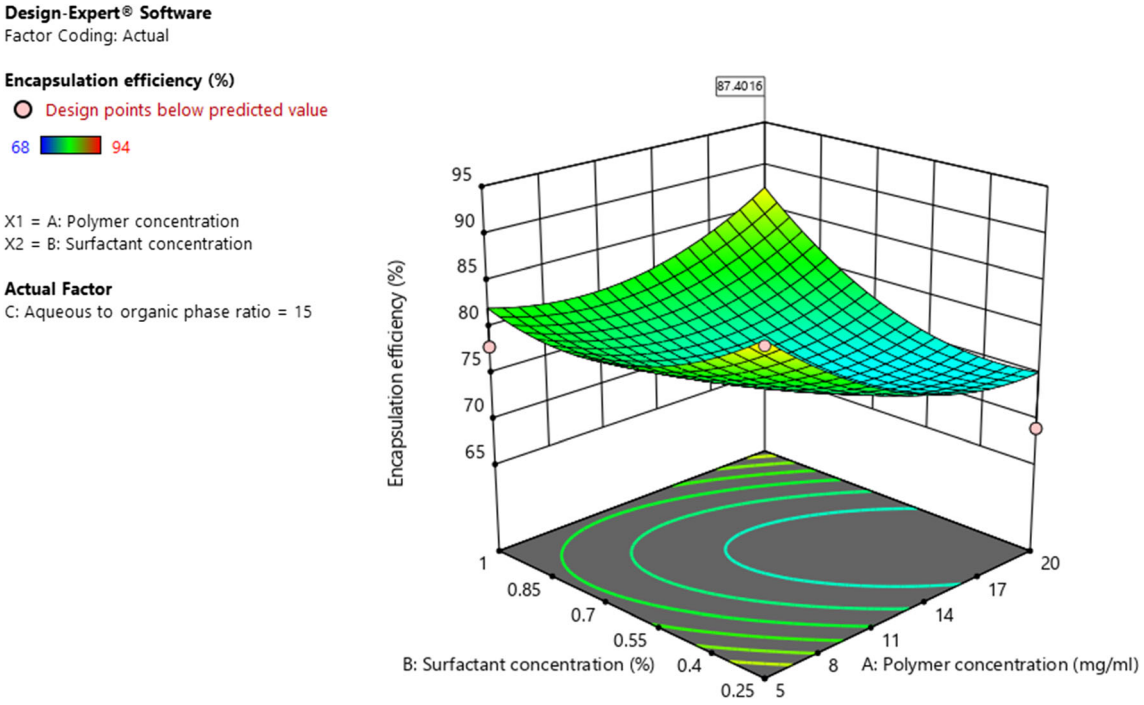

Fig. 3 Three-dimensional (3D) response surface plots showing the effect of the variable on response. The effect of polymer concentration and surfactant concentration on the encapsulation efficiency

efficiency enhances with a boost in polymer concentration. Decline in encapsulation efficiency of the drug was seen with enhancing surfactant concentration. A rise in bit particle size with rise in surfactant concentration is noted. The particle size showed an initial reduction when the concentration of the organic phase was boosted. On additional boosting the concentration, the particle size raised. Greater encapsulation efficiency noted with enhanced organic phase. The optimized variables revealed an excellent fit to the second-order polynomial equation. After model simplification with backward elimination, the r value decreased a little to 0.9545, 0.5866 and 0.7280 respectively. The lack of fit was not significant at 95% self-confidence level. All the remaining parameters were significant at $p \leq 0.05$. The statistical analysis of the results produced the adhering to polynomial equations and also provided in Table 3. Anticipated optimum ranges of the independent variables are detailed in Table 4. The fitting outcomes showed that the optimized nanoparticles with high entrapment efficiency, minimal particle size and ideal zeta potential were acquired with the polymer concentration of 5% w/v, surfactant concentration of 1% w/v and aqueous to organic phase ratio of 10:1 v/v, respectively. Table 4 programs that the experimental values of both sets prepared within the optimum range were extremely near to the anticipated values, with low percent bias, recommending that the optimized formulation was trustworthy and also sensible. It can be ended that a high desirability value might be gotten with a polymer concentration of 5% w/v, surfactant concentration of 1% w/v and aqueous to organic phase ratio of 10:1 v/v. The desirability acquired was 0.736 and also the same is represented in Fig. 4.

