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Development and Formulation of Nanostructured Zein Based Drug Delivery System for Sorafenib Tosylate

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ABSTRACT
Nanotechnology, specifically the production of nanoparticles, has attracted unprecedented attention from various scientific fields. Nanoparticles can be tailored for targeted drug delivery, enhance bioavailability, and offer a controlled release of medication from a single dose. The size, hydrophobicity, hydrophilicity, and surface charge distribution of polymeric nanomedicines all affect their capacity to reach the target organ. Zein, a protein found in maize kernels, has special thermoplastic and hydrophobic qualities that set it apart from other proteins. It has been commercially available since 1938 and has a variety of industrial uses. Here an antisolvent nanoprecipitation method was used for the formulation of the zein based nanoparticle of Sorafenib. Where the organic phase was constituted with ethanol and the aqueous or antisolvent phase was deionised water with pluronic and PVA. With the help Box-Behnken design the formula of final preparation was optimized.
Keywords: *Biopolymer, Nano Particle, Targeting, Sorafenib Tosylate, Zein, Antisolvent Method*

INTRODUCTION

When comparing the practice of medicine today to that of the previous century, it is impossible to ignore the countless advances made to treat diseases that were thought to be incurable. In order to effectively treat complex illnesses, many new drugs have been developed; however, some of these drugs have serious side effects, and the benefits may not always warrant the risks. Yet, certain medications are practically useless in vivo since they have been shown to be highly successful in vitro but are unable to withstand the endogenous enzymes present in the gastrointestinal (GI) tract (if given orally). Even though amazing strides have been achieved in finding therapeutic targets and creating better drug molecules, there is always opportunity to enhance drug delivery methods and targeting.[1] Over the past few decades, nanotechnology—specifically, the production of nanoparticles—has attracted unprecedented attention from a wide range of scientific fields. Nanoparticles that mimic or change biological processes have been developed through the application of nanotechnology in medicine. The preferred size for nanomedical applications is less than 200 nm, whereas nanoparticles are solid, colloidal particles with sizes ranging from 10 nm to less than 1000 nm. One of the most important research topics in recent years has been the development of medication delivery systems using nanoparticles.[2] Notably, particle size directly affects the effectiveness of the majority of medication delivery methods (with the exception of intravenous and solution). Drug nanoparticles have increased solubility and, thus, improved bioavailability due to their tiny size and huge surface area. They also have the capacity to penetrate the pulmonary system, penetrate the blood brain barrier (BBB), and be absorbed via the skin's tight endothelial cell junctions. [3] Particularly, because they can be tailored for targeted

drug delivery, enhance bioavailability, and offer a controlled release of medication from a single dose, nanoparticles made from natural and synthetic polymers—both biodegradable and non-biodegradable—have drawn increased attention. By adapting the system, the drug can be protected from degradation by endogenous enzymes. [4]

