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Original Article

DEVELOPMENT OPTIMIZATION OF SORAFENIB-LOADED PLGA NANOPARTICLES GUIDED BY *IN SILICO* COMPUTATIONAL TOOLS

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ABSTRACT

Objective: The purpose of this study was to develop, characterize, and optimize sorafenib-loaded Poly (lactic-co-glycolic acid) PLGA polymeric nanoparticles for prolonged delivery of sorafenib for improved hepatic cancer treatment

Methods: The drug-excipient interaction was explored by molecular docking studies within silico tools. The drug-loaded polymeric nanoparticles were prepared by single emulsion solvent evaporation method using box-bhenkan design and characterized for particle size, zeta potential, and entrapment efficiency. Shape and surface morphology was analysed by Transmission Electron Microscopy (TEM). In vitro drug release study was performed by using a diffusion membrane.

Results: The docking analysis inferred that the drug has interacted well with PLGA and PF-68, which could prevent the drug crystal formation. The optimized polymeric nanoparticles had a particle size of 175 nm, Entrapment Efficiency (EE) of 85.1% and zeta potential of-23.8mV were found to be within 95% of CI of the predicted value, which is acceptable. TEM studies showed that the formed polymeric nanoparticles were smooth, spherical in shape and uniform in size. *In vitro* drug release study of optimized formulation showed extended release for sorafenib.

Conclusion: Based on the computational studies and in vitro release studies, the developed Sorafenib loaded in PLGA nanoparticles could be a promising formulation in oral drug delivery for the treatment of liver cancer.

Keywords: Sorafenib, In silico, PLGA, Polymeric nanoparticle

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INTRODUCTION

Computational modelling is a valuable tool that can overcome the limitations of experiments. It can independently vary parameters and identify the interactions that are responsible for the observed results. Computational studies have shown that it is an effective and convenient way to simulate interactions under different conditions, even beyond what is possible in the laboratory [1]. The introduction of computational approaches like Quantitative Structure-Activity Relationships (QSARs), molecular modelling, molecular mechanics, computational fluid dynamics, and Physiologically Based Pharmacokinetics (PBPK) model [2], Design of Experiment (DoE) [3] etc., reduces complex experimental efforts and thereby expedite the drug innovations and its regulatory process. Polymeric Nanoparticles (NPs) encapsulating biologic molecules within protective carriers can effectively address several challenges associated with oral delivery [4]. These carriers can shield the encapsulated biologics from the harsh Gastro Intestinal environment, including acidic pH and digestive enzymes, thereby enhancing their stability and absorption [5]. Hepato Cellular Carcinoma (HCC) is the fourth most common cause of cancer-related death worldwide [6]. Risk factors for HCC include chronic hepatitis B and hepatitis C, alcohol addiction, metabolic liver disease (particularly non-alcoholic fatty liver disease) and exposure to dietary toxins such as aflatoxins and aristolochic acid [7]. HCC result in a state of chronic liver inflammation and fibrosis, which is thought to cause HCC development. Over 80% of patients with HCC initially have cirrhosis of the liver, which is characterized by immature hepatocytes and disorganized liver histology [8]. Sorafenib is a multikinase inhibitor that directly connects to DNA and impedes the formation of nucleic acid, and this leads to impairment of molecular structure and further steric effects [9]. As a consequence, the growth and proliferation of cancer cells in the body are hampered. However, sorafenib is poorly soluble, metabolized rapidly in the liver, and is a known substrate for p-glycoprotein [10, 11]. It, therefore, has low bioavailability when administered orally. To overcome these limitations, several types of drug delivery systems have been developed, including cubosomes [12], nanosuspensions [13], Self Nano Emulsifying Drug Delivery System SNEDDS [14], Magnetic nanoparticles [15], nanofibers [16], micelles [17], PLGA nanoparticles [18] PLGA is a synthetic, biodegradable and biocompatible polymer [19]. Krebs cycle is responsible for the removal of PLGA from the body without affecting the normal physiology of the body [20].

