



“FORMULATION OF SIMVASTATIN LOADED CHITOSAN NANOPARTICLES BY USING CENTRAL COMPOSITE DESIGN”

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Abstract: The goal of this study is to design and develop a Simvastatin particulate drug delivery system utilizing the ionic gelation method in order to improve the drug's solubility and sustain its release. Chitosan (77% DA), non-toxic, biodegradable and biocompatible polymer were used as matrix material while Sodium tripolyphosphate used as the crosslinking agent. Central composite design was chosen as statistical tool for analysis study and to optimization of formulation variables like CS percentage, TPP percentage and DR. The formula that was found to be the most efficient was chosen and characterized for particle size, polydispersity index, zeta potential, EE%, drug loading, in-vitro drug release, DSC, XRD and SEM. The stability study of optimal SIM CS-TPP NPs was carried out for one month at room temperature. Optimized formulations had PS 281.4nm, a narrow PDI 0.441, ZP -17.7mv, sustain drug release 71.48% over 12 hrs and EE% of 99.94%. SEM study confirmed that successful fabrication of nearly spherical shaped particles in nanometer range. There was no physical change and no huge disparity in PS and ZP after one month of stability testing, showing that the formulation was stable. As a result, nanoparticulate systems such as PNP might be used to improve the bioavailability of poorly soluble drugs such as Simvastatin.

Keywords: Simvastatin, Chitosan, Sodium Tripolyphosphate, Central Composite Design.

1.Introduction:

Recently, substantial efforts have been made to target a medication or drug delivery system in a particular section of the body for a longer duration to achieve desired benefits, not only for local drug targeting, but also for better control of systemic drug delivery. Traditional dosage forms are frequently associated with challenges in delivering the prescribed dose to the intended location. Many drugs experience major presystemic metabolism in the liver when taken orally, resulting in GI discomfort, poor absorption, and bioavailability. Limitations to parenteral drug delivery are high production cost and poor patient compliance, thus it becomes necessary to explore other novel routes of drug delivery [1]. One approach to improving the physicochemical characteristics of a medicament is to use a particle drug delivery system. It is based on altering physical nature of the drug to alter its properties like solubility, permeability etc. Particulate drug delivery system comprises with microparticles, microsphere and nanoparticle etc. Nanotechnology is at the forefront of these new technologies for drug delivery [2]. Nanocarriers can improve the bioavailability of loaded drugs by allowing them to stay in systemic circulation for longer periods of time and by providing a controlled drug release profile, resulting in steady-state plasma levels and fewer side effects [3]. Various formulation components of this system are stabilizer, organic solvents, buffers, cross linking agent and cryoprotectant. Most important component of this system is polymer which can be classified as natural or synthetic polymer. This polymer can combine with drug or other API in a way that drug release can be controlled and give ideal pharmacokinetic profile according to need of dosage form. Biodegradable polymers have the advantage of being completely eliminated from the body via normal metabolic processes. Besides having biocompatible and biodegradable properties of natural polymer; nevertheless, due to batch-to-batch variability in characteristics and the possibility of mild immunogenicity, their usage has been limited. On the contrary, synthetic polymer are well-known for their well-regulated chemical makeup [4,5]. Ischemic heart disease (ICH) is the leading cause of death worldwide, accounting for 16% of all casualties. Since 2000, this disorder has had the most significant rise in mortality, increasing by more than 2 million to 8.9 million fatalities in 2019. Stroke and chronic obstructive pulmonary disease (COPD) are the second and third leading causes of death, respectively accounting for 11 percent and 6% of all deaths [6]. Simvastatin is an HMG-CoA (Hydroxy-3-methylglutaryl coenzyme A) reductase inhibitor that lowers lipid levels and lowers the risk of cardiovascular events including myocardial infarction and stroke. Simvastatin is an anionic lipophilic drug having a poor bioavailability in the oral route [7]. Because it is practically insoluble in water (maximum solubility of 30 g/mL), shows poor absorption in GI tract, with less than 5% absorption, after a 40 mg oral dosage and first-pass metabolism. As a result, this is an excellent candidate for the current study [8]. A substance that can be derived from natural sources and has the capacity to manufacture polymeric nanoparticles is preferable to synthetic polymer. Using a cross linking agent, this agent can be cross linked. Cross-linking agents can be found in a variety of forms like Sodium tripolyphosphate, Glutaraldehyde and Formaldehyde etc. [9]. Chitosan is a natural polysaccharide that is a cationic copolymer of glucosamine and N-acetylglucosamine units connected by 1–4 glucoside linkages. It is the most widely used and distributed biomaterial after cellulose. CS is a useful drug delivery system in pharmaceutical formulations because of its in-situ gelation, biodegradability, biocompatibility, excellent adhesion qualities, and lack of toxicity [10]. Intramolecular and intermolecular cross-linking is mediated by electrostatic attraction between the positively charged amino groups of CS and the negatively charged phosphates of TPP when CS is combined or homogenised with TPP, a multivalent anionic polymer, resulting in the formation of hydrogel [11,12]. Because of its nontoxic properties and fast gelling capacity, sodium tripolyphosphate is commonly employed. It can also create a reasonably high-density stable complex with CS [13]. A two-factor, three-level (3^2) Central Composite Design of Response surface study was selected for statistically optimize the formulation variables of SIM CS-TPP NPs preparation [14]. It is a valuable statistical experimental tool for the study of for the main effect and interactions of different factors. Design Expert® software was used to construct and estimate the experimental design (Version 13, Stat-Ease). The independent variables were: 1. Chitosan percentage 2. Sodium Tripolyphosphate percentage. The levels of factors selected as (-1,0,+1) and their representative actual values are shown in Table 1. The dependent variables were chosen as PS(Y_1), ZP(Y_2) and DR(Y_3).

2. Materials and methods:

2.1 Materials

Simvastatin (Sim) was obtained from Aarti Pharmaceuticals, Mumbai. Sodium tripolyphosphate (TPP) was procured from Kemphasol Pvt. Ltd. Mumbai. Chitosan (CS) (77%DA) was supplied as gift sample from Pelican Biotech Pvt. Ltd. Kerala. Poloxamer 188 was obtained from BASF Ltd. Mumbai. All other chemicals & reagents were of extra pure analytical grade.

Table 1: Independent variables and respective levels in the CCD for SIM CS-TPP NPs preparation and their levels for the optimized SIM CS-TPP NPs formula.

Factors (Independent variables)	Levels of variables			Optimized level
	Low	Medium	High	
1.Chitosan(% w/v)	0.6	0.8	1	0.948
2.Sodium Tripolyphosphate(% w/v)	1.8	2.4	3	2.985

2.2 Experimental design

A two-factor, three-level 3^2 CCD was conducted to statistically optimize the formulation variables of SIM CS-TPP NPs preparation. Construction and estimation of the experimental design was performed using Design Expert[®] software (Version 13, Stat-Ease). The independent variables were: 1. Chitosan percentage 2. Sodium Tripolyphosphate percentage. The levels of factors selected as (-1, 0, +1) and their representative actual values are shown in Table 1. The dependent variables were chosen as PS(Y_1), ZP(Y_2) and DR(Y_3).

The low and high level of CS and TPP percentages were selected based on our preliminary study i.e. saturated solubility of SIM in presence of CS. According to this study, the solubility of SIM rises when the concentration of CS increases up to the certain threshold i.e. 320mg after that it get declines.

According to the CCD followed, with face centered, 15 formulae were generating out of which eliminating repeated formulae, 9 formulae were prepared. The 9 formulae of the 3^2 CCD with their composition are shown in Table 2. The estimation of model and term significance was performed by analysis of variance (ANOVA). Probability p-value ($p < 0.05$) denoted significant.

Table 2: Composition of the SIM CS-TPP NPs.

Formula	Factors level in actual value	
	Chitosan(% w/v)	Sodium Tripolyphosphate(% w/v)
F1	0.6	1.8
F2	0.6	2.4
F3	0.6	3
F4	0.8	1.8
F5	0.8	2.4
F6	0.8	3
F7	1	1.8
F8	1	2.4
F9	1	3

2.3 Preparation of Simvastatin loaded CS-TPP NPs by ionic gelation method

According to the above-mentioned design, nine formulae of SIM CS-TPP NPs were prepared by the ionic gelation method [15] using TPP as a crosslinking agent and Poloxamer 188 as stabilizer [16,17]. Different weight of CS (77%DA) was dissolved in 1.5% of (v/v) acetic acid and stirred at 350 rpm on magnetic stirrer for 24 hours. The Poloxamer 188 1.5%(w/v) prepared solution were added in CS solution and stirred for 5min,at 600rpm.Briefly SIM 40mg was dissolved in 1.5ml of ethanol and prepared solution were added in CS-TPP solution dropwise and stirred at 600rpm for 15min.Finally 2ml of TPP solution(prepared by dissolving different weights of TPP in distilled water)was added dropwise to the prepared CS-SIM solution and sonicated with Ultra probe sonicator(BIO-TECHNIQUES INDIA) with 10s on time,5s off time with total time 7minutes.Were amplitude set as 25%.Homogenized SIM-CS-TPP NPs stirred on magnetic stirrer for 2hours at 600rpm for hardening and to achieve complete interaction between CS and TPP. The resulted NPs were separated by using cooling centrifuge (Bio-era) at 14000rpm for 15min at 20⁰C.Separated pellets were dried in hot air oven at 80⁰C.