The target organ, the intended medication, and the drug's compatibility with the biodegradable polymer all affect how a nano drug delivery system should be formed. The size, hydrophobicity, hydrophilicity, and surface charge distribution of polymeric nanomedicines all affect their capacity to reach the target organ. The size of polymeric nanoparticles is crucial for getting past various biological barriers and arriving at the intended location. [5] Zein is a protein found in maize kernels is called zein. Gorham gave it its name in 1821 after it was initially separated from entire white maize. Albumins, globulins, prolamins, and glutelins are the four main groups of proteins that can be distinguished based on their solubilities. Zein is a member of the prolamins, which are distinguished by their solubility in aqueous alcohol and by the fact that they produce a sizable quantity of proline and amide nitrogen when hydrolyzed. Zein is present in maize endosperm as protein bodies, which are cytoplasmic deposits with a diameter of 2-4 um. Zein proteins make up between 50 and 60 percent of all endosperm proteins. Zein is recognized to serve only as a nitrogen storage component in the growing seed. [6] Zein possesses special thermoplastic and hydrophobic qualities that set it apart from the majority of other proteins that are readily available. It has a special ability to make durable, transparent, tasteless, and odorless films and fibers. It is also very resistant to water and oil. Consequently, zein has been commercially available since 1938 and has a variety of industrial uses. Com Products Refining reached its peak production in 1956, producing approximately 6.3 million kg. The nonfood markets for zein virtually vanished overnight around 1960 when more affordable synthetic fibers and petroleum-based materials became available. Nonetheless, customers have grown more environmentally conscious in recent years, supported the creation of botanical alternatives to chemicals and fuels derived from fossil fuels, and are prepared to pay more for products that are more ecologically friendly. Zein is classified into four groups based on its solubility and sequence homology: α-zein (19 and 22 kDa), β-zein (14 kDa), γ-zein (16 and 27 kDa) and δ-zein (10 kDa). The percentages of α-zein, β-zein, and γ-zein in total zein are 75%–80%, 10%–15%, and 10%–15%, respectively. Only in the presence of a reducing agent can β, γ, and δ-zein be extracted. Zein is distinguished by its insolubility in water, with the exception of alcohol, high urea concentrations, alkali (pH 11 or higher), and anionic detergents. Its amino acid makeup is the cause of this. Zein lacks basic and acidic amino acids but is especially high in glutamic acid (21–26%), leucine (20%), proline (10%), and alanine (10%). Zein's poor dietary nitrogen balance is explained by its conspicuous lack of tryptophane and lysine. Zein's solubility characteristic is caused by a large percentage of nonpolar amino acid residues and a lack of basic and acid amino acids. The high number of α-helix structures and highly homologous repeat units make up α-zein. The majority of the sequence of β-Zein is β-sheet and aperiodic (β-turn and random coil) with very few α-helix structures. In its physiological state, γ-Zein has 31% β-sheet structures and 33% α-helix structures. There are no repeating sequences or distinct domain structures in δ-Zein. [6].

MATERIALS AND METHODS

Sorafenib Tosylate (MAC -CHEM Products (India) private limited, Thane, India. Batch No: SRF 022100, Pharmacopeial grade: IP), Zein (Sigma Aldrich company (India) Z3625 lot #SLC9723), Pluronic F 127 (Sigma Aldrich company (India) Batch #0000131309), TEA (Triethylamine) (Sigma Aldrich company (India), DCC (1, 3, dicyclohexyl carbodiimide) NHS (N-hydroxy succinimide), Folic acid (all from Sigma Aldrich company, India),

Calibration Curve of Sorafenib Tosylate

Begin by precisely weighing 10 mg of sorafenib tosylate and dissolving it in ethanol. After dispersing the drug, transfer it to a 10 mL volumetric bottle and top it off with ethanol. Pipette the generated stock solution into separate 100 mL volumetric flasks in different volumes to obtain concentrations of drug within a range of 20 to 100 µg/mL, adjust the contents in each flask. The absorbance of each diluted solution should then be measured using a UV-visible spectrophotometer at 264 nm.

Preformulative and Compatibility Studies

Fourier Transform Infra-Red Spectral Analysis

Pressed pellet technique was used in the FTIR analysis of drug and excipients. The samples were mixed with IR grade potassium bromide and converted to a homogenous transparent pellet. FTIR scanning was performed using Jasco FTIR -4100 type A in the range of 400 to 4000 cm^{-1}

Differential Scanning Calorimetry (DSC)

DSCQ 2000Thermal Analyzer V24.11 Build 124 was used for analysis of samples. Argon gas flow was adjusted to 60ml/min at a heating rate of 20.00°C/min. The reference material used was Alumina. The behavior of the material and reference with respect to temperature is plotted on a DSC thermogram.

Thermogravimetric Analysis (TGA)

The Sample was loaded on the top of the weighing stem. About 50 mg of the sample was taken in a ceramic pan and the temperature scanning rate was 20^0 C/min from 0 -700 ^{0}C.

Formulation and Optimization of Zein Nanoparticles

Preparation of Zein-folic acid particles

Every step was done in the dark to prevent folic acid from degrading. In short, 0.5 mL of TEA(Triethylamine) was introduced to a round-bottom flask containing 1 g of folic acid that had been dissolved in 20 mL of DMSO (dimethyl sulfoxide) while being stirred. The solution was then agitated overnight at room temperature after adding 520 mg of NHS (N-hydroxy succinimide) and 940 mg of DCC (1, 3, dicyclohexyl carbodiimide) molar excess. Dicyclohexylurea, the by-product, was then eliminated using filtration. After that, cold anhydrous ether containing 30% acetone precipitated the yellow NHS-FA (N-hydroxy succinimide- folic acid) which was subsequently cleaned three times using the same solution. After that, the product was left to dry overnight at room temperature in a vacuum oven. Then, 10 mL of DMSO was used to dissolve 100 mg of NHS-FA ester. After adding 100 mg of zein in total to the mixture, 0.5 mL of TEA was added while being stirred. At 35 °C, the reaction was conducted for 24 hours. To eliminate unreacted products, the covalently bound FA-ZN was purified by dialysis against 70% ethanol aqueous for two days. After that, the mixture was vacuum-dried for a full night at room temperature. [10, 11]