Perturbation plots exist in Figs. 5, 6 and 7 for anticipated models to get a much better understanding of the examined treatment. These sorts of plots reveal the impact of an independent factor on a particular response, with all

Table 3 Reduced response models and statistical parameters obtained from ANOVA

Responses	Regression model	Adjusted R^2	Model p value	%CV	Adequate precision
Particle size	PS = 209.87–5.11A–3.41B–0.7655C+4.63AB–0.3750AC+ 1.13BC–8.33A2–11.16B^2–17.88C^2	0.8126	0.0001	3.28	21.28
Zeta potential	ZP = –30.50–6.68A+3.29B–9.26C+6.37AB–6.88AC– 4.88BC+13.14A^2+10.67B^2+3.42C^2	0.8563	0.0001	2.86	10.42
Encapsulation efficiency	EE = 68.27–1.65A+1.84B+1.84C+4.62AB–0.3750AC– 0.3750BC+3.70A^2+4.23B^2+5.29C^2	0.8824	0.0001	3.12	14.26
Acceptance criteria		1	< 0.05	< 4	> 10

Table 4 Comparison of experimental and predicted values under optimal conditions for final formulation

Polymer concentration	Surfactant concentration	Aqueous to organic phase ratio	Particle size	Zeta potential	Encapsulation efficiency
10 mg	0.5 mg 1%	10:1			
Predicted			118	−24	90
Experimental			121	−25	89
Bias %			2.5%	4.1%	1.0%
Acceptance criteria 6%					

Bias was calculated as (predicted value–experimental value)/predicted value × 100

various other aspects held consistent at a referral factor [18] A steepest incline or curvature suggests sensitiveness of the response to a specific factor. Figure 5 shows that surfactant concentration had one of the most crucial impact on particle size followed by polymer concentration and aqueous to organic phase ratio. Figure 6 shows that surfactant concentration had one of the most vital impact on zeta potential followed by polymer concentration and aqueous to organic phase ratio. Figure 7 reveals that aqueous to organic phase ratio had the most crucial result on encapsulation efficiency followed by polymer concentration and surfactant concentration.

3.2 Effect of Aqueous/Organic Phase Ratio

The particle size of nanoparticles showed an initial reduction when the concentration of the organic phase was boosted. On additional raising the concentration, the particle size increased. These findings might be described on the basis of change in the viscosity of the emulsion developed. Boosted viscosity of the emulsion by alternation of aqueous/organic phase ration caused high viscous resistance versus the shear pressure throughout the nanoparticle formulation. The encapsulation efficiency was within a narrower range. Greater encapsulation efficiency of formulations might be due to raised viscosity of the emulsion created which would certainly have caused high viscous resistance versus the shear pressure throughout the emulsification. The cumulative amount of drug release raised with raising concentration of organic phase made use of in the preparation work of nanoparticles (data not provided). The difference in the release profile can be attributed to the difference in the surface area of nanoparticles despite the difference of particle size.