2.4 Characterization of Prepared SIM CS-TPP formulations.

2.4.1 PS and PDI analysis of SIM-CS-TPP NPs

The particle size (PS) and particle size distribution (PDI) were determined through dynamic light scattering (DLS) with a HORIBA scientific SZ-100 instrument as a particle size analyzer. The samples were appropriately dispersed within distilled water and sonicated by using bath sonicator for 5 minutes. The reading was taken at detection angle 90⁰ and at 25⁰C temperature for 80s.

2.4.2 Measurement of ZP of SIM-CS-TPP NPs

The zeta potential (ZP) measures the overall charge carry by the particle and formulations stability. The electrophoretic mobility measurement determined using HORIBA scientific SZ-100 instrument. The sample were dispersed in appropriate distilled water. All measurements were carried out at 24.8⁰C for 120s.

2.4.3 Determination of EE% of SIM-CS-TPP NPs

The quantity of un-entrapped drug in the aqueous solution, i.e., supernatant after centrifugation at 14000 rpm for 15 minutes at 20⁰C, was used to calculate the EE percent of the NPs. Compared with the total quantity of drug added to the formulation. The supernatant was diluted and examined at 239 nm using a UV Spectrophotometer (LABINDIA[®] ANALYTICAL UV 3000+) The amount of free SIM estimated by comparing absorbance of filtered solution to preconstructed SIM calibration curve(R²=0.9947).

For the purpose of calculating entrapment efficiency, the following formula was used:

Entrapment efficiency (EE) % =

Initially used amount of drug in formulation (Wi) – Drug amount found in the supernatant (Wf)

Initially used amount of drug in formulation (Wi)

2.4.4 Determination of Percentage yield and Drug loading of SIM-CS-TPP NPs

The percentage of yield and drug loading was determined by indirect method. After drying of formulation, weight was calculated. Further calculation done by using following formula:

$$\% \text{ Nanoparticle's yield} = \frac{\text{Mass of nanoparticles recovered}}{\text{Mass of polymer, drug and excipients}} * 100$$

$$\% \text{ Drug loading} = \frac{\text{Mass of drug in nanoparticles preparation}}{\text{Mass of nanoparticles recovered}} * 100$$

2.4.5 In-vitro Drug release:

The dialysis bag diffusion method was used for to check in-vitro drug release of the F1 to F9 batches [18]. An amount of dried NPs equivalent to 10mg of simvastatin were suspended in dialysis bag(Dialysis Membrane-135,HIMEDIA®) with 2ml pH phosphate buffer 7.4, after which both ends were kept closed. And kept in beaker with 100ml of release medium (phosphate buffer pH 7.4 80ml + 20ml ethanol). The beaker was placed over a magnetic stirrer(BIO TECHNIQUES INDIA) at 100 rpm where temp. was maintained at 37°C. 5 ml sample withdrawal at definite time intervals(15min,30min,1,2, 3,..12hr),the sink condition was maintained each time. The amount of drug released was analyzed spectrophotometrically at 252nm using UV spectrophotometer.

The release mechanism was studied by using mathematical model.

2.5 Formulation Optimization

After imposing specific constraints on particle size, zeta potential, and drug release, shown in Table 3, the best SIM CS-TPP NPs formula was developed utilizing Design-Expert 13 software, surface response study, and Central Composite Design. The resulting optimal formula was then produced and examined for additional characterization such as Particle size, PDI, Zeta potential, DSC, XRD ,in vitro drug release, and SEM analysis.

Table 3: Model summary statistics of model given by software for tested responses, constraints for SIM CS-TPP NPs formula and the predicted and observed value of the responses.

Responses	r ²	Adjusted r ²	Predicted r ²	Constrains	Predicted	Observed	95% prediction interval
Y ₁ : Particle size(nm)	0.7727	0.6363	-0.2088	Minimum	206.67	281.4	328.134
Y ₂ : Zeta Potential(mv)	0.6616	0.0975	-2.9778	In Range	-20.63	-17.7	-4.53864
Y ₃ : DR (%)	0.8723	0.8297	0.7156	Maximum	78.08	71.48	83.6974

2.6 Characterization of optimal SIM CS-TPP NPs formula

2.6.1 PS analysis, PDI, ZP, EE% and drug loading

The PS, PDI, ZP, EE% and drug loading of optimized formula was carried out by the same method as previously described.

2.6.2 Fourier Transformation Infrared Spectroscopy (FTIR)

The structural features of pure drug and optimized formula were estimated by FTIR. The spectra of SIM, CS, TPP, Poloxamer 188, Physical mixture and optimal formula were recorded for using Bruker ATR-IR spectrophotometer. The sample is directly placed on clean ATR crystal and hold in probe. The 4000-450 cm^{-1} range were used for scanning.

2.6.3 Differential scanning Calorimetry (DSC)

The thermal stability of drug and optimized formula were determined by DSC. The DSC thermograms of pure SIM and optimal formula were recorded by using Differential scanning Calorimeter (Universal V4.5A TA Instrument ((SDT Q600 V20.9 Build 20)). Sample 4.09mg were placed in aluminium pan and heated within the range 0-200 $^{\circ}\text{C}$ at the rate of 10 $^{\circ}\text{C}/\text{min}$. in N_2 atmosphere.

2.6.4 Powder XRD (X-ray powder diffractometry):

The crystalline habit of pure drug plays a critical role in assessing the stability of nanoparticles as they may undergo polymorphic changes during storage. The Powder XRD patterns of pure drug SIM and optimized formula were obtained using X-ray powder diffractometry (PW 1719, Philips, The Netherlands) with Cu as anode material operated at a voltage of 46 kV and a current of 40mA. The sample were analyzed in the 2θ angle range of 10-90 $^{\circ}$.

2.6.5 In-vitro Drug release:

The Optimized formulations in vitro drug release was determined using dialysis bag diffusion method as previously described. Same procedure was followed for pure drug SIMs in-vitro drug release.

2.6.6 Scanning Electron Microscopy (SEM):

The surface morphology of the optimized batch was examined by scanning electron microscopy the sample were mounted onto an aluminium stub using double sided carbon adhesive tape and coated with gold palladium at 50mA for 6min. The particles observed via SEM under high vacuum at an ambient temperature.

2.6.7 Stability studies:

The optimized SIM loaded CS-NPs formation was transferred to glass vial and was kept in desiccator. Stability study was carried out at room temperature for one months. The nanoparticles were evaluated for its physical appearance, particle size and zeta potential.

3.Result and Discussion

3.1 Statistical Analysis of the 3²CCD

Design-Expert 13 software and Central composite design are used to do statistical analysis of the data received from the trial. After imposing precise restrictions on particle size, zeta potential, and drug release, the optimum Sim CS-TPP NPs formula was found. The resulting optimal formula was then produced and examined for further assessment using particle size, PDI, Zeta potential, DSC, XRD, in vitro drug release, and SEM.

Two independent variables and three response variables were used in the Design research. Particle size (Y1), Zeta potential (Y2), and Drug release (Y3) are response variables, with Chitosan concentration (A) and Sodium Tripolyphosphate concentration (B) as independent factors. These responses were fitted using linear regression to a 2-factor interaction (2FI) for particle size, a quadratic for Zeta potential, and a linear for drug release to get the model of choice with the greatest adjusted and projected r^2 . The significance of the difference was determined using a one-way ANOVA with a 0.05 probability threshold.

Particle size (Y_1): $-1,211.09 + 1,902.25 A + 656.389 B - 828.75 AB$ (Equation 1)

Zeta potential (Y_2): $-137.267 + 315 A + 5.5 B - 12.0833 AB - 180 A^2 - 0.277778 B^2$ (Equation 2)

Drug release(Y_3): $33.8644 + 18.5 A + 8.93056 B$ (Equation 3)

3.1.1 Influence of the investigated factor on PS and PDI

The particle size and particle density index (PDI) of nanoparticles are key variables in their performance. It was discovered that smaller particles have more surface area and are more efficient at entrapment. While the particle size distribution of nanoparticles was revealed by PDI. For mono dispersed particles, the PDI value starts at 0.01. While sample with very broad size distribution have PDI value ≥ 1 . As shown in Table 4, the PDI values of formulations was found within range 0.571 to 2.811.

The prepared SIM CS-TPP NPs must be small enough to cross the biological membrane with enhanced penetration and to sustain the drug release. As shown in Table 4, the PS of SIM CS-TPP NPs ranged from 209.2nm to 436.6nm, suggesting the capacity to producing small NPs.

ANOVA analysis revealed that CS percentage and TPP percentage were the significant factors affecting mean PS($p < 0.5$). As shown by 3D surface plot illustrated in Fig.1a and equation 1, increasing both factors (CS and TPP concentrations) lead to increase in particle size. While combined effect of CS and TPP concentration was inverse. Higher the concentration of CS and TPP leads to larger -sized particles resulting from the accumulation of CS molecules themselves on the surface of the molecule.