In the present study, we attempted to improve the release profile of sorafenib by developing PLGA nanoparticles using the Quality by Design (QbD) approach. Box-Behnken design (BBD) was adopted to investigate the effect of formulation variables (PLGA, Polyvinyl Alcohol and Sonication time) on the quality attributes of nanoparticles (particle size, EE% and zeta potential). Computational modelling approach is used to ensure higher degree of compatibility between sorafenib and PLGA.

MATERIALS AND METHODS

Materials

Sorafenib was gift sample from MSN lab Hyderabad, India, PLGA and Polyvinyl Alcohol (PVA) were procured from SD Fines Mumbai, India respectively. All other chemicals and solvents used in the study were of analytical grade and others were of pharmaceutical grade.

Computational method

The entire computational research work was carried out on a linux/Ubuntu 20.04 long Term Support (LTS) system. We computationally analysed the binding affinity and interaction of avidin with biotin and PLGA with sorafenib in a complex structure to test the hypothesis. Accordingly, the protein structure of avdin from the RCSB-PDB (PDB ID: 2AVI) and the chemical structures of biotin (PubChem CID: 171548), PLGA (23111554), and Sorafenib (PubChem CID: 216239) were retrieved from PubChem databases. Further, all chemical structure was converted to pdb (.pdb) format

with clean geometry using Avogadro 1.2 and BIOVIA Discovery Studio Visualizer (BIOVIA-DSV) software [21]. Further, an individual, as well as a double docking study was carried out using Auto Dock 4.2 software, and the docking complexes were visualised with BIOVIA-DSV, respectively [22].

Formulation of sorafenib-loaded PLGA nanoparticles

PLGA nanoparticles were prepared by single emulsion solvent evaporation method with slight modifications [23, 24]. Briefly, 100 mg of PLGA polymer was dissolved in 3 ml of organic solvent (Dichloromethane along with to form a primary emulsion, which was further emulsified in an aqueous PVA solution (2% w/v) to form an oil-in-water emulsion using a microtip probe so nicator (VC 505, Vibracell Sonics, Newton, USA) set at 55W of energy output for 2 min over an ice bath. The emulsion was stirred overnight for the evaporation of the organic solvent. Excess amount of PVA was removed next day by ultracentrifugation at 50, 602×g, 4 ℃ for 20 min (Sorvall Ultra speed Centrifuge, Kendro, USA) followed by washing thrice with double distilled water. The recovered nanoparticulate suspension was lyophilized for two days (−80 °C and b10 μM mercury pressure) to get the lyophilized powder for further use.

Experimental design

Response Surface Methodology aims to establish the relative importance of two or more factors and also to indicate whether or not interaction occurs between the factors and thereby affects the magnitude of the response [25]. Box Behnken design. A 3-level, 3-factor, 17 run experimental design was adopted to optimize levels of variables in the nano formulations. The selected independent variables were amount of Polymer i. e PLGA (X1), PVA (X2), and Sonication Time (X3) as shown in (table 1). The dependent variables were Particle size (Y1) EE%(Y2) and Zeta potential (Y3). The generation of experimental runs, Analysis of Variance (ANOVA) study and optimization were carried out by Design expert® software 12.

Table 1: Experimental design parameters

Independent variables (X)	Coded value		
	Low (-1)	Mid (0)	High (+1)
PLGA (X1) (mg)	100	150	200
PVA(X2) (mg)	2	3	4
Sonication Time (X3) (mg)	5	7.5	10
Responses (Y)	Constraint		
Particle size(Y1)	Minimize		
Entrapment efficiency (Y2)	Maximize		
Zeta Potential (Y3)	Between-20 to-30		