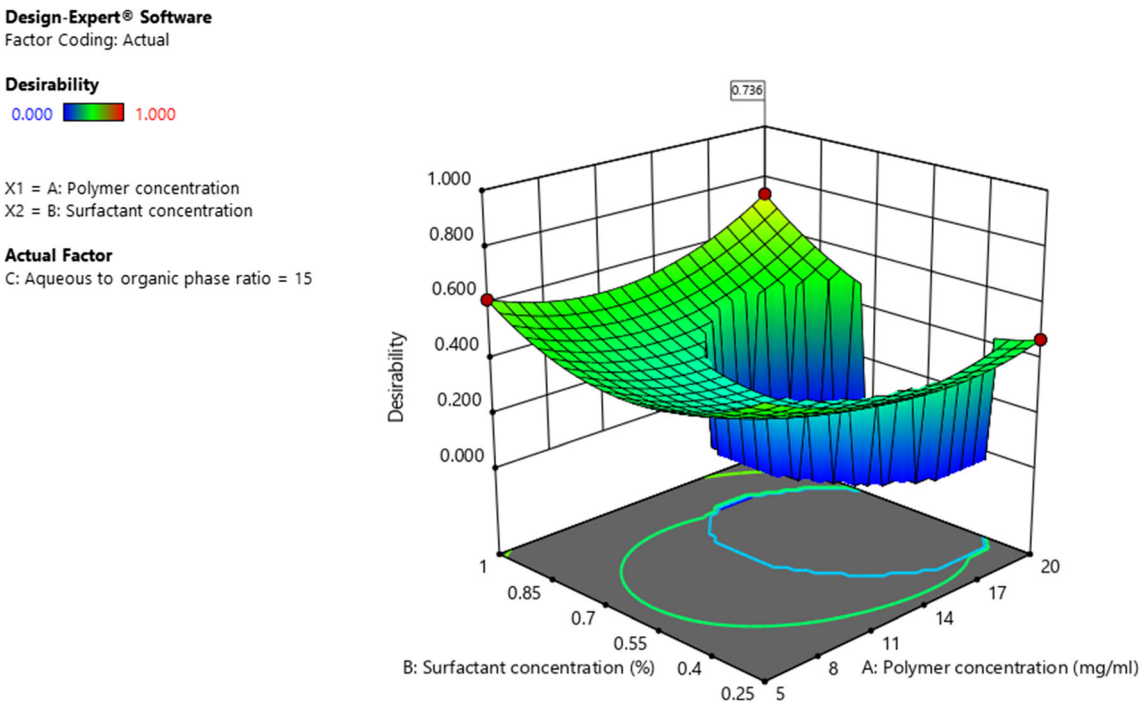

Fig. 4 Three-dimensional (3D) response surface plots showing the desirability with a value of 0.736

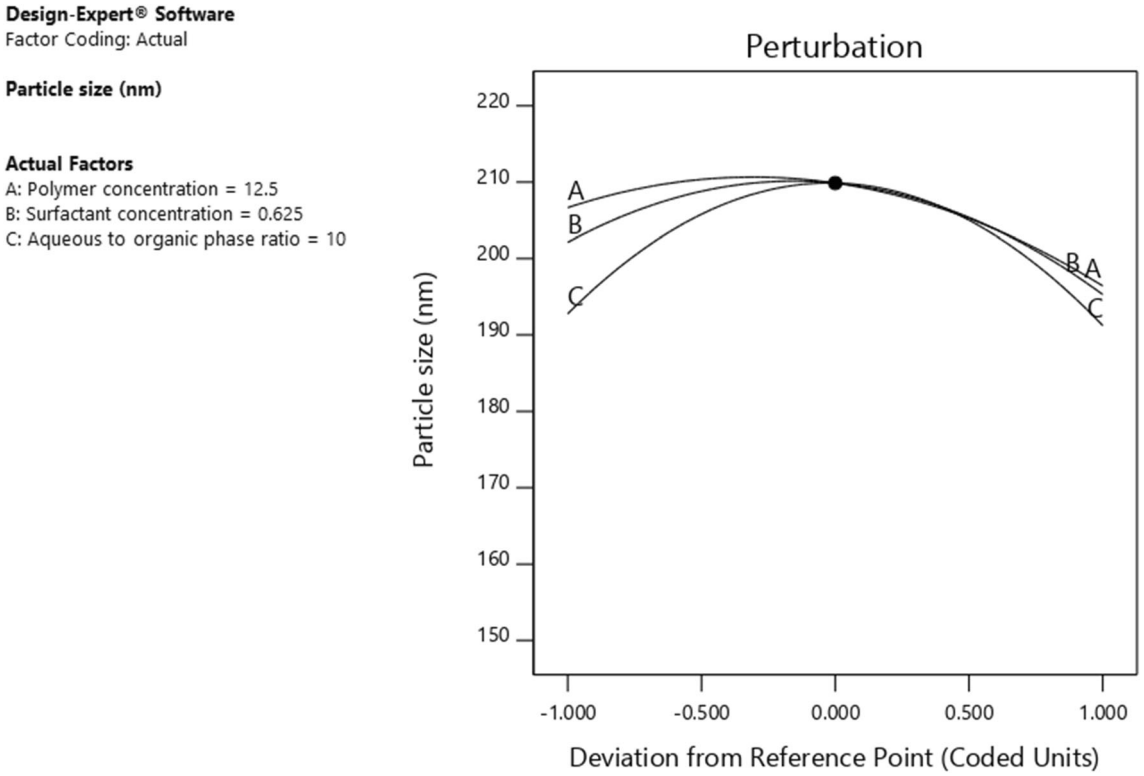

Fig. 5 Perturbation plot showing the effect of each of the independent variables on particle size where A, B and C are polymer concentration, surfactant concentration and aqueous to organic phase ratio respectively

3.3 Effect of Surfactant Concentration

In this examination, poloxamer was picked as well as utilized as the surfactant. It was observed that at 0.25% poloxamer concentration, the emulsion created was not stable as well as phase separation took place after a couple of hours of emulsification causing the development of polymer accumulations. Raising poloxamer concentration from 0.25 to 1.0% caused lesser particle sizes. Boost in particle size was seen with more boost in concentration from 1.0 to 2.0%. A rise in PLGA nanoparticle size with boost in poloxamer concentration has actually likewise been reported in the literatures [19]. In the emulsion solvent diffusion method, the emulsification and also stabilization of the globules are two vital aspects. The quantity of surfactant plays an essential function, due to the fact that it can stay clear of the coalescence of the oil globules. The surfactant molecules have a tendency to align themselves at the droplet surface lowering the free energy at the interface between two phases and also withstanding coalescence of the droplets. Smaller nanoparticles have large surface area and also hence require much more surfactant to stabilize the emulsion nanodroplets. Much less quantity of surfactant might cause development of unstable emulsion suggesting that 0.25% poloxamer might not suffice to stabilize the emulsion nanodroplets, resulting in phase separation after a couple of hours.