3.1.2 Influence of the investigated factors on ZP

The zeta potential (ZP) of a particle indicates the particle's total charge and formulation stability. The particle stability in dispersion is significantly influenced by the surface charge, which reduces agglomeration formation through electrostatic repulsion between particles. The ZP range of ± 30 mv indicates formulation stability. The zeta potential was determined to be between -3 and -21.6 mv as shown in Table 4. The utilization of NaTPP as a crosslinking agent is indicated by the negative zeta potential.

According to the statistical data obtained from the design expert tool, ANOVA analysis showed that the factors CS and TPP concentration do not show any significant effect on ZP. The 3D surface graph Fig 1b, and equation 2 revealed that zeta potential increases as the concentration of Chitosan increases up to a point and then decreases, whereas the zeta potential increases as the concentration of Sodium Tripolyphosphate increases. While on combination shows inverse effect.

3.1.3 Influence of the investigated factors on DR

According to the aforementioned findings, cumulative drug release during 12 hours ranged from 62.62 % to 77.42 %. Because of their smaller particle size, batches F3, F6, F8, and F9 had the greatest drug release (71.16 %, 75.68 %, 77.15 %, and 77.42 %, respectively) as shown in Table 4. Batches indicate an initial burst release for the first 15 minutes, omitting F9, and then a gradual release over the next 12 hours. The first burst release was caused by untrapped drug on the nanoparticles' surface. Due to appropriate encapsulation of the medication within nanoparticles, the release continues to be sustained.

The 3D surface graph Fig 1c and equation 3 indicate that the drug release rises as the concentration of both Chitosan and NaTPP increases, based on the statistical data collected from the design expert tool.

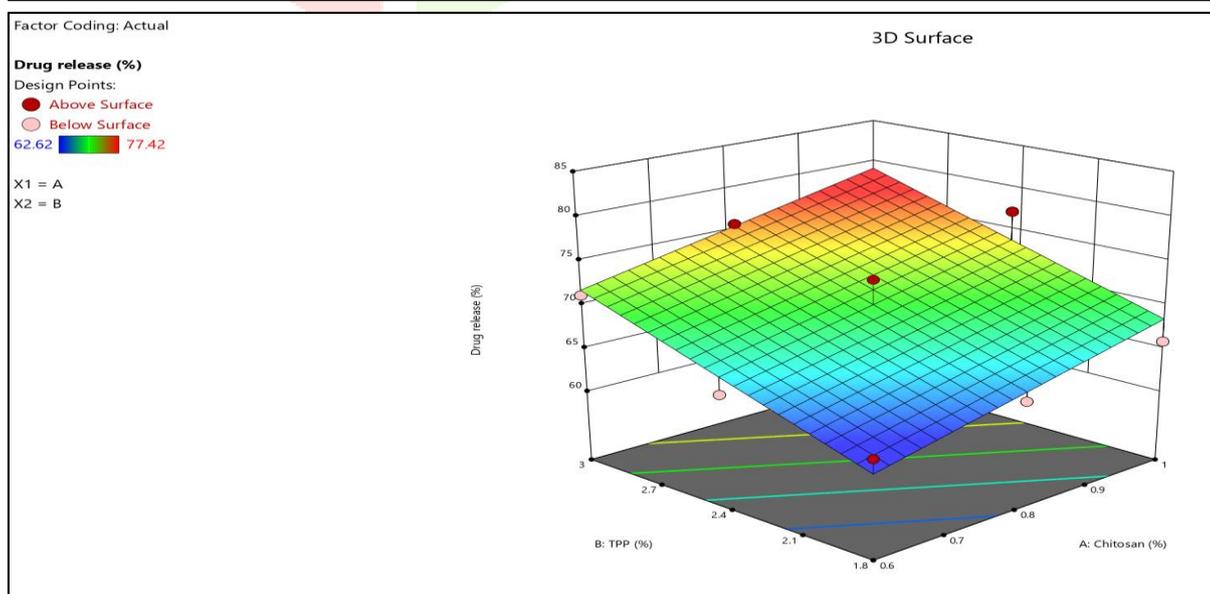
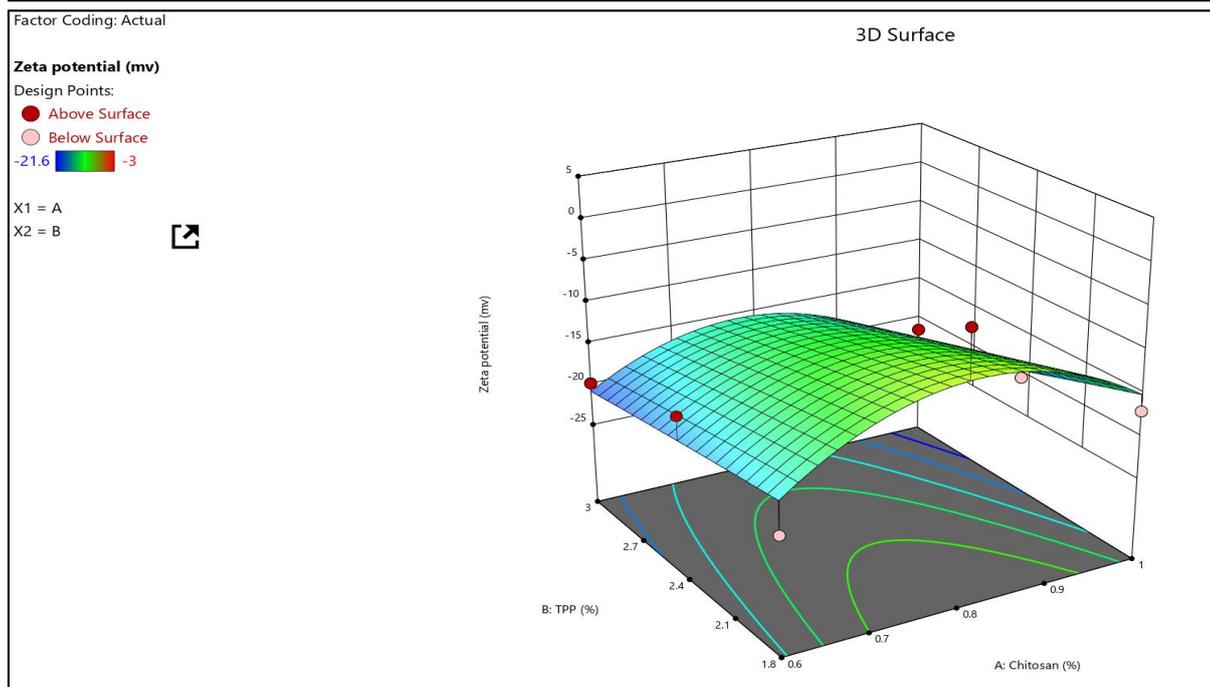
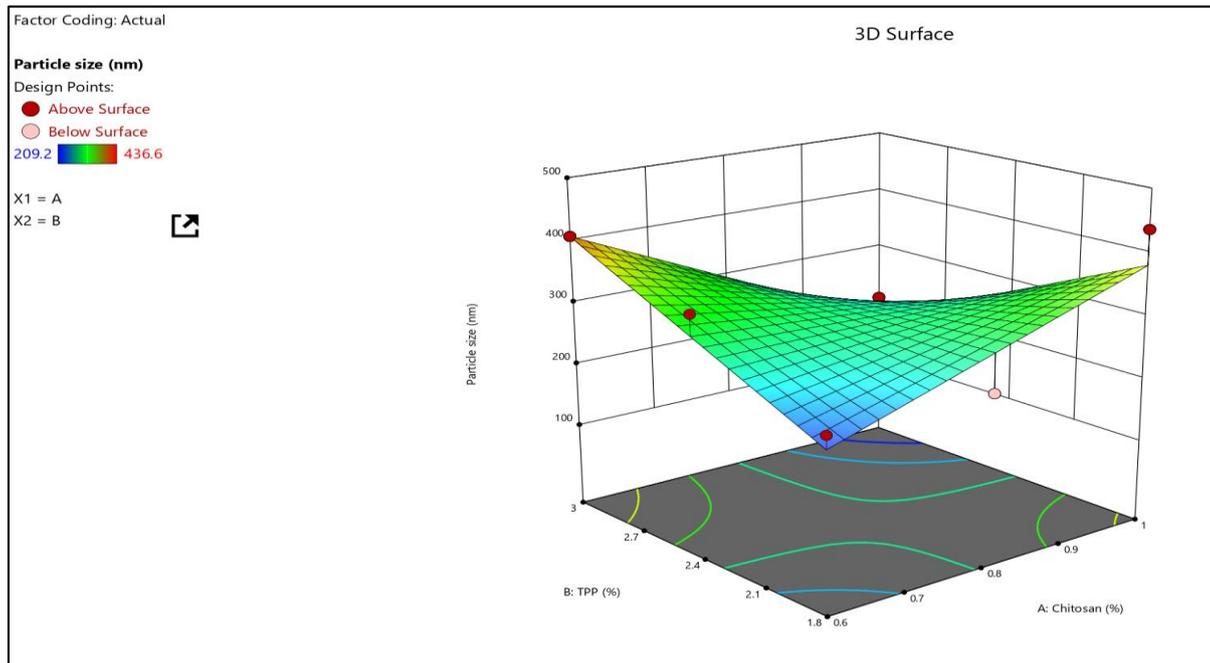


Fig 1 3D surface plot of a) Particle size analysis b) Zeta potential analysis c) Drug release analysis.