Preparation of Zein-sorafenib tosylate Nanoparticles

The antisolvent nanoprecipitation method was adopted for preparation of Nanoparticles. Zein and drug along with tween 80 were dissolved in 20 ml of 85% ethanol and stirred for 15 minutes on a magnetic stirrer. In a separate beaker PVA and Pluronic F 127 were dissolved in deionised water with the aid of gentle heat. After cooling the aqueous phase, the alcoholic zein-drug mixture was added dropwise through a syringe into a 30 ml of the solution for a period of 15 minutes at 20 KHz frequency in a probe sonicator by keeping the mixture on an ice bath.

Optimization of Zein nanoparticles by Box-Behnken Design

The design expert stat ease version 13 was employed in conducting the optimization study of zein nanoparticles. Three independent parameters amount of zein, amount of stabilizer and pH was selected for optimizing the dependant parameters, particle size, zeta potential, and poly dispersibility index of the preparation.

Table 1: data obtained from design expert software

Run	Independent Variables		
	Amount of Zein (mg)	**Amount of stabilizer (mg)**	**pH**
1	295	475	7
2	500	475	4
3	295	900	10
4	295	475	7
5	295	900	4
6	90	900	7
7	500	900	7

8	295	475	7
9	295	475	7
10	90	50	7
11	295	50	10
12	500	50	7
13	295	475	7
14	295	50	4
15	90	475	4
16	500	475	10
17	90	475	10

RESULTS AND DISCUSSION

Calibration Curve of Sorafenib Tosylate

The calibration curve of Sorafenib Tosylate was performed by using a UV-visible spectrophotometer at 264nm. The regression coefficient value of Sorafenib Tosylate was found to be 0.9912, which indicates a linear curve (Figure 1).

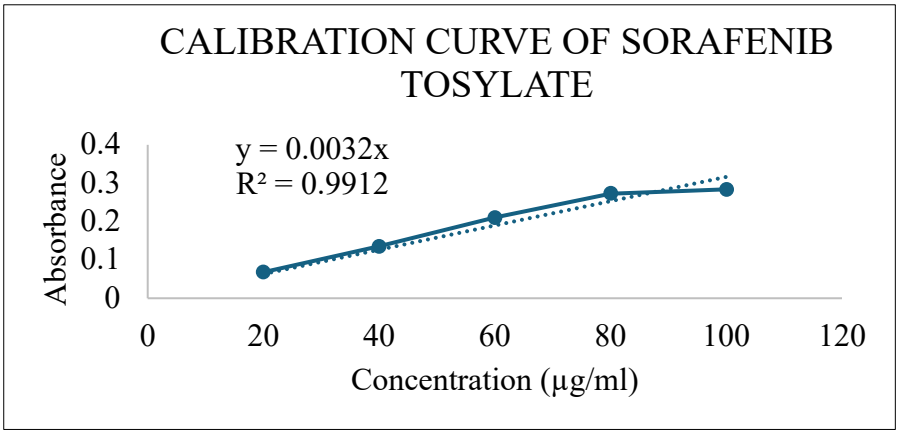

Figure 1: Calibration curve of Sorafenib Tosylate

FTIR Analysis

The FTIR analysis of Sorafenib tosylate and excipients were performed using pressed pellet technique and the spectra obtained was given below (figure 2).