The encapsulation efficiency at first boosted and after that reduced when the surfactant concentration enhanced from 0.25 to 01.0% as well as additional to 2.0%, respectively. Decline in encapsulation effectiveness of the drug was seen with raising surfactant concentration. Ansamycin being a hydrophobic molecule will certainly often tend to remain in the oil nanodroplets. Yet with a rise in poloxamer concentration in the external aqueous phase, ansamycin might diffuse out from the oil nanodroplets as well as solubilize as micelles in the aqueous stage. A lot more solubilization of the drug in the external aqueous phase will certainly lead to lowered quantity of surfactant offered at the aqueous/organic phase interface and also therefore agglomeration of nanodroplets might happen. An additional description can be on the basis of gelatinization of poloxamer molecules. As a result of solid hydrogen bonds using hydroxyl group between inter- or intra-molecules of poloxamer, gelatinization of poloxamer at the oil/water interface might happen throughout the nanoparticle formulation [20]. Consequently, enhancing particle size and reducing encapsulation effectiveness were observed with enhancing surfactant concentration. The quantity of drug release lowered with a boost in the surfactant concentration (data

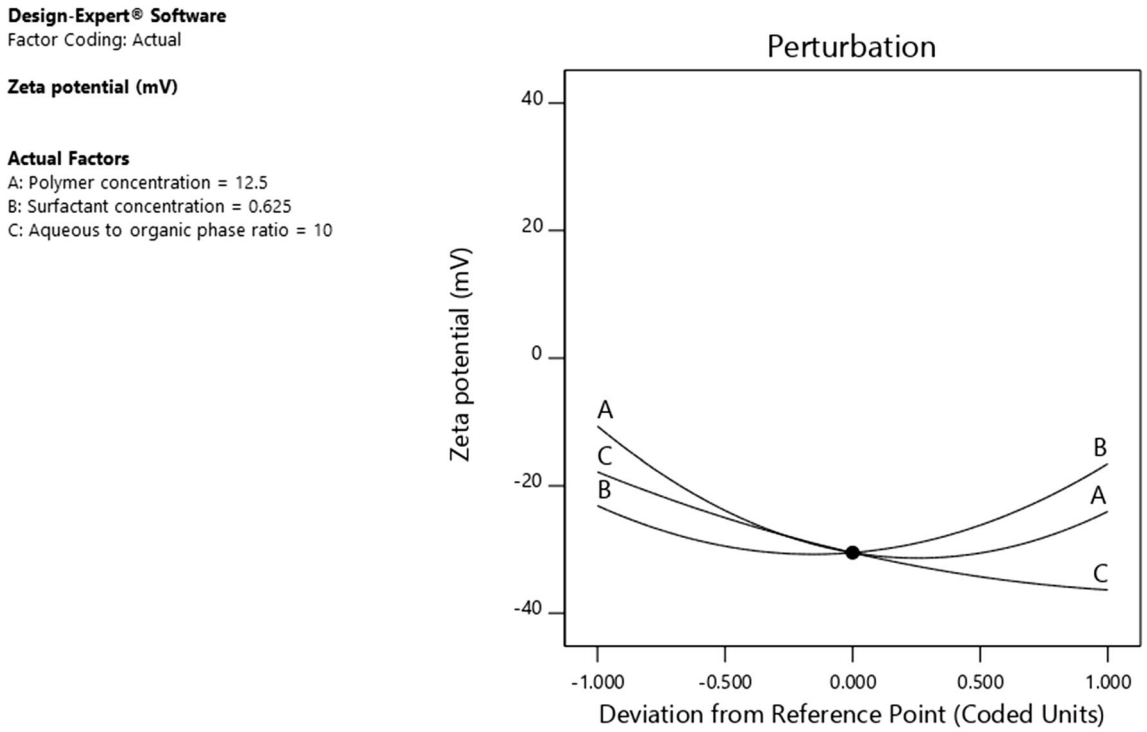

Fig. 6 Perturbation plot showing the effect of each of the independent variables on zeta potential where A, B and C are polymer concentration, surfactant concentration and aqueous to organic phase ratio respectively

not provided). This phenomenon might be attributed to the difference in the particle size at various concentrations. As the surfactant concentration enhanced from 0.25 to 1.0%, the particle size raised. The boost in surface area because of smaller sized nanoparticles can have been adding to a greater release.