PLGA-Poly(lactic-co-glycolic acid),PVA-Poly vinyl Alcohol

Table 2: Optimization design using box–behnken design

Formulation	PLGA(X1)	PVA(X2)	Sonication time (X3)
SF1	200	3	10
SF2	100	2	7.5
SF3	150	3	7.5
SF4	150	3	7.5
SF5	150	3	7.5
SF6	200	2	7.5
SF7	100	3	5
SF8	200	4	7.5
SF9	100	3	10
SF10	150	3	7.5
SF11	150	4	5
SF12	150	4	10
SF13	150	2	10
SF14	200	3	5
SF15	150	3	7.5
SF16	150	2	5
SF17	100	4	7.5

X1: PLGA-Poly(lactic-co-glycolic acid),X2:PVA-Poly vinyl Alcohol X3:Sonication Time, SF: Sorafenib-loaded polymeric nanoparticles

Particle size, polydispersity Index (PDI) and zeta potential

Particle size and PDI was determined by Zeta sizer by dynamic light scattering (Nano ZS, Malvern Instruments, UK). The Zeta potential of a particle is the overall charge that the particles obtain in a particular medium [26]. Zeta potential values help to assess the stability of the formulation

Entrapment efficiency

Take the required ml of the polymeric nanoparticle suspension in Tarsus centrifuge tube of 15 ml capacity and it is centrifuged by cold centrifugation at 10000 rpm for 30 min at 4 °C. After centrifugation, the supernatant and the sediment are separated. The concentration of Sorafenib present in the supernatant was analysed by Ultra Violet spectroscopic method at 263 nm. The percentage entrapment efficiency was calculated using the following formula [27].

$$EE\% = \frac{\text{Total amt of drug} - \text{Amt of free drug}}{\text{Total amt of drug}} \times 100$$

Transmission electron microscopy

The morphology of formulation was observed under TEM (TECNAI 200Kv TEM, Fei, Electron optics Oregon USA) by using negative staining method [28]. A drop of NPs, diluted with water (1/50 times), was spread on a 200 mesh copper grid coated with carbon film and kept for about 3 min. A drop of phosphor tungstic acid (2% w/w) was dripped on the grid for 30 sec and excess droplet was removed using a filter paper. Finally, the grid was air-dried for about 2h and then used for microscopic analysis.

Fourier transform infrared study

The Fourier Transform Infrared (FTIR) analysis was performed to know the chemical interaction between the drug and polymer inside the prepared nanoparticles. Fourier Transform Infrared spectroscopy was performed using a Shimadzu FTIR in scanning region from 4000 to 400 cm-1 region [29].

In vitro drug release study of sorafenib from NPs

The dialysis bag was used to study the release profile of Sorafenib from Opt-SVF-PLGA-NPs and Pure-SVF-Dispersion in an *in vitro* environment. The prepared NPs were placed in dialysis bags (12,000–14,000 DM–27, Millipore, Burlington, MA), which were kept at 37°C with constant magnetic stirring at 50 rpm and immersed in 50 ml of PBS with a pH of 7.4. 1 ml samples were taken from the receptor compartment at predefined intervals (0, 2, 4, 6, 8, 10, 12, 14, 16, 18, and 24 hours) and replaced with an equivalent volume of fresh medium [30]. Similarly, the process is done for Pure-SVF-Dispersion. The tests were run three times. the amount of released SVF was determined spectrophotometrically at a wavelength of 263 nm

RESULTS AND DISCUSSION

In silico studies

Based on the individual docking score (kcal/mol), avidin-biotin complexes showed a docking score of-6.24 kcal/mol, while PLGA-Sorafenib showed-3.45 kcal/mol, respectively. Further, both avidin-biotin and PLGA-SO showed a comparatively higher docking score of-7.60 kcal/mol. According to molecular analysis, the PLGA-Sorafenib complex has three hydrogen bonds with five Pi-alkyl bonds, one Pi-sulphur bond, and one van der Wall bond interaction, whereas the PLGA-Sorafenib complex has four hydrogen bonds with one Pi-alkyl bond (fig. 1). Furthermore, the formulation contained two individual complexes with higher binding affinity than individual compounds, implying that it could be a novel approach to long-term controlled release drug delivery. Briefly, as per the theoretical chemistry point of view, an ideal embolism between polar organic molecules with a polymer coat is expected to control the drug delivery, as validated in the experimental section. Moreover, theoretically, bioinformatics tools were an indispensable part of early drug discovery, drug chemistry, and drug delivery analysis [31]. However, molecular docking, a type of artificial intelligence technique that can predict the molecular interaction of any biological substance, needs more validation in experiments for further translational application.