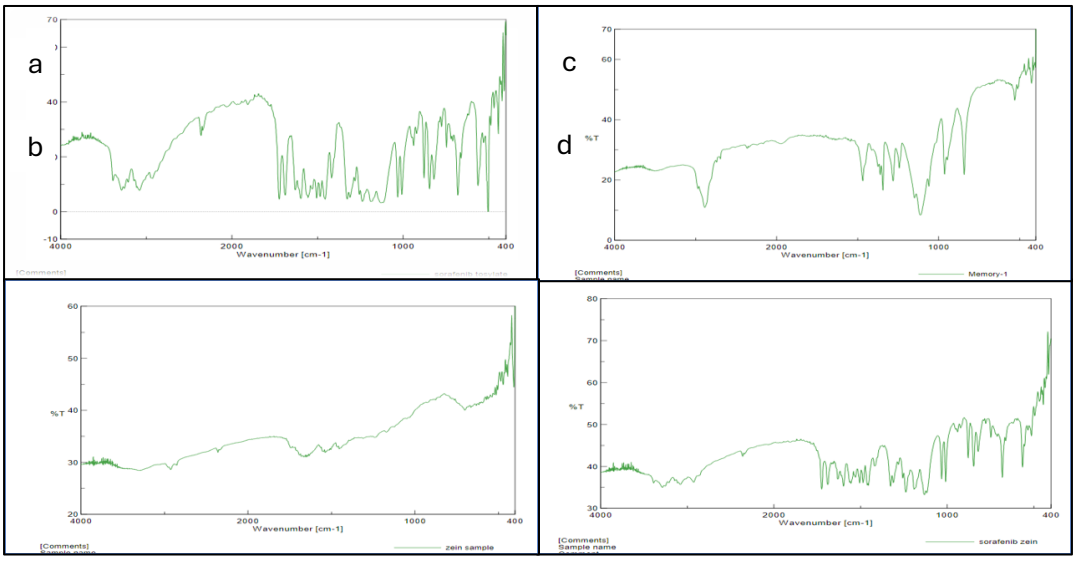

Figure 2: FTIR spectra of a) Sorafenib Tosylate, b) Zein, c) Pluronic F 127, d) drug excipient physical mixture.

The distinctive peaks at 3134.7 cm^{-1}, 1727 cm^{-1},1699.13 cm^{-1} shows the presence of N-H, C-H, and C=O groups respectively in Sorafenib Tosylate (figure 2a). FTIR of zein showed distinctive peaks of amino groups and carbonyl groups at 1639 cm^{-1} 3304 cm^{-1}, 2959 cm^{-1}. The spectra obtained is shown in figure 2b. The peaks at 2889 cm^{-1}, 1342 cm^{-1} and 2111 cm^{-1}in the FTIR spectra (figure 2c) of Pluronic F 127 confirms the presence of CH, OH and COC groups in the structure. The drug excipient physical mixture was subjected to the FTIR analysis and spectra (figure 2d) showed distinctive peaks of groups of Sorafenib and Pluronic F 127 indicating that there is no physical interaction between the drug and excipients.

Differential Scanning Calorimetry

It is a technique in which the energy necessary to establish a zero-temperature difference between the sample & reference material is measured as a function of temperature. Here, sample & reference material are heated by separate heaters in such a way that their temperature kept equal while this temperature increased or decreased linearly. During heating two types of reactions can be take place one is the endothermic and the other is the exothermic. If sample absorbs some amount of heat during phase transition, then reaction is said to be endothermic. If sample released some amount of heat during phase transition, then reaction is said to be exothermic.