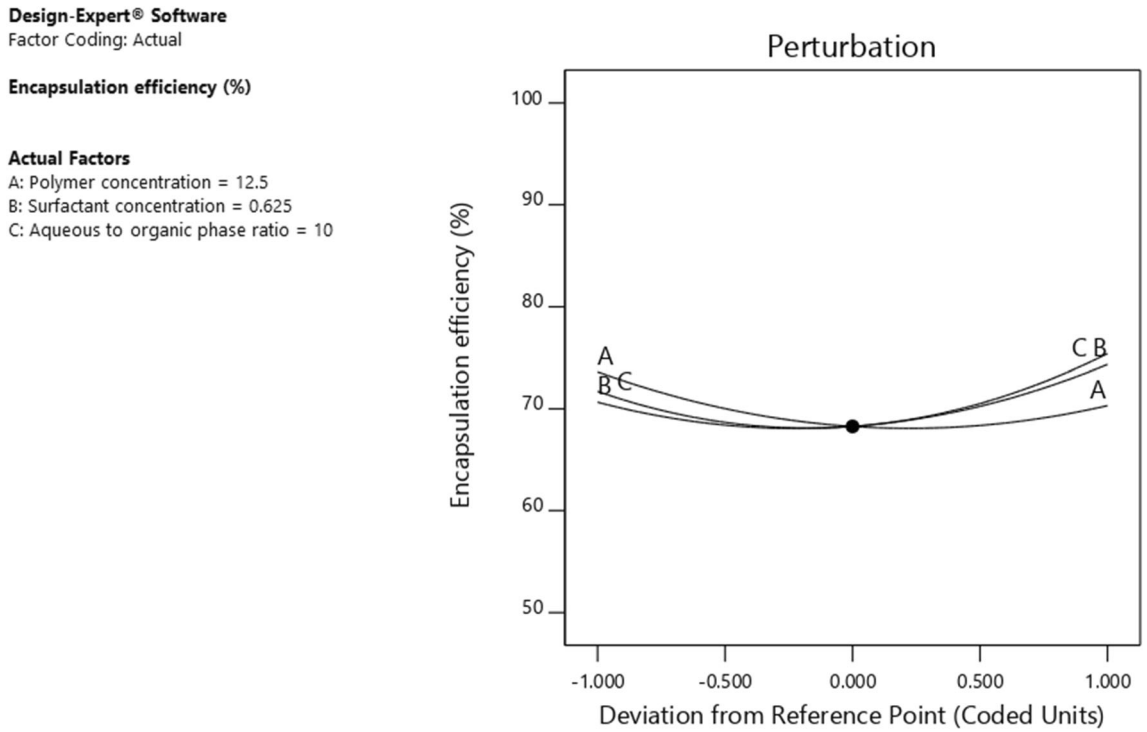

Fig. 7 Perturbation plot showing the effect of each of the independent variables on encapsulation efficiency where A, B and C are polymer concentration, surfactant concentration and aqueous to organic phase ratio respectively