Characterization of sorafenib-loaded PLGA nanoparticles

Sorafenib-loaded polymeric nanoparticles (SF) were successfully formulated by employing box-bhenkam design and constituents' effect (PLGA, PVA and sonication time) on its attributes was analysed. The independent variables, i.e., polymer, PVA and sonication time at three levels, were evaluated for their concomitants on particle size; % entrapment efficiency and zeta potential. The results obtained are given in table 3.

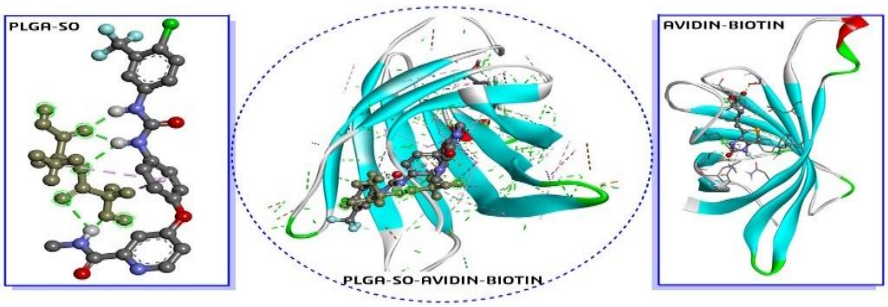

Fig. 1: Molecular docking analysis of PLGA-SO, AVIDIN-BIOTIN, and PLGA-SO-AVIDIN-BIOTIN in a single complex. The interface was visualised using BIOVIA-DSV software and an image assembled by Chem Draw 21.0 software

Table 3: Characterization of sorafenib-loaded PLGA nanoparticles

Formulation	Particle size (nm)	Entrapment efficiency %	Zeta potential (mV)	PDI
SF1	182±13.42	90±5.42	-24.1±1.23	0.143±0.01
SF2	166±11.02	76.5±43.44	-22.9±1.52	0.145±0.09
SF3	168±15.12	82±4.48	-23.5±1.65	0.146±0.08
SF4	170±15.13	82.1±6.20	-23±1.20	0.147±0.06
SF5	168±10.11	81.8±4.12	-23.9±1.11	0.150±0.05
SF6	181±13.08	89±5.47	-21.5±1.18	0.155±0.08
SF7	164±11.07	76.2±3.22	-23.9±1.64	0.149±0.05
SF8	181±12.47	88±4.41	-26.9±1.23	0.154±0.01
SF9	163±13.78	75.6±4.42	-24.2±1.22	0.157±0.04
SF10	167±10.25	82.4±4.86	-23.6±1.34	0.143±0.02
SF11	170±14.32	83±5.35	-26.5±1.46	0.152±0.01
SF12	169±12.47	83.9±4.26	-25.9±1.55	0.146±0.03
SF13	175±11.24	85.1±6.24	-23.8±1.32	0.147±0.07
SF14	182±10.58	89.5±5.40	-23.1±1.21	0.157±0.04
SF15	168±11.25	84±5.23	-23.2±1.22	0.148±0.02
SF16	169±13.22	84.3±4.21	-21.9±1.09	0.144±0.03
SF17	162±13.04	75.1±3.25	-24.9±1.25	0.151±0.06