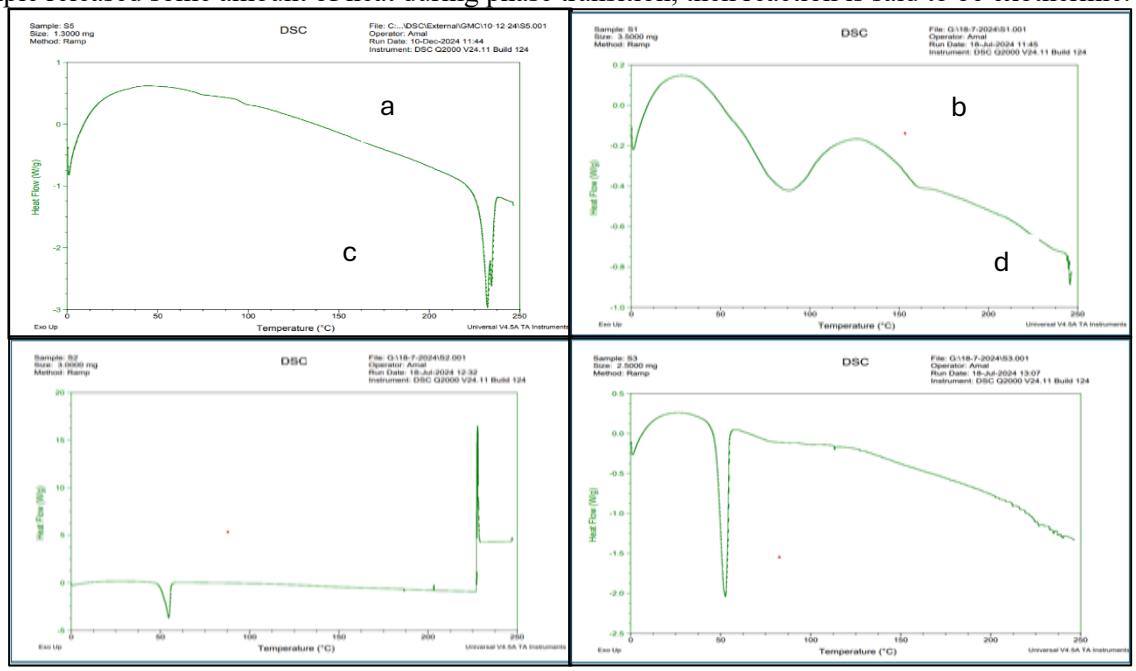

Figure 3: DSC thermogram of a) Sorafenib tosylate b) Zein, c) Zein and Pluronic F 127, d) Sorafenib tosylate, zein and Pluronic F 127 physical mixture

Thermogravimetric Analysis

Thermogravimetric (TG) analysis can be defined as the change in mass of a sample as a function of temperature. Derivative of the TG curve, which represents the rate of mass change with respect to time is called as the DTG or Derivative Thermogravimetry. While Differential Thermal Analysis (DTA) measures the temperature difference

between a sample and a reference material. In the thermograms given below the blue, red, and green graphs shows TG, DTG and DTA respectively.

In the TG thermogram of Zein (figure 4a), the mass decreased steadily from about 100 to 500°C, indicating thermal degradation or decomposition likely due to the evaporation of bound water or solvents and the breakdown of the protein structure. The major mass loss between 250 and 350°C suggested the primary decomposition of the zein protein. The DTG shows prominent peak around 300°C, indicating the temperature at which the rate of mass loss is highest. This peak corresponded to the main decomposition event of zein, where the protein rapidly degrades.

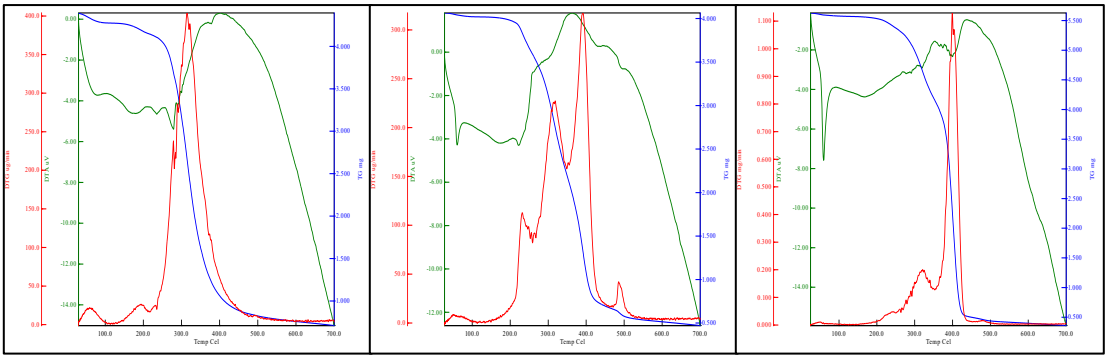

Figure 4: the blue, red and green curves are TG, DTG and DTA thermograms of a) Zein, b) Pluronic F 127, c) Sorafenib tosylate, Zein and Pluronic F 127 physical mixture

The DTA of Zein shows a broad endothermic peak around 300°C was observed, followed by a smaller peak around 350°C. The endothermic peak around 300°C corresponded to the major decomposition event identified in the DTG curve, where the energy is absorbed to break down the protein structure. The smaller peak at higher temperatures could indicate further degradation or the formation of more stable residues.