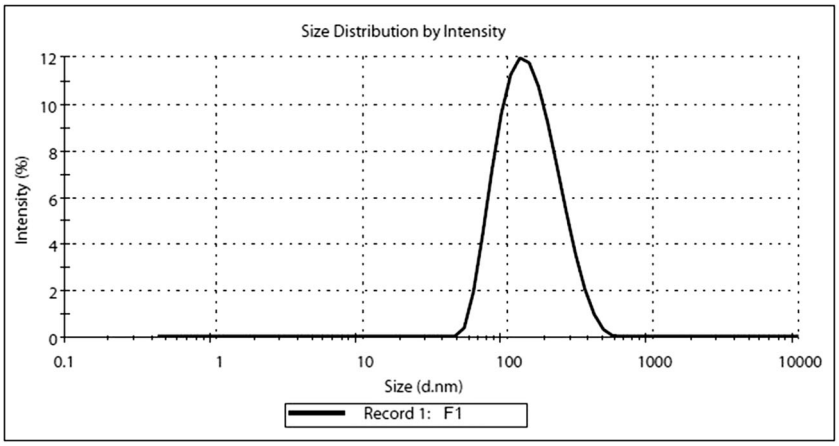

Fig. 8 Particle size of optimized ansamycin-loaded polymeric nanoparticle

3.4 Effect of Polymer Concentration

The mean particle size of formulations prepared utilizing 5, 10 and 20% of PLGA led to a raising pattern. As the polymer concentration boosted from 5 to 10% as well as 20%, the mean encapsulation efficiency increased. The drug release reduced with rise in the polymer concentration as well as recommended that polymer concentration plays a considerable role in figuring out the drug release from the ansamycin-loaded PLGA nanoparticles (data not provided). Enhancing the polymer concentration, while maintaining the quantity of organic phase consistent at 10 ml, the viscosity of the organic phase is enhanced, which causes boost in the viscous forces withstanding droplet breakdown as well as therefore larger oil droplets are created, leading to boosted particle size [19]. Likewise the boost in polymer concentration raises the organic phase viscosity, which boosts the diffusional resistance to drug molecule from organic phase to aqueous phase, thus alluring even more drug in the polymer nanoparticles. Enhancing polymer concentration likewise boosts particle size and also drug content is understood to raise with particle size in various other systems [21]. A boost in particle size boosts the length of diffusional pathways right into the aqueous phase, consequently lowering the drug loss via diffusion as well as raising the drug content. Additionally, the moment needed for polymer precipitation reduces at greater polymer concentration, so there is much less time for drug particles to diffuse out of nanoparticles, which raises the drug content [22]. One more factor might be the accessibility of a better quantity of polymer to envelop the drug, therefore not creating saturation of encapsulation [23]. The decreased percentage of cumulative drug release can be because of the enhanced particle size and also hence smaller sized surface area at greater polymer concentration. An additional description for reduced cumulative drug release at greater polymer concentration might be the enhanced concentration of the polymer existing which impedes the drug release by diffusion [23].

3.5 Characterization

3.5.1 Particle Size, Zeta Potential and SEM Measurement

The mean particle size of ansamycin-loaded polymeric nanoparticles was 121 nm (Fig. 8). The zeta potential of the same was found to be − 25 mV (Fig. 9), and it is sufficiently high to form stable pharmaceutical preparation. In order to provide

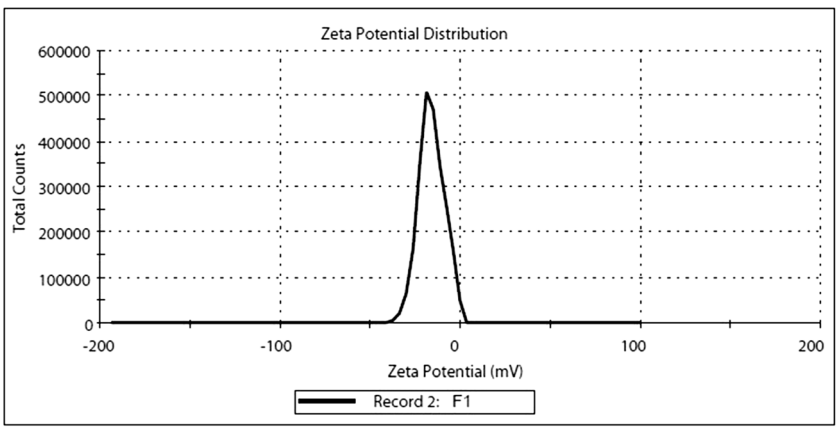

Fig. 9 Zeta potential of optimized ansamycin-loaded polymeric nanoparticle

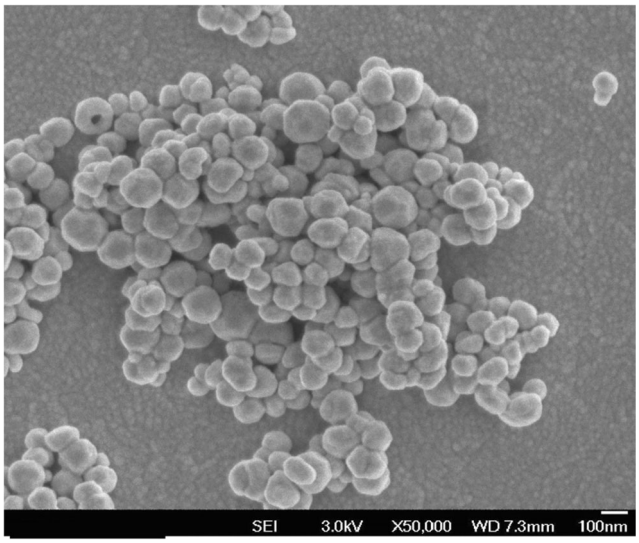

Fig. 10 SEM image of optimized ansamycin-loaded polymeric nanoparticle

information on the morphology of the optimal ansamycin-loaded polymeric nanoparticles, SEM was used to take photos, as shown in Fig. 10. The optimized nanoparticles are spherical in shape.