(n=3 mean ± SD), SF: Sorafenib-loaded polymeric nanoparticles

Particle size

The generated quadratic model for particle size was suggested by the Design Expert software and the statistical analysis of the model leads to the given model fitting reduced polynomial equations for particle size,

$$Particlesize=168.54+11.51X1-1.16X2-0.4250X3-5.17X1X2+4.15X1X3+1.00X2X3-0.5708X1^2-2.17X2^2+3.00X3^2$$

As the concentration of PLGA increases, there is an increase in the particle size suggests the Positive effect of PLGA (X1) on particle size. reason could be that during emulsification, the viscosity of organic phase increases due to the huge amount of polymer and led to the formation of nanosized droplets with a large, similar work was reported by Neelam l. Dashputre *et al.* 2023 [31] and the interaction of PLGA and PVA suggests the positive influence on the size of nanoparticles. The model is

suggested to be significant by the model's F-value of 57.24. Therefore only 0.01% chance are there that this large F-value might happen due to noise. P-values is 0.0001 which is less than 0.0500 which indicates that the model terms are significant. Here, X1, X2 are significant model terms. The value of anticipated R^2 0.9986 is in close accord with the adjusted R^2 of 0.9881 The effect of independent variables on responses is depicted by 2D counter plot in fig. 2.

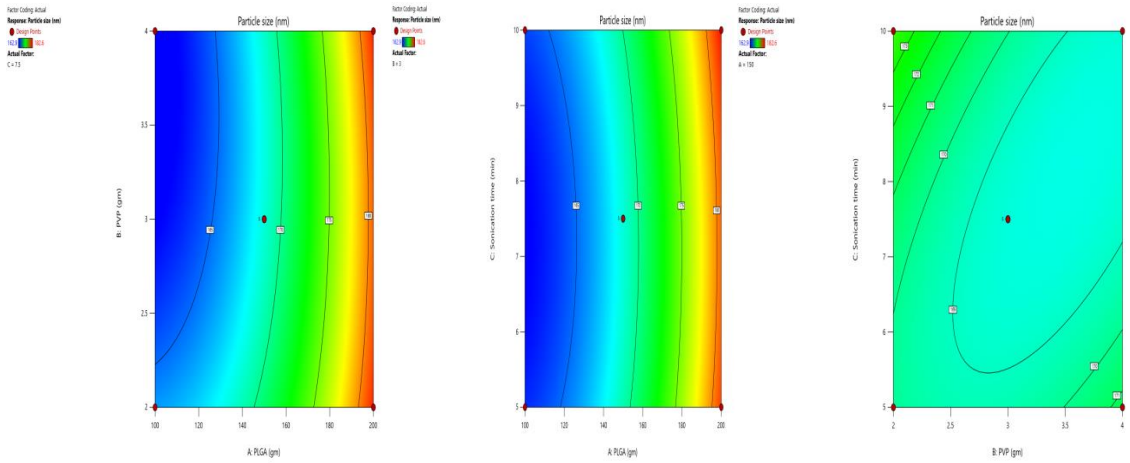

Fig. 2: (a) 2D Counter plot depicting the effect of PLGA, PVA and Sonication time on the particle size

Entrapment efficiency %

The quadratic model fitting of Entrapment efficiency was done by applying regression statistics and the reduced polynomial equation for %EE was found to be,

$$EE\%=82.46+6.64X1-0.6125X2+0.2000X3+0.1000X1X2+0.2750X1X3+0.0250X2X3-0.7800X1^2+0.4700X2^2+1.15X3^2$$

The polynomial equation gives a positive influence of coefficient X1 and the positive influence of coefficient X2 and the polynomial terms suggests the effect of polymer concentration on the Entrapment Efficiency. it describes with the increase in polymer concentration, the entrapment efficiency of polymeric nanoparticles formulations significantly increasing. The present result is supported by Mohanty *et al.* in 2022 [32] The Model F-value of 67.25 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 is<0.0001, which is less than 0.0500 indicate model terms are significant. In this case, X1, X2 are significant model terms. The Predicted R^2 of 0.9404 is in reasonable agreement with the Adjusted R^2 of 0.9739**.** The effect of PLGA and PVA concentration on the entrapment efficiency of formulations obtained from the design shown in table 3 and fig. 3. This is due to increased polymer concentration increase the viscosity of organic phase, which will resist the diffusion of drug into aqueous phase leading to the incorporation of more drugs inside nanoparticle.