Table 4:PS, ZP and DR for the prepared SIM CS-TPP NPs

Batch code	Particle Size (nm)	Poly Dispersity Index	Zeta Potential	EE%	Percentage yield	Drug loading	%DR
F1	237.6	0.749	-21	99.88	39.65	34.78	62.62
F2	345.9	2.811	-16	99.91	41	35.08	64.41
F3	408	0.75	-19.8	99.92	49.22	35.95	71.16
F4	236.9	0.848	-3	99.92	51.54	33.39	63.67
F5	267.7	0.88	-14	99.91	46.09	35.27	72.95
F6	270.1	0.773	-19.4	99.91	40.89	37.91	75.68
F7	436.6	1.428	-17	99.88	42.1	35.71	65.82
F8	241.6	0.855	-20.6	99.89	50.65	36.90	77.15
F9	209.2	0.571	-21.6	99.88	51.98	37.96	77.42

3.2 Drug release Kinetics Study of F1 to F9 formulations

The kinetic study is useful to determine the release mechanism of drug from the formulation. Release data were fitted to kinetic models in order to identify the DR kinetics. It was found that in- vitro DR significantly fitted to Higuchi model, as the plot shows that maximum linearity regression coefficient (R^2) than others. It reveals that drug released through diffusion mechanism.

Table 5: Drug release kinetic Study of F1to F9 batches

KINETIC MODEL	F1	F2	F3	F4	F5	F6	F7	F8	F9
ZERO ORDER MODEL	0.9223	0.8985	0.8681	0.8699	0.8829	0.8829	0.9426	0.7239	0.8797
FIRST ORDER MODEL	0.9368	0.1618	0.1629	0.166	0.165	0.1635	0.1605	0.1679	0.1644
HIGUCHI MODEL	0.9442	0.9356	0.9061	0.9478	0.9315	0.8931	0.9284	0.8443	0.9162
HIXON CROWELL MODEL	0.9344	0.9046	0.8756	0.8773	0.8895	0.8612	0.9453	0.9453	0.8827
KORSMEYER-PEPPAS MODEL	0.7269	0.5718	0.4928	0.5499	0.5327	0.4749	0.6034	0.3321	0.5525

3.3 Determination of EE%, Percentage yield and drug loading of F1 to F9 batches

The ratio of experimentally measured percent of drug content to real or theoretical mass of drug utilized in nanoparticle production is known as entrapment efficiency. The findings reveal as table 4 and fig 2, that all nine batches had the maximum entrapment efficiency. Batches F3 and F4 have 99.92 percent EE, whereas batches F1 and F8 have 99.88 percent. Because of the hydrophobic character of the medication Simvastatin and the hydrophilic nature of the polymer Chitosan, the formulation has the maximum entrapment efficiency. The percent weight of active ingredient encapsulated to the weight of nanoparticles is known as drug loading. The effectiveness of loading is determined by the polymer drug blend and technique utilized. The drug loading was determined to be in the range of 33.39 % to 37.96 % as shown in table 4. While the percent yield was discovered to be 39.65% -51.98 percent. The drug loading rises as the particle size decreases, and as the polymer concentration increases from F1 to F9, the amount of chitosan accessible for entrapment increases.

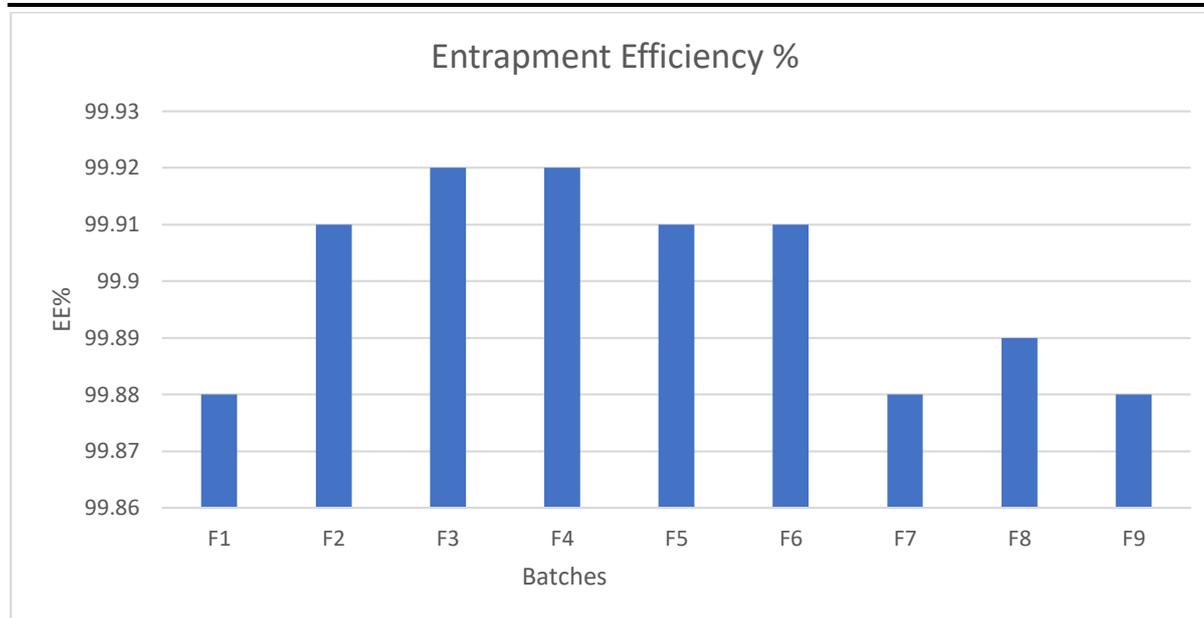
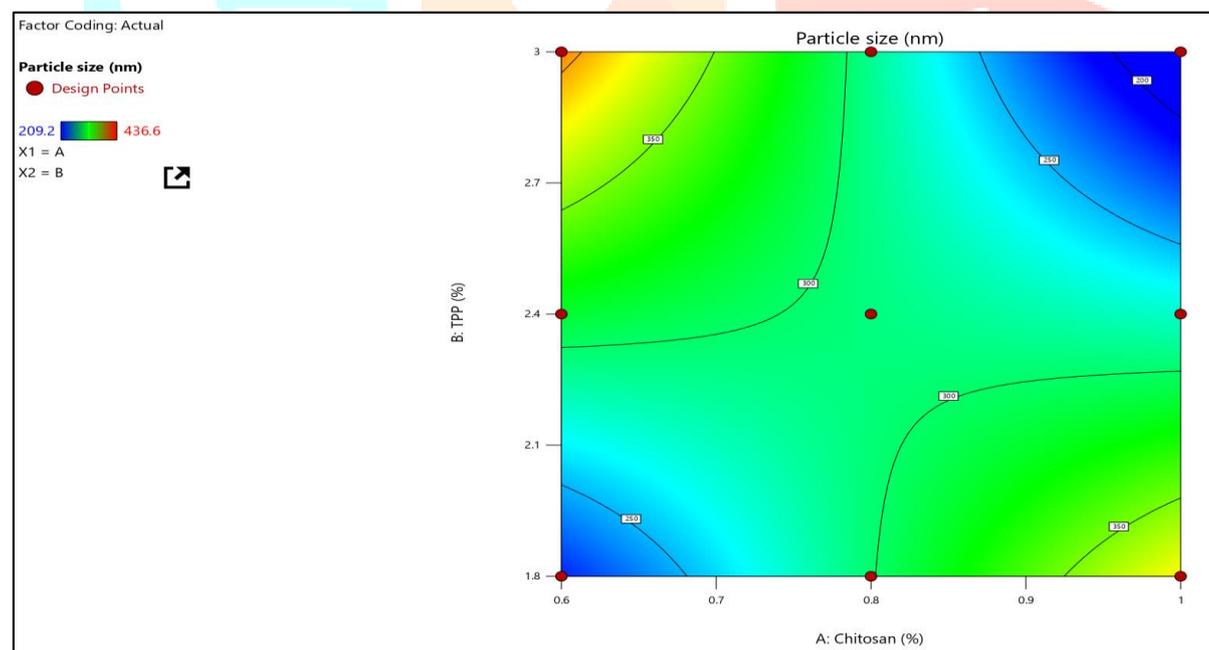


Fig. 2 Entrapment Efficiency of F1 to F9 batch

3.4 Formulation Optimization

After applying constraints on PS, ZP and DR the optimized formula was chosen by Design Expert® software, with an overall desirability 1. The factor level of the optimized formula as shown in table 1. The optimum formula was prepared for the assessment of the optimization process and further characterization on the basis of Contour plots and Overlay plot Fig 3 and Fig.4 respectively .