3.5.2 Fourier Transmission Infrared Spectroscopy Analysis

FTIR analysis is used to study the interactions between Ansamycin and the polymer PLGA used in the formulation. The infrared spectra of Ansamycin, the polymer used, their physical mixture and the formulation of the same are shown in Fig. 11. The IR of the mixture of drug sample and PLGA were found to be within the specified range. Hence, there is no interaction between the drug sample and the polymer likely to be used in the formulation and hence can be used in the formulation. Ansamycin procured their entire characteristic peak in physical mixture. That is significant peak 2400–3100 were retained in the physical mixture. Peak at 3055 i.e. O–H stretching was prominent in Ansamycin along with the physical mixture. In fingerprint region of Ansamycin, the characteristic band at 1578 C–O stretching, 1550–1468 C–C stretching and 1243 C–N stretching was retained in the physical mixture. On the basis of FTIR spectra investigation, no chemical interaction was observed between drug and polymer.

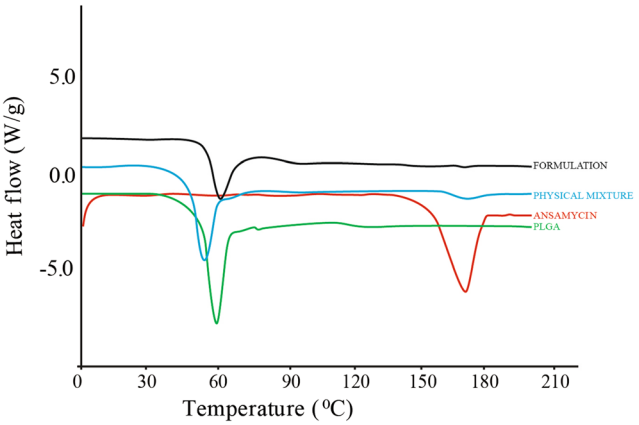

Fig. 12 DSC of ansamycin, PLGA, physical mixture and nanoformulation

3.5.3 Differential Scanning Calorimetry Analysis

The thermal behaviour of ansamycin was investigated by DSC. The pure ansamycin shows a sharp endothermic peak that corresponds to melting point at 170 °C. Thermogram of PLGA showed a sharp endothermic peak at 58 °C (Fig. 12). The DSC thermogram of ansamycin was compared with DSC thermogram of mixture of Ansamycin and polymers used in the formulation and there should be no interference in the peak of drug and polymers. The DSC of the mixture of drug sample and polymers was found to be within the specified range. Hence, there is no interaction between the drug sample and

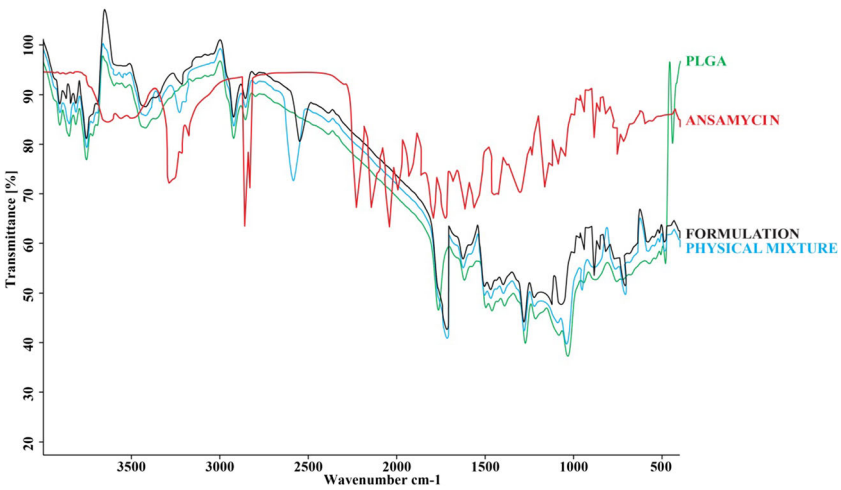

Fig. 11 FTIR of ansamycin, PLGA, physical mixture and nanoformulation

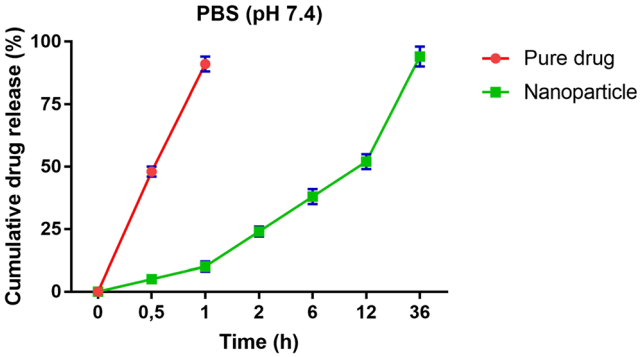

Fig. 13 Cumulative percentage drug release of pure drug ansamycin and ansamycin-loaded polymeric nanoparticles in PBS (pH 7.4)

the polymers likely to be used in the formulation and hence can be used in the formulation.