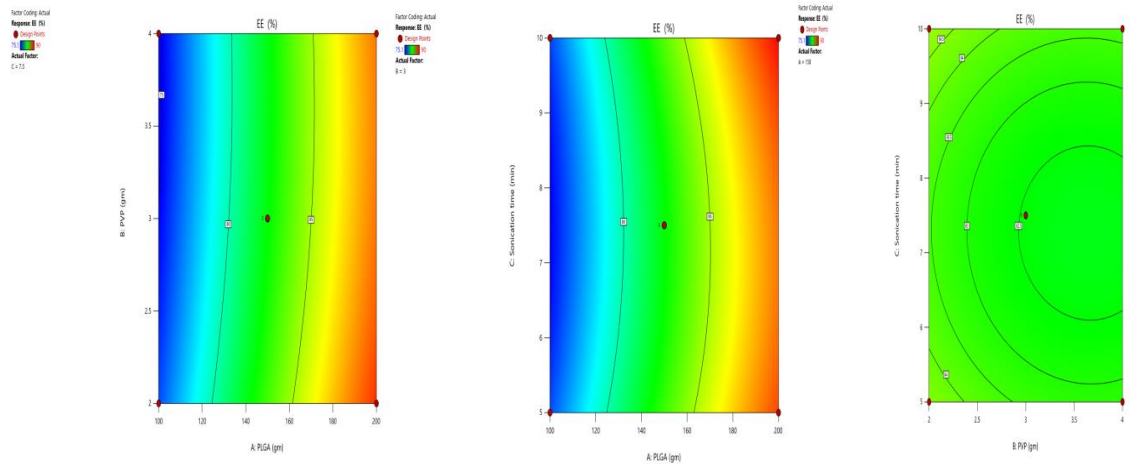

Fig. 3: (a) 2D Counter plot depicting the effect of PLGA, PVA and sonication time on the EE%

Zeta potential

The zeta potential analysis for the prepared polymeric nanoparticle formulations was also done using Malvern zeta sizer. The higher Zeta potential values of a formulation indicate increased stability of the polymeric nanoparticles. Regression Statistics was implemented and fitting to Quadratic model of zeta potential. Reduced polynomial equation for zeta potential obtained was:

$$ZetaPotential=23.44+0.0375X1-1.76X2+0.2000X3+0.1000X1X2+0.2750X1X3+0.0250X2X30.7800X1^2+0.4700X2^2+1.15X3^2$$

The negative sign in the polynomial equation for coefficient X1 and coefficient X2 suggests the conc. of PLGA (X1) decrease in zeta potential values. The model is suggested to be significant by the model's F-value of 30.76. There is only 0.01% chance that this large

F-value might happen due to noise. P-values is<0.0001, which is less than 0.0500 reflects model values are significant. Here X1, X2 are significant model terms. The value of anticipated R^2 0.8153 is in close accord with the adjusted R^2 of 0.9436.

The zeta potential for the prepared polymeric nanoparticle was found within the range-21.5 to-26.9. The negative value shows that the carboxylic groups in the end of PLGA polymer will allow the passing of molecules across lipid barrier and prolong the circulation time [33].