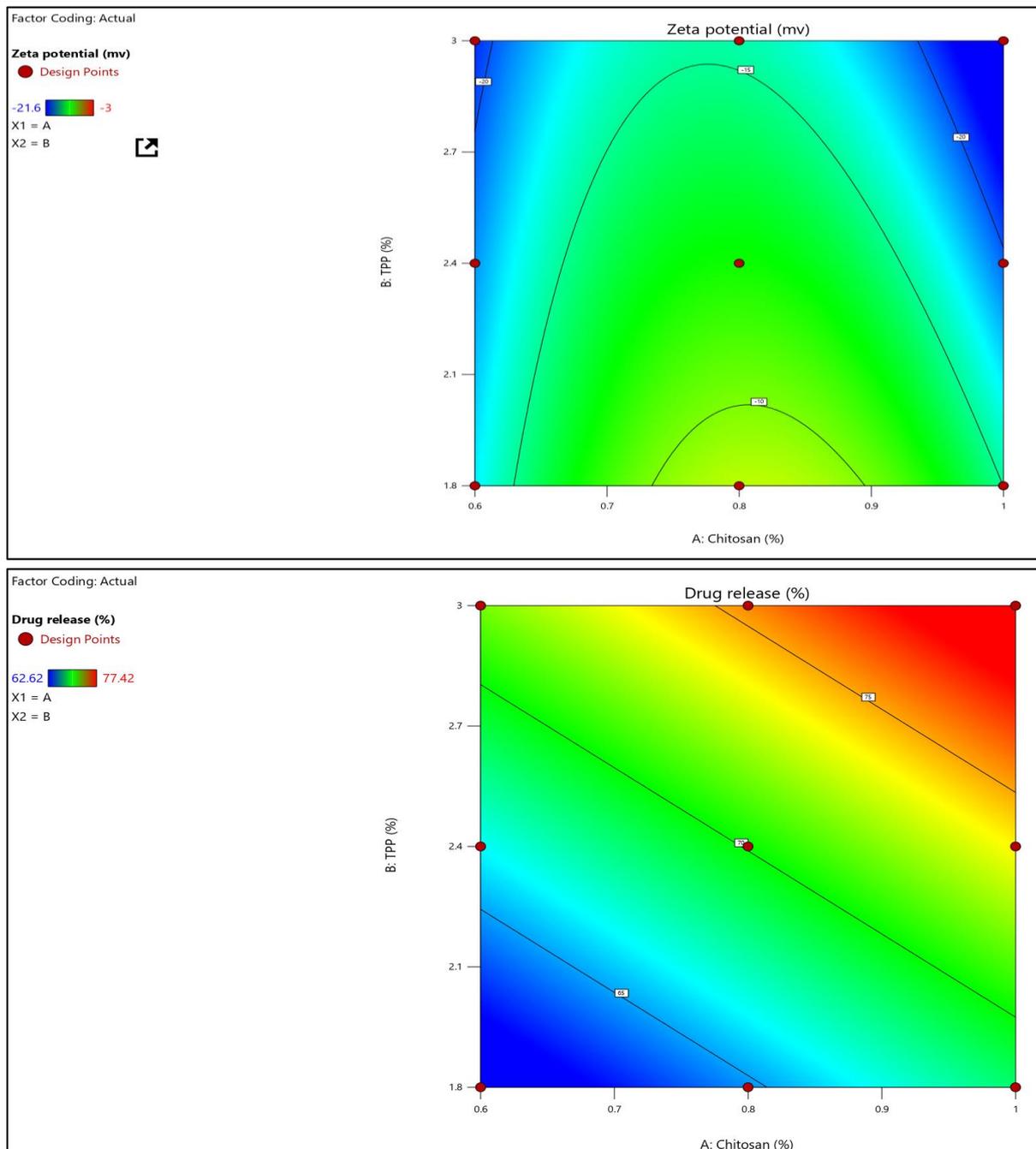


Fig. 3 Contour graph of Particle size, Zeta potential and Drug release

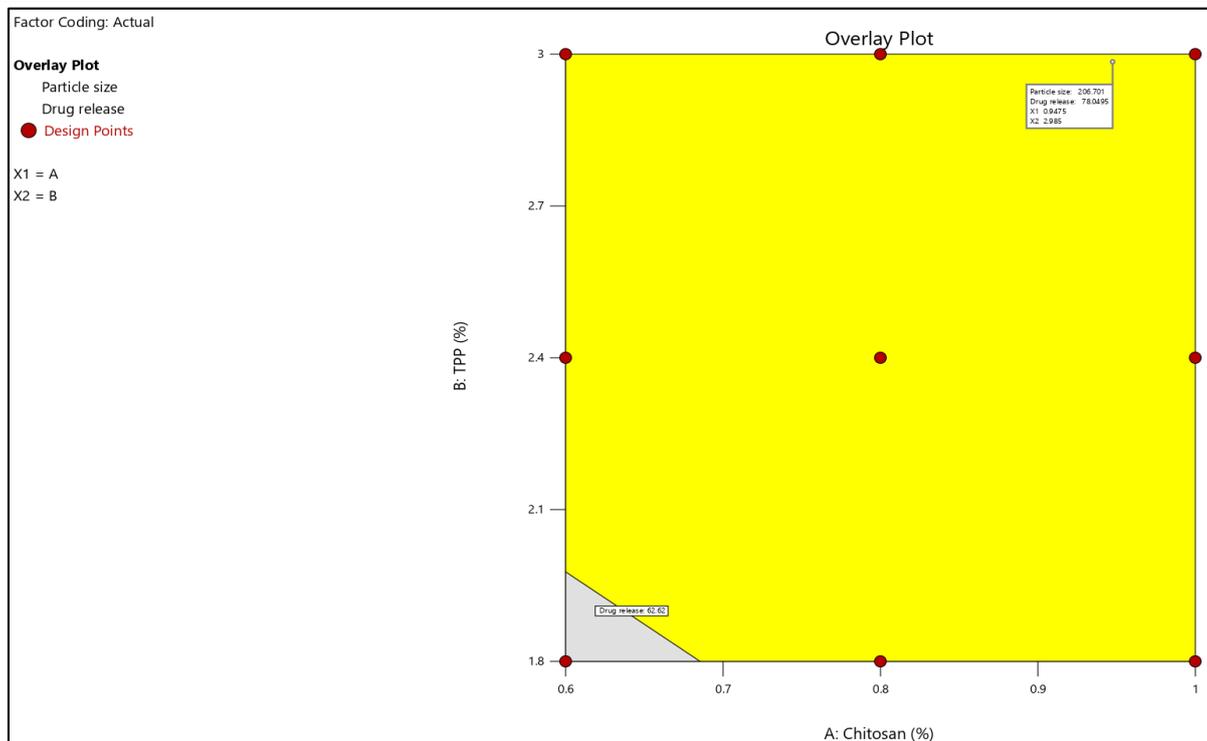


Fig. 4 Overlay plot

3.5 Characterization of the optimal SIM CS-TPP NPs

3.5.1 PS, PDI, ZP, EE% and drug loading

The suggested SIM CS-TPP NPs formula was prepared and evaluated. The validity of the optimization process was confirmed since the observed P, ZP and DR exist between the low and high confidence intervals of the predicted value, as shown in Table 3 and Fig.5 and Fig.6. In addition, EE% and drug loading was found to be 99.94% and 36.93% respectively. Due to the successful incorporation of medicament into the polymers shows formulation had best entrapment efficiency.