The TG curve of Pluronic F 127 (Figure 4b) showed a gradual decrease in mass from around 200 to 450°C, indicating the thermal degradation. Decomposition in multiple stages, which is common in polymeric materials. There were distinct peaks in the DTG curve, particularly one major peak around 400°C. The peak at 400°C indicated the primary degradation phase where the rate of mass loss is highest. While the DTA curve showed endothermic peaks around 40 and 220°C, followed by an exothermic peak near 370°C. The endothermic peaks could be associated with the melting or softening of Pluronic F-127. The exothermic peak corresponded to the major decomposition event. But in the TG thermogram of physical mixture of Sorafenib Tosylate, Zein and Pluronic F 127, the mass decreased sharply from about 250 to 410°C, indicating thermal degradation or decomposition (figure 4c). There were distinct peaks in the DTG curve, particularly one major peak around 400°C. The DTA curve showed a sharp endothermic peak around 50°C, followed by exothermic peaks around 350 and 450°C.

Powder X-Ray Diffraction (PXRD)

Powder Xray diffractograms of the drug Sorafenib tosylate, zein, Pluronic F 127, a physical mixture of the polymer zein and the drug and another mixture of zein, pluronic along with sorafenib tosylate were obtained and is shown in the figure 5

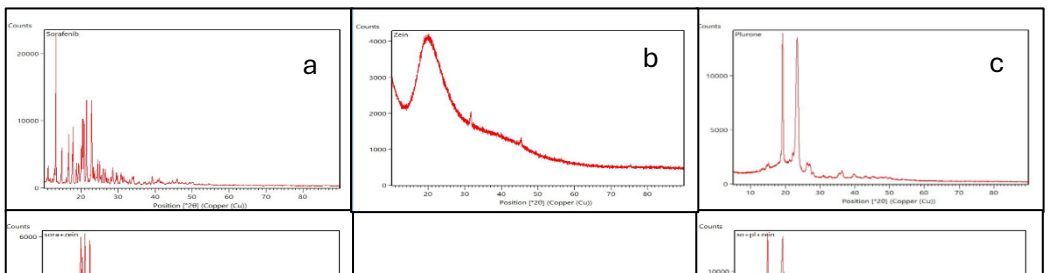

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Figure 5: PXRD of a) Sorafenib Tosylate, b) Zein, c) Pluronic F 127, d) zein & drug physical mixture, e) zein, pluronic and drug physical mixture

The diffractograms of the drug and excipients shows the crystalline nature of their physical forms. The diffractogram of the drug (figure 5a) exhibits highly intense, sharply distinctive peaks at 2θ angles of 12, 12.1, 16.72, 22.19, and 27.31 indicating that the drug is monoclinic in nature. Similarly, the diffractogram of the polymer zein (figure 5b) also proves to monoclinic in nature. While from the diffractogram of Pluronic F 127 (figure 5c) it was confirmed that it exhibits a hexagonal structure. The mixture of the drug and polymer (figure 5d) also possess a monoclinic structure while the mixture of sorafenib, zein and pluronic gives an orthorhombic arrangement with sharp peaks in 2θ angles.

Optimization of Formulation

The zein nanoparticles were prepared by the pH-controlled nanoprecipitation method. The formulation was optimized via the response surface method using a 3 level, 3 factor Box-Behnken Design (BBD) provided by design expert software. 17 trial runs were suggested by the software and each of the combination was performed and analyzed for particle size, zeta potential and poly dispersity index (PDI). The following table 2 shows the data provided by the software and the observations for each run.

Table 2: data table provided by BBD and the responses observed for each run

Run	Independent Variables			Dependent Variables		
	Amount of Zein (mg)	Amount of stabilizer (mg)	pH	Particle size (nm)	Zeta potential (mV)	PDI
1	295	475	7	440	-12	0.271
2	500	475	4	678.9	-15.2	0.287
3	295	900	10	645.6	-13.9	0.338
4	295	475	7	459.1	-11.1	0.271
5	295	900	4	857.3	-0.12	0.265
6	90	900	7	1646.9	-0.6	0.297
7	500	900	7	766.5	-13.8	0.424
8	295	475	7	421.7	-10.3	0.274
9	295	475	7	458.6	-11.5	0.287
10	90	50	7	1996.3	-14.7	0.291
11	295	50	10	775.8	-8.7	0.266
12	500	50	7	464.2	-13.2	0.176
13	295	475	7	488.5	-11.5	0.269
14	295	50	4	1004.6	-22.1	0.227
15	90	475	4	1654.2	-10.4	0.211
16	500	475	10	404	-16.4	0.226
17	90	475	10	1616.2	-10.5	0.389