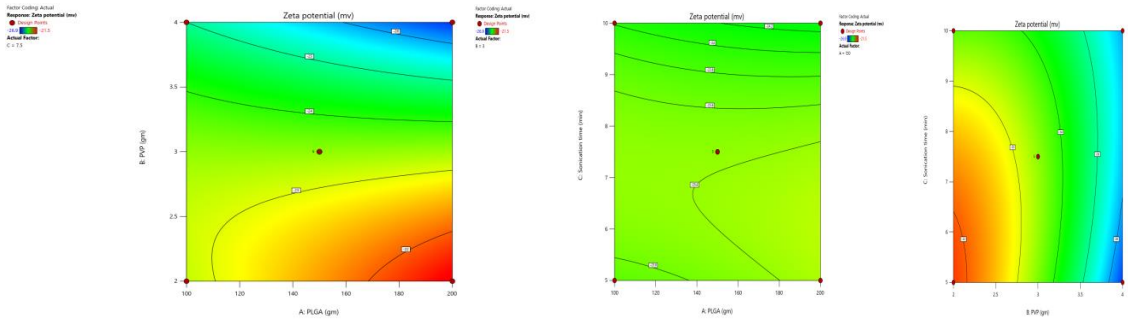

Fig. 4: (a) 2D Counter plot depicting the effect of PLGA, PVA and sonication time on the EE%

Optimization

The optimum Opt-SVF-PLGA-NPs formulation was selected by applying constraints on the dependent factors as shown in table xx. Point prediction of the Design Expert software 12 was used to determine the optimized NPs on the basis of closeness of desirability factor close to 1, which predicted the optimized process parameters to be PLGA(⍰1)150 mg, PVA(⍰2)3 mg, Sonication time(⍰3) 7.5 minute with predicted values of responses Particle size(⍰1)178.53 nm, Entrapment Efficiency%(Y2)86.05% and zeta potential (⍰2)-23.98mV The optimized formulation (Opt-SVF-PLGA-NPs) was developed and characterized for Particle size, Entrapment Efficiency % and zeta potential. The experimental value for responses Particle size(⍰1)175 nm, Entrapment Efficiency%(Y2)85.1% and zeta potential (⍰3)-23.8mV of optimized formulation was found in good agreement with the predicted values generated by the Response Surface Model RSM and the result assures the validity of RSM

TEM study

The optimized formulation Opt-SVF-PLGA-NP has a particle size of 175 nm, as shown in TEM image fig. 5. This is the hydrodynamic size of the particles, which is the size they appear to be when they are suspended in water. TEM images can provide a more accurate representation of the actual geometric size of the particles. They can also be used to qualitatively observe the relationship between process factors and particle size [34].

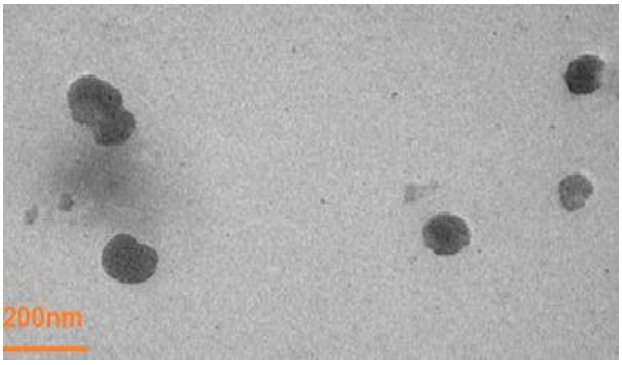

Fig. 5: TEM image of optimized formulation Opt-SVF-PLGA-NPs

FTIR studies

The FTIR spectra of free Sorafenib shows two characteristic bands at 3281.02 and 3250 cm−1 due to the N-H stretching. The observed peaks at 3082 cm−1 and 2955.04 cm−1 are related to the C-H stretching band of aromatic and aliphatic CH, respectively. The peaks at 1691 and 1714 cm−1 are characteristic peaks of the amide C=O group.