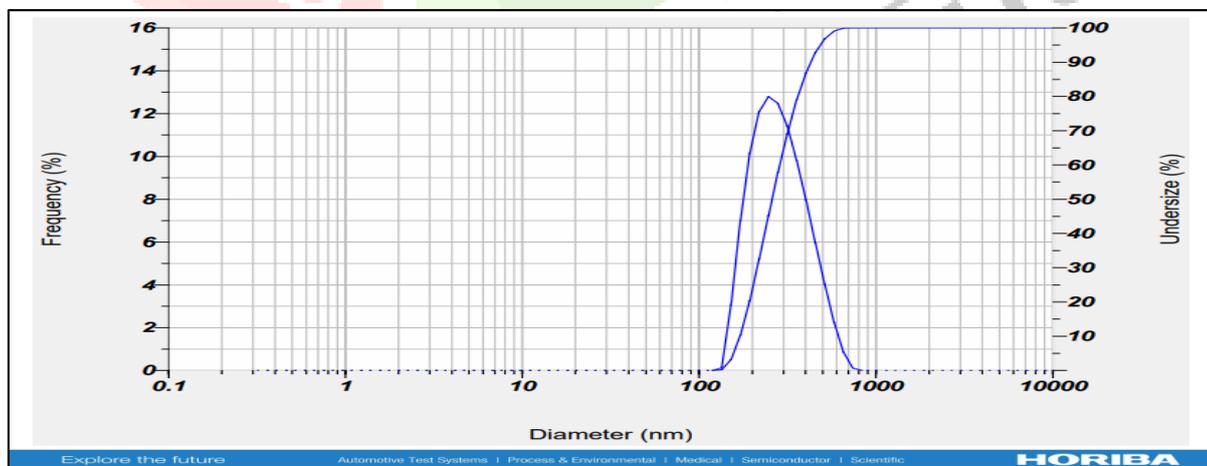


Fig. 5 Particle size distribution of optimal SIM CS-TPP NPs

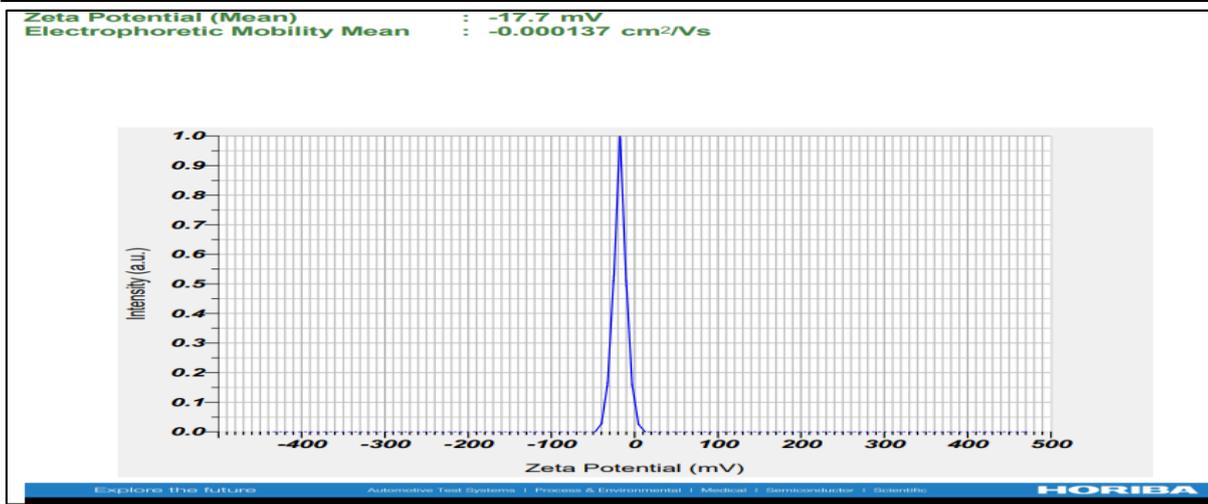
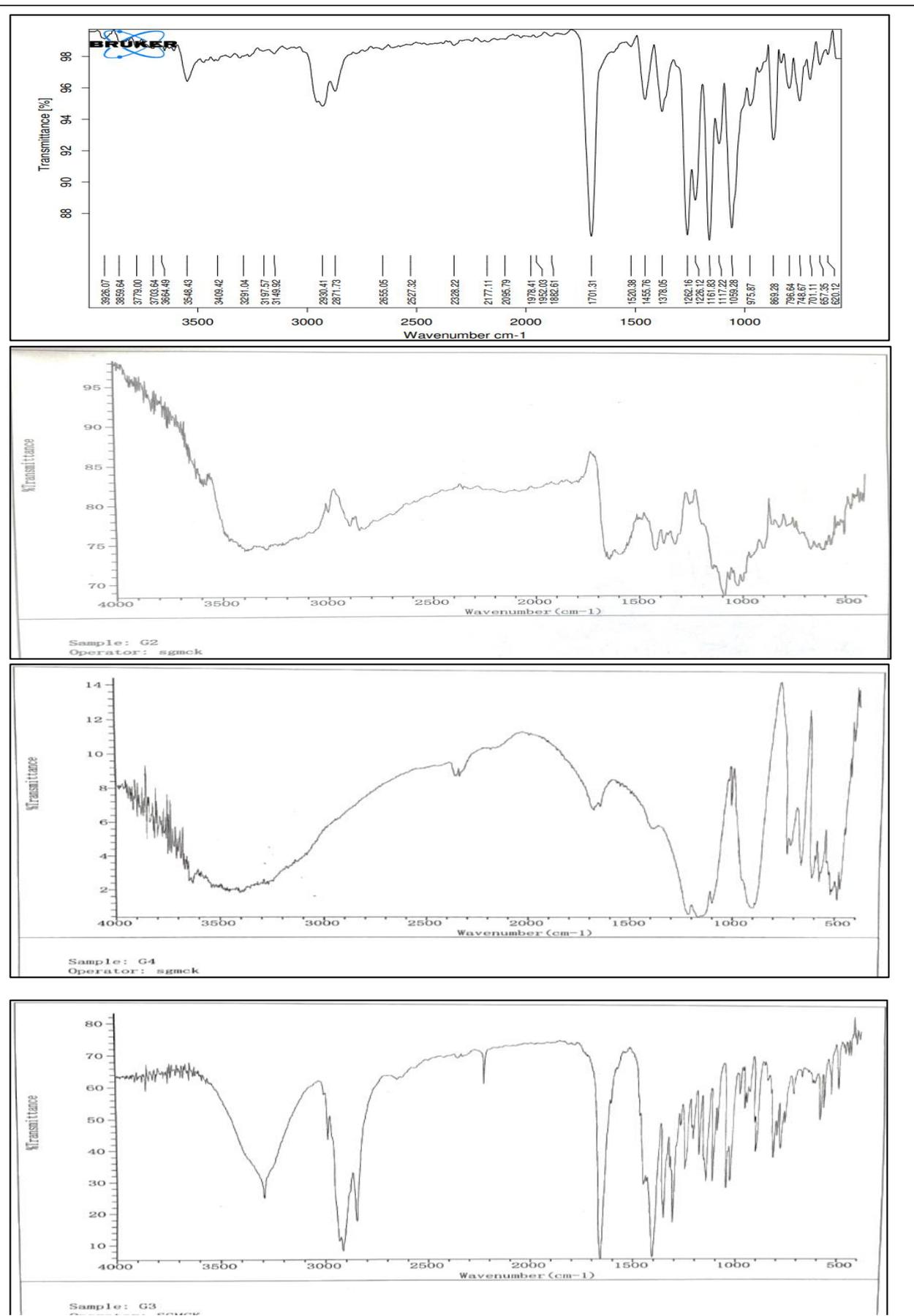


Fig. 6 Zeta potential Graph of optimal SIM CS-TPP NPs



3.5.2 FT-IR spectroscopy



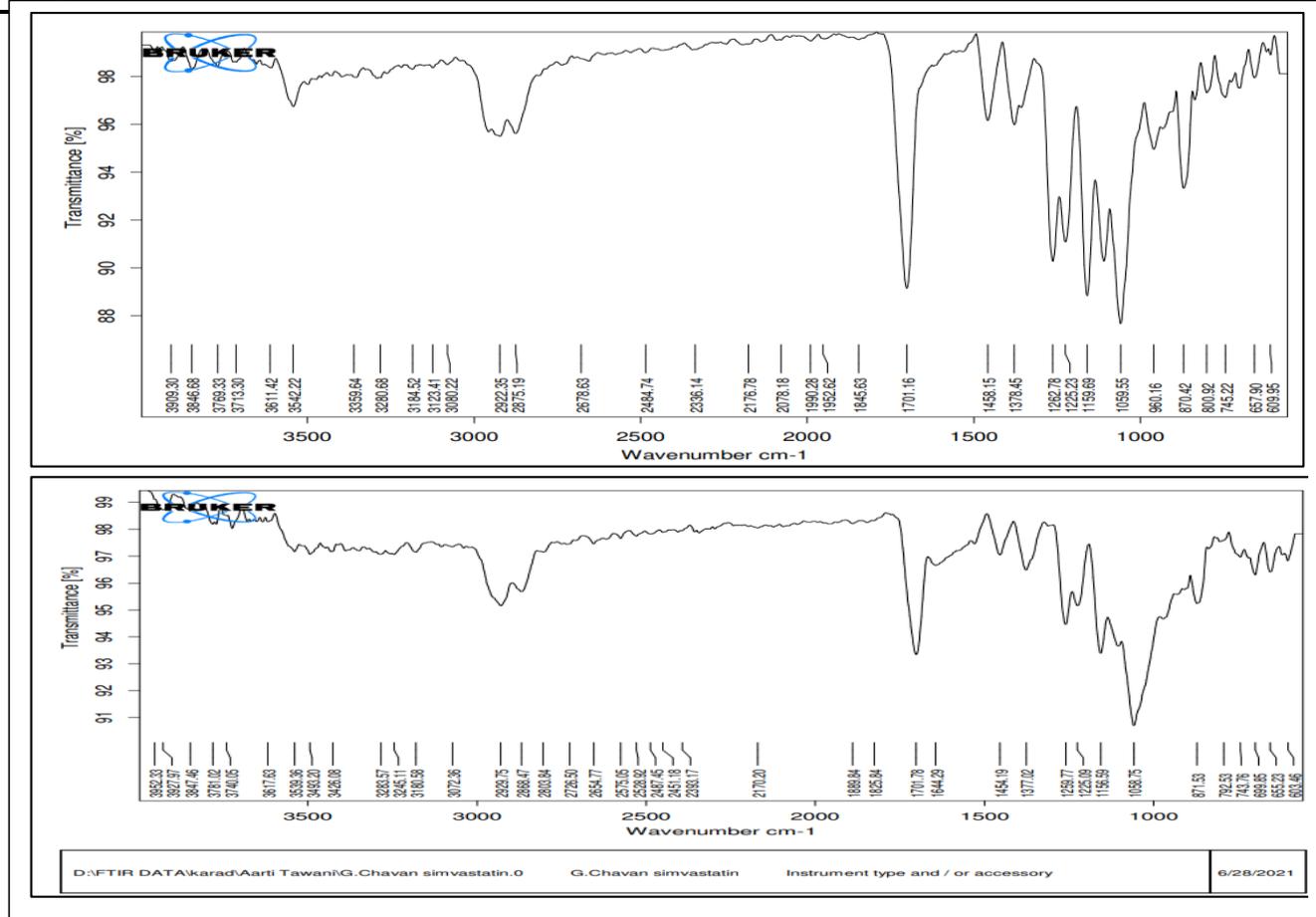


Fig. 7 FT-IR spectra of SIM; CS; TPP; Poloxamer 188; Physical mixture and optimum SIM CS -TPP NPs.