The process optimization was performed to achieve certain criteria in the formulation. The goal was to minimize the particle size and PDI and to maximize the zeta potential while confining the amount of zein and stabilizer in a range at pH 7 to get a stable formulation. Figure 6 shows the ANOVA data of model showing the effect of independent factors on the particle size, zeta potential and poly dispersity index. From the data of particle size analysis, the model F-value of 573.88 implies the model is significant. There is only a 0.01 % chance that an F value this large could occur due to noise. P values less than 0.005 indicates the model terms are significant. The lack of fit value of 2.81implies the lack of fit is not significant relative to the pure error. From the predicted vs actual plot and the contour plot it was confirmed that the lowest particle size comes with the increased concentration of zein.

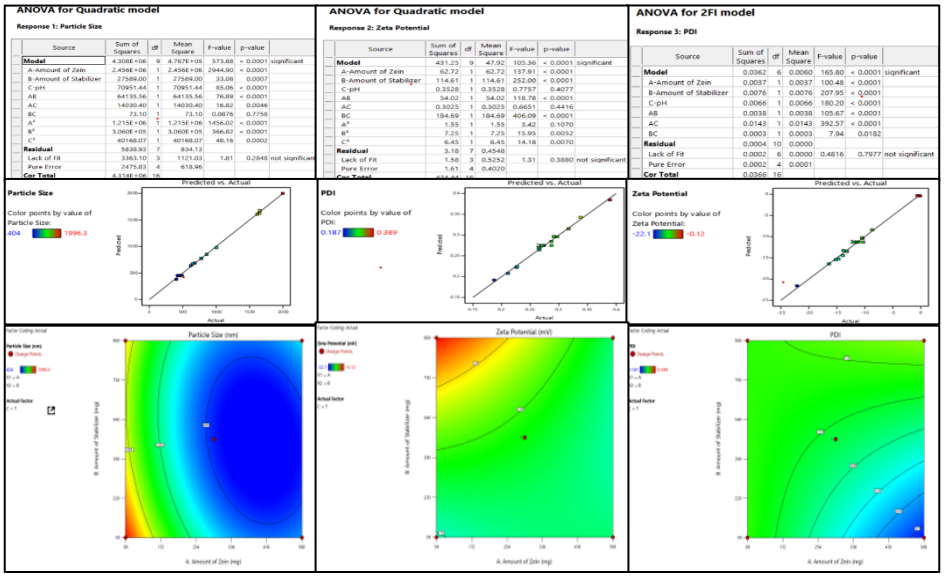

Figure 6: shows the ANOVA table and model graphs for particle size, zeta potential and PDI

From the contour plots it was clear that the concentration of zein influences the particle size, zeta potential and PDI of the formed zein nanoparticles. As the amount of zein used in the preparation increased it causes the formation of nanoparticles at a range of 400 nm. While the higher concentration of zein causes low PDI and satisfactory value of zeta potential indicating the enhanced stability of the nanoparticles formed. Final formulation of the nanoparticles was based on the formula suggested by the software.

CONCLUSION

Zein nanoparticles of sorafenib tosylate are a promising method for improving the anticancer medication sorafenib's solubility, bioavailability, and therapeutic effectiveness. In order to increase the medicine's stability and enable regulated drug release, researchers have turned to zein, a naturally occurring polymer made from corn protein. Better medication delivery to specific areas is made possible by the encapsulation of sorafenib tosylate in zein nanoparticles, which may lessen side effects and enhance patient outcomes in the treatment of cancer. Here the formulation of zein nanoparticles of Sorafenib tosylate follows antisolvent precipitation method where an organic phase constituting the drug and the polymer in dropwise added into the aqueous antisolvent system with stabilizers.

In order to optimize the formulation Box Behnken design was used. The optimized formulation had shown better stability in terms of lower particle size and satisfactory PDI and zeta potential values.

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