3.5.4 In Vitro Drug Release Study

The release pattern of nanoparticles planned for IV management is of prime significance. The drug-loaded nanoparticles need to expose minimal cargo leakage in the blood flow with managed drug release at the target site. Fast release of drug in the blood stream is unwanted as it can result in systemic poisoning [24, 25] Continual Ansamycin release from the nanoparticles is taken into consideration as a preferable release behaviour.

To clarify the in vitro drug release behaviour of free drug and also nanoformulation of ansamycin, we examined the drug release characteristics in PBS (pH 7.4) at 37 °C by the dialysis technique and also fluorometric evaluation. Release of free drug was extremely fast with 48% as well as 91% drug release following with 0.5-h and also 1-h incubation, respectively. Under the exact same condition, a burst release of 24% from nanoformulation was located at 2 h followed by 38% at 6 h, 52% at 12 h and 94% at 36 h (Fig. 13). The initial burst release in the delivery system could be accounted for any free or surface bound drug.

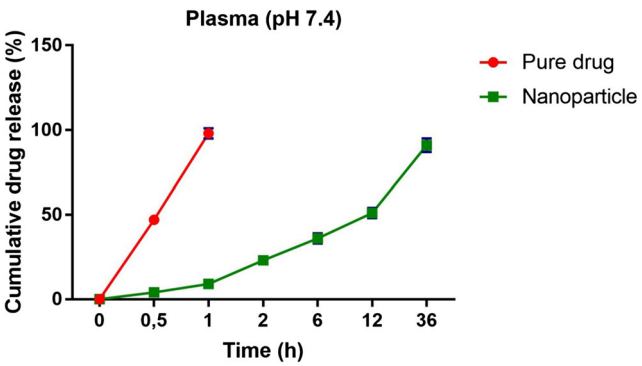

Fig. 14 Cumulative percentage drug release of pure drug ansamycin and ansamycin-loaded polymeric nanoparticles in plasma (pH 7.4)

To resemble the possible in vivo release of ansamycin, in vitro release was additionally examined in human plasma. There was no much deviation noted with the release pattern in the plasma medium. A release of 23% at 2 h, 36% at 6 h, 51% at 12 h and also 91% at 36 h (Fig. 14) was observed. This release may further decrease in an in vivo environment due to first pass effect. Although in vitro release studies in the presence of plasma try to mimic in vivo condition, it may not exactly simulate the harsh in vivo environment.

3.5.5 Stability of Ansamycin-Loaded Polymeric Nanoparticles

To explore the impact of storage temperature on the stability of ansamycin-loaded polymeric nanoparticles, the nanoparticles were stored at 2–8 °C as well as 25 °C in the dark over a duration of 180 days. A rise in particle size and also reduction in zeta potential as well as encapsulation effectiveness were observed with storage time at both the storage conditions. To examine any changes in the drug release profile during storage, drug release studies were done and also compared with the preliminary formulations. A marginal difference in the release rate was observed from both the formulations stored at different conditions. A sustained drug release was noticed with both the formulations stored at different storage conditions.

4 Conclusion

An emulsification solvent diffusion method was employed to prepare the polymeric nanoparticles. The ansamycin-loaded polymeric nanoparticles were optimized using the central composite rotatable design–response surface methodology by fitting a second-order model to the response data. The experimental values of the nanoparticles prepared under the optimum conditions were mostly close to the predicted values. Ansamycin-loaded polymeric nanoparticles under the optimized conditions gave rise to the EE of 89%, mean particle size of 121 nm and zeta potential value of −25 mV. SEM showed that the nanoparticles are spherical, loading with drug microcrystal uniformly on the surface of and inside the nanoparticle. The drug release behaviour from the nanoparticles exhibited a biphasic pattern with burst release at the initial stage and sustained release subsequently. The drug release experiments in the nanoparticles in vitro exhibited a sustained release over 36 h. These results indicated that the polymeric nanoparticles obtained in this study could potentially be exploited as a carrier with an initial dose and prolonged plasma level in vivo when therapeutically desired.

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Authors' Contributions KGS formulated, optimized and characterized the nanoforumulations and also writing the manuscript. RV was again a major contributor in reviewing the manuscript. SS approved the final manuscript. All authors read and approved the final manuscript.

Compliance with Ethical Standards

Competing Interests The authors declare that they have no competing interests.

Research Involving Human Participants or Animals None.

Informed Consent None.

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