Fig.7 shows the FT-IR spectra of SIM, CS, TPP, Poloxamer 188, Physical mixture and optimum SIM CS -TPP NPs. The model drug SIM exhibits characteristics peaks at 3548.43 cm^{-1} (O-H free stretching), 1701.31 cm^{-1} (Carbonyl C-O Ester stretching), 1262.16 cm^{-1} (Lactone C-O-C stretching), 869.28 cm^{-1} , 2930.41 cm^{-1} , 2871.73 cm^{-1} (C-H stretching) and 1059 ($\text{C}=\text{O}$ carbonyl group). These peaks were in agreement with literature which confirms that drug used was Simvastatin. The FT-IR spectra of CS showed a peak at 3400 and 3550 cm^{-1} (O-H free stretching), and characteristic peak at 1600-1700 cm^{-1} (NH_2 group), 1100 cm^{-1} (C-O stretching), 2800-2900 cm^{-1} (C-H stretching). The spectrum of TPP demonstrated a distinctive peak 1200-1250 cm^{-1} ($\text{P}=\text{O}$ stretching) and 1100-1200 cm^{-1} (PO_2 stretching). While Poloxamer 188 showed the characteristic peaks at 2800-2900 cm^{-1} (C-H stretching aliphatic), 1400 cm^{-1} (O-H bend) and 1050-1100 cm^{-1} (C-O stretching). At the physical mixture IR spectra, the distinctive functional groups of Chitosan, Sodium tripolyphosphate, Poloxamer 188, and SIM as mentioned above were found in the same position. This demonstrated that the drug and other excipients was not incompatible. In the optimal formula spectra typical peaks of SIM occurred in, indicating that SIM was entrapped within NPs without altering its functional groups. In the spectra, additional peaks were produced at 1644.29 cm^{-1} and 1454 cm^{-1} , indicating crosslinking between the phosphoric and ammonium groups in the NPs formulation, demonstrating that SIM was fully entrapped within CS-TPP NPs.

3.5.3 DSC (Differential Scanning Calorimetry)

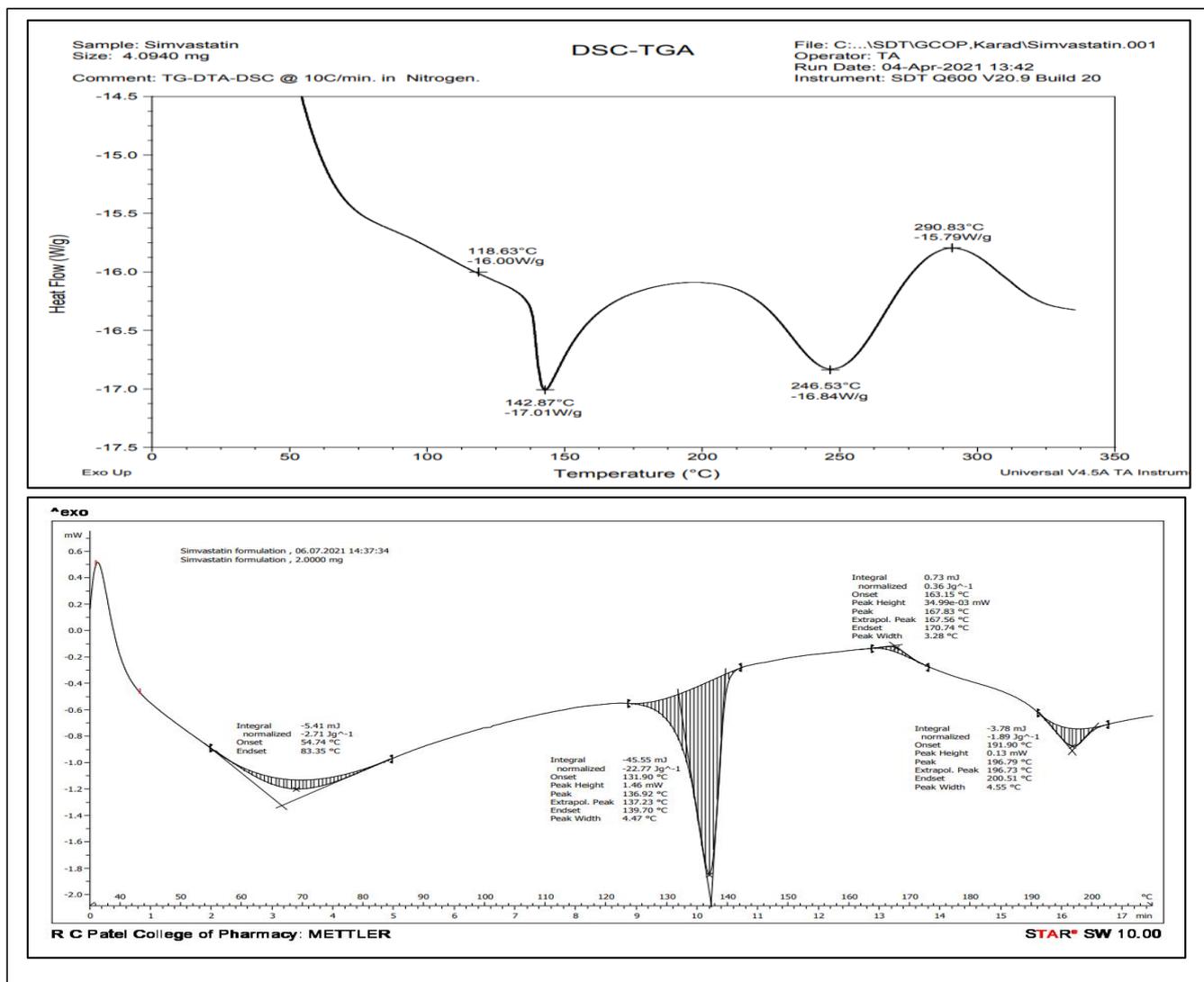


Fig.8 DSC graph of a) Pure SIM and b) optimal SIM CS-TPP NPs formula

DSC was used to assess the drug's thermodynamic behavior as well as its physical state (amorphous or crystalline). Fig.8 a) The drug has a strong intensity endothermic peak at 142.87^oC, which corresponds to the melting point of SIM which is 138^oC. It reveals that crystalline form of SIM. Whereas b) The thermogram of Sim CS-NaTPP NPs shows the sharp endothermic peak at 136. 92^oC.which was near to the actual melting point of Simvastatin that i.e., 138^oC reveals that Simvastatin entrapped successfully within NPs without any interaction.