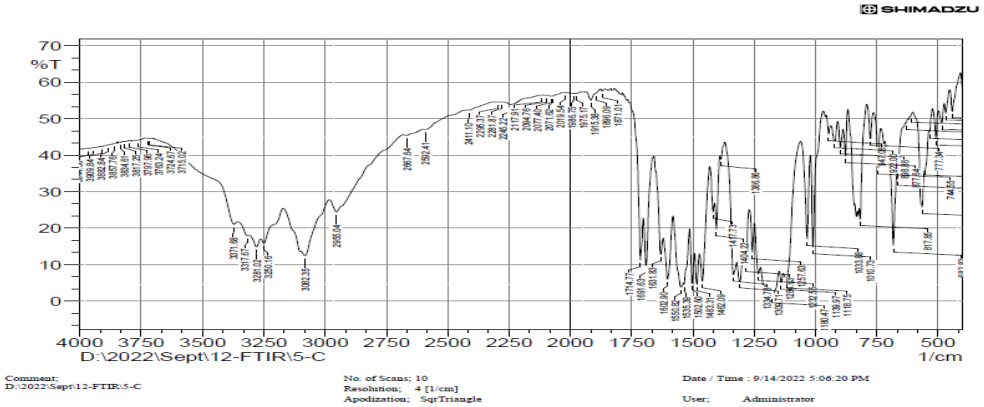

Fig. 6: FTIR image of sorafenib

In vitro drug release study of SVF from NPs

In vitro drug release profile of the polymeric nanoparticle was given in fig. 7 from this, it was concluded that Opt-SVF-PLGA-NPs nanoparticle showed slower drug release in comparison with pure drug dispersion. The release design of drug revealed a biphasic pattern where they initially showed bust release, followed by sustained release. The initial burst of the release of Sorafenib was

due to the immediate dissolution and release of drug adsorbed on the surface of the nanoparticle, followed by slow and sustained release of drug present on the core of the polymer matrix. Similar results were found by lu B *et al.,* and Kamajar N *et al.,* The optimized formulation Opt-SVF-PLGA-NP released 98% of sorafenib in 24h, where as the pure drug was released completely in 7h, this could be due to PLGA nanoparticles restricted the release of the drug rapidly from nano formulation.

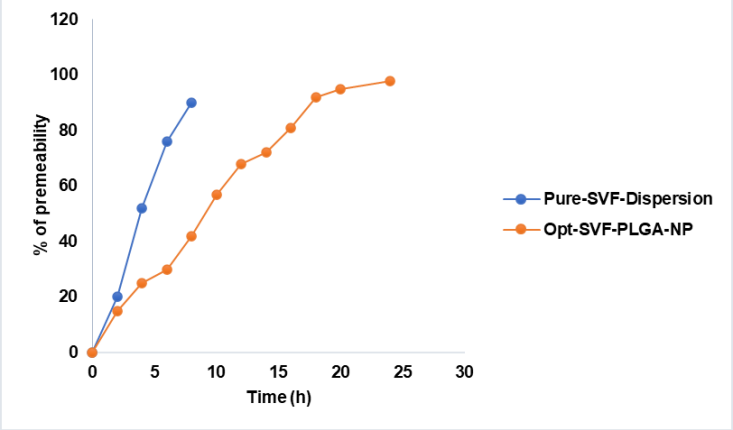

Fig. 7: *In vitro* drug release of Opt-SVF-PLGA-NP and pure-SVF-dispersion

CONCLUSION

The drug-excipient interaction was explored by molecular docking studies. The Polymeric nanoparticles containing Sorafenib was prepared by single emulsion solvent evaporation using PLGA as polymer and PVA as surfactant. Box-Bhenkam designs were adopted for optimization using "Design Expert" software. Different microscopic images showed that the formed polymeric nanoparticles were smooth, spherical in shape and uniform in size with a size less than 200 nm. *In vitro* drug release study of the optimized PLGA nanoparticle showed sustained release for prolonged time period., the developed Sorafenib loaded in PLGA nanoparticles could be promising formulation in oral drug delivery for the treatment of liver carcinoma.

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Nil

AUTHORS CONTRIBUTIONS

All authors have contributed equally

CONFLICT OF INTERESTS

Declared none

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