3.5.4 Powder XRD (X-ray powder diffractometry)

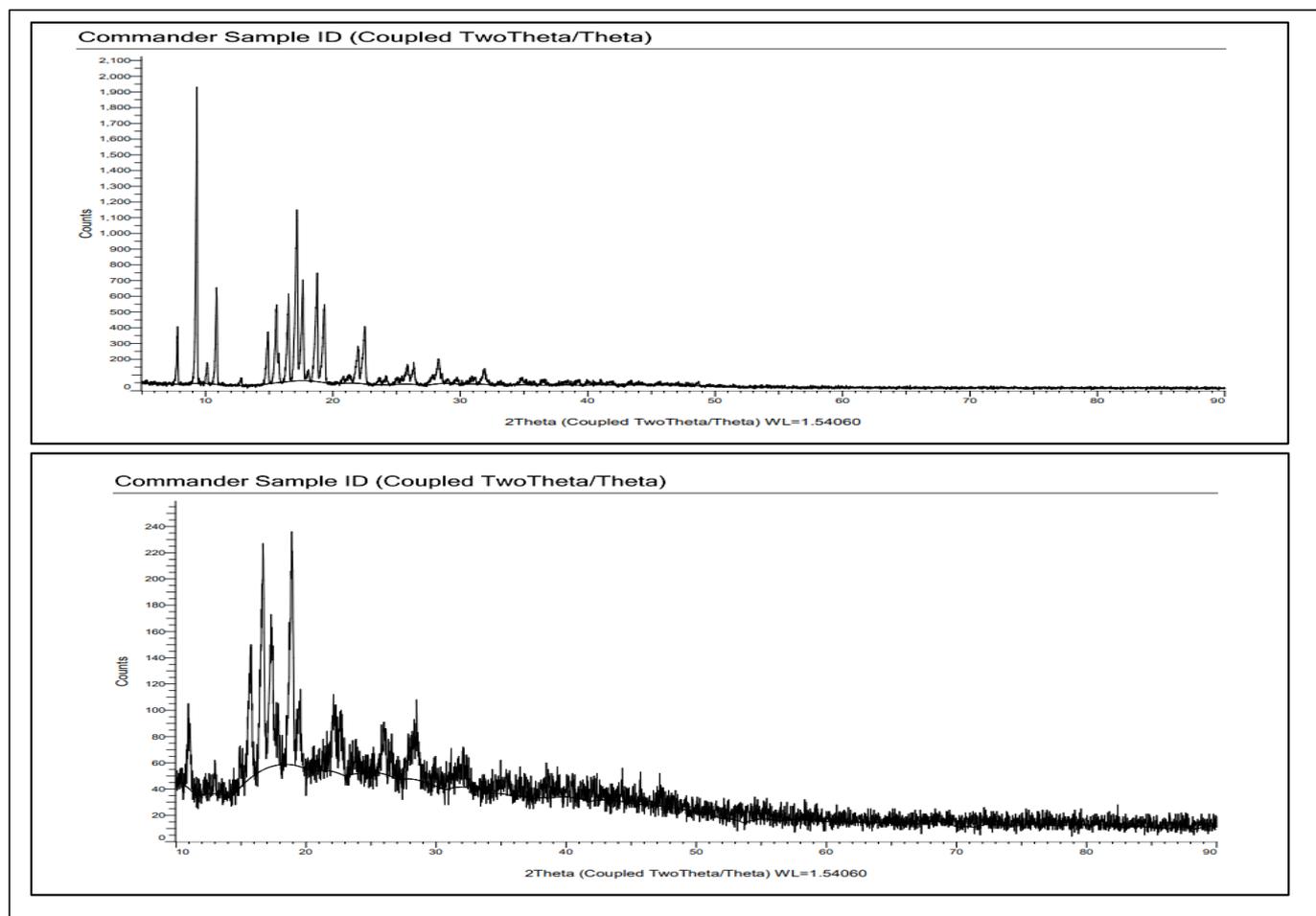


Fig 9. XRD graph of Pure SIM and optimal SIM CS-TPP NPs

X-ray Diffractometry is a technique for determining a substance's crystal habit. Differences in intensities of peak after and before formulation give the idea about transformation. Throughout the scanning range, the diffraction pattern of pure SIM revealed a highly crystalline structure Fig 9a), as shown by several sharp distinct peaks at the diffraction angles of 2θ (9.2, 11, 17.5, and 18.7). The crystalline character of the pure drug was demonstrated by the sharp and high intensity diffraction peaks in Fig. 9a), whereas the optimized batch was amorphous. As demonstrated in Fig. 9b), peak intensities are lower in the improved formulation. Amorphization was observed with a relative crystallinity of 31%.

3.5.5 In-vitro drug release

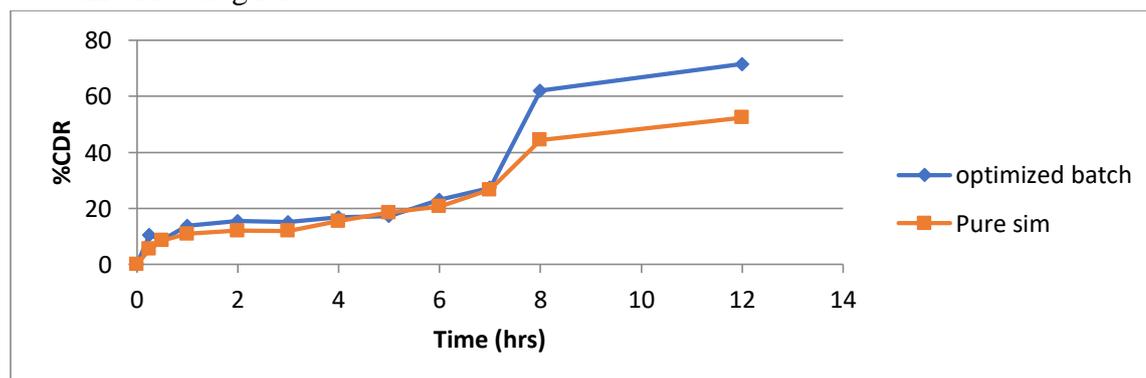


Fig. 10 Dissolution profile of Optimal SIM CS-TPP NPs and Pure SIM

The optimized formula gave a CDR of 71.48 % after 12 hours, whereas pure SIM gave a CDR of 52.41 %. According to the above findings Fig.10, the optimized batch has a different drug release than raw Simvastatin. This was due to Pure SIM being entrapped in chitosan and having a smaller particle size. The findings of the DSC and XRD of the formulation indicate amorphization, which leads to improved drug release and solubility.

3.5.6 Scanning Electron Microscopy (SEM)

SEM is the technique which describes the surface morphology of the particles. For this study particles were observed under high magnification.

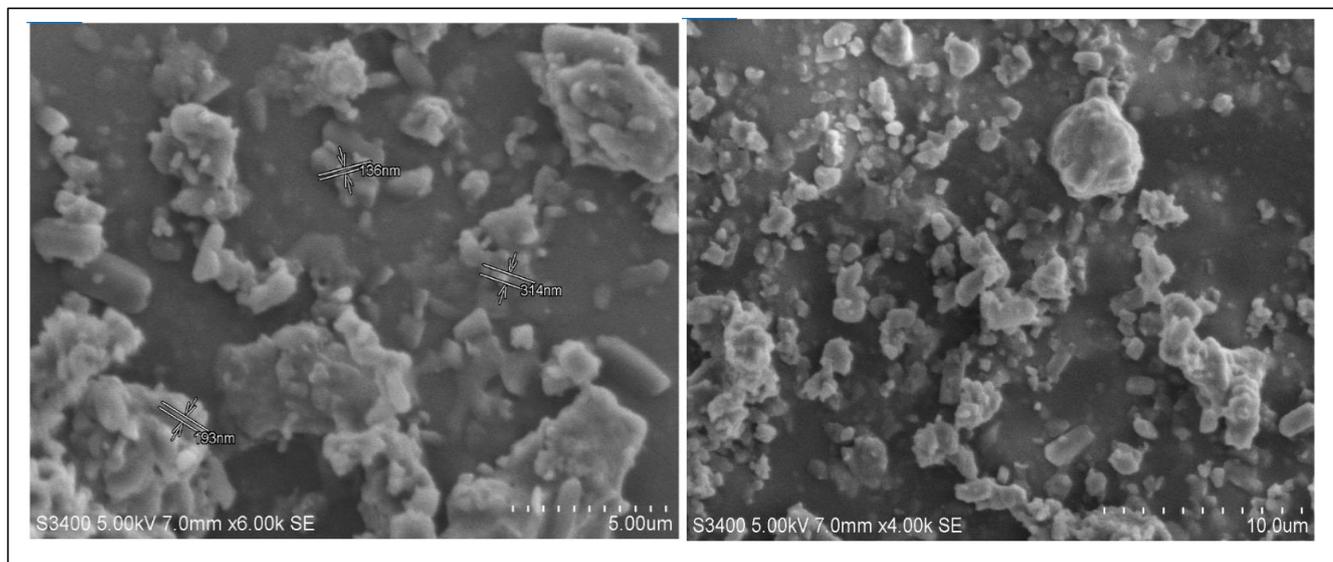


Fig. 11 Scanning Electron Microscopy (SEM) images of optimal SIM CS-TPP Formula

From the above SEM images Fig. 11, it was confirmed that successful fabrication of nearly spherical shape Sim loaded CS NPs. The needle shape of pure simvastatin get completely disappeared and smooth and nearly spherical shaped NPs were formed. The first image also confirms the nanometer range of particles.

3.5.7 Stability Study

The stability studies performed for one month at room temperature. Further tested for physical change, particle size and zeta potential.

Table 6: Characterization of optimal SIM CS-TPP NPs after stability studies

Formulation	Physical change	Particle size	Zeta potential
Optimal SIM CS-TPP	No	297.6nm	-17.8mv

After one months of stability study there was no any physical change in case of Colour occurs. The particle size was found to be 297.6nm and zeta potential -17.8mv. Showed that there was no significant difference in the results indicating that the formulation was stable.

4. Conclusion

Based on the results of this study, it was determined that Simvastatin-loaded nanoparticles are effective carriers for improving the delivery of antihyperlipidemic agents. Simvastatin was effectively entrapped in Polymeric nanoparticles using the ionic gelation technique in this work. The purpose of this study was to create and develop PNPs for improving the solubility of water-insoluble drugs. Chitosan and sodium tripolyphosphate were employed to make the NPs. On the Simvastatin loaded nanoparticles, the influence of the formulation composition and process parameters was examined and optimised. Formulations that have been optimised have the optimal PS 281.4nm, a narrow PDI 0.441, ZP -17.7mv and sustain drug release

71.48%. Because of the effective crosslinking of chitosan and sodium tripolyphosphate, as well as the modified and sustained drug release caused by the smaller particle size, the greatest entrapment efficiency is observed. We may get desirable PNP properties by choosing the right polymer, crosslinking agent, and surfactant. The formulations were tested for physicochemical properties and drug release in vitro. Design expert 13 proven to be a highly effective tool for analysing experimental data that reveals that CS and TPP percentage shows significant effect on PS and DR. In vitro drug release tests revealed an enhanced rate of dissolution. The work offers up the possibility of producing Simvastatin-loaded PNPs at a commercially competitive cost. As a result, nanoparticulate systems like PNPs may appear as a potential delivery method for increasing bioavailability of poorly soluble drugs like Simvastatin.

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