RESEARCH ARTICLE

Application of Box-Behnken Design for Formulation Optimization of Sorafenib loaded Nanoparticles Targeting to Liver Carcinoma: *Ex-vivo* Intestinal Permeation and Cytotoxicity Study

Gnyana Ranjan Parida[1], Gurudutta Pattnaik[1], Amulyaratna Behera[2], Dibya Lochan Mohanty[3], Suraj Sahoo[4]

[1]*School of Pharmacy and Life Sciences, Centurion University of Technology and Management, Odisha, India.*
[2]*School of Pharmacy, Driems University, Cuttack, Odisha, India.*
[3]*Centre for Nanomedicine, School of Pharmacy, Department of Pharmaceutics, Anurag University, Hyderabad, India.*
[4]*Sri Jayadev College of Pharmaceutical Sciences, Bhubaneswar, Odisha, India.*

Received: 12[th] March, 2024; Revised: 03[rd] May, 2024; Accepted: 2[nd] August, 2024; Available Online: 25[th] September, 2024

ABSTRACT

The anticancer drug sorafenib (SFB) was formulated as a polymeric nanoparticle using Box-Behnken design. The solvent evaporation method was utilized to develop the sorafenib polymeric nanoparticles. Formulations were then evaluated in terms of their morphological structure, entrapment efficiency (EE%), zeta potential, polydispersity index (PDI), and particle size. *Ex-vivo* intestinal permeation studies for pure drugs, as well as optimized formulation, were performed in rats. Furthermore, the anticancer activity was evaluated using the HepG2 cancer cell lines. Transmission electron microscopy (TEM) revealed that the improved formulation had a particle size of 175 nm, PDI of 0.134, zeta-potential of -23.8 mV, and the sorafenib-loaded PLGA NP were recognized as spherical particles. Over 90% of the drug was released in less than 24 hours, with an EE% of 85.1%. Sorafenib-loaded PLGA NP efficiently inhibited HepG2 cells.

Keywords: Sorafenib, Nanoparticles, Hep G2 cell lines, Anticancer activities.

International Journal of Drug Delivery Technology (2024); DOI: 10.25258/ijddt.14.3.25

How to cite this article: Parida GR, Pattnaik G, Behera A, Mohanty DL, Sahoo S. Application of Box-Behnken Design for Formulation Optimization of Sorafenib loaded Nanoparticles Targeting to Liver Carcinoma: *Ex-vivo* Intestinal Permeation and Cytotoxicity Study. International Journal of Drug Delivery Technology. 2024;14(3):1426-1434.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

The most prevalent kind of primary liver cancer and the principal cause of cancer-related mortality globally is hepatocellular carcinoma (HCC).[1] HCC, in the context of cirrhosis and chronic liver disease (CLD), accounted for 830,000 cancer-related deaths and 906,000 new cases globally in 2020, making it the sixth most frequently diagnosed cancer and the third most prevalent cause of cancer-related death, with a sharp increase in incidence.[2] The primary cause of HCC worldwide, and especially in Asia, is chronic hepatitis B (CHB) infection. The primary causes in Western nations are non-alcoholic steatohepatitis (NASH), chronic hepatitis C, and alcoholic cirrhosis. The incidence of HCC has risen in the US within the last 10 years.[3] Sorafenib is one of the well-established molecularly targeted medications that show significant promise in increasing cancer patients' overall survival and progression-free survival rates in clinical settings.[4] Research on cumulative mechanistic elucidation has demonstrated that sorafenib's anticancer activity is due to its capacity to inhibit cell growth, trigger cell death, and stop angiogenesis.[5] When compared to chemotherapeutic drugs, sorafenib exhibits noteworthy efficacy profiles for cancer therapy; nonetheless, it is crucial to recognize that sorafenib still has a number of disadvantages in clinical therapy. (i) Sorafenib's limited bioavailability (~8.43%) severely limits its clinical applicability because of its low water solubility, fast metabolism, and rapid elimination;[6] (ii) A lot of cancer patients usually have to temporarily stop taking sorafenib or reduce their dosage because of severe side effects such diarrhea, skin toxicity, hypertension, and hand-foot syndrome. To overcome the aforementioned issues and create the desired sorafenib delivery system with major therapeutic benefits, a number of sorafenib-loaded nano-delivery systems have been created and carefully investigated.[7] These intelligent sorafenib nano-delivery systems have several benefits over conventional formulations, including increased tumor accumulation

*Author for Correspondence: gurupharma2121@gmail.com

capacity, regulated drug release, and better targetability and bioavailability.[8] One important benefit of sorafenib-based nano-delivery systems is that, before entering tumor tissues, they remain dormant. This helps to prevent sorafenib from leaking into the systemic circulation too soon, which lowers the risk of side effects.[9]

One potential strategy to lessen the severe side effects of using anticancer drugs is to develop a nanocarrier that allows for targeted drug delivery and controls the release of therapeutic substances. It has been discovered that polymeric nanoparticulate drug delivery techniques are useful for maintaining drug release and focusing it on particular tissue.[10]

Traditional sorafenib nano-delivery methods, including liposomes,[11] solid-lipid nanoparticles,[12,13] micelles,[14] gold nanoparticles,[15] and inorganic nanoparticles,[16] have significantly improved therapeutic effects over the past few decades when compared to free sorafenib. Overall data, however, indicated that there are still certain issues or problems with the conventional sorafenib nano delivery methods, such as poor targetability, uncontrolled drug release, and unguided blood circulation.

This study aimed to improve the prepared composition by varying the sonication time, polymer, and surfactant concentrations in order to construct and define sorafenib polymeric nanoparticles. The effect of individual components on formulation attributes was investigated using a Box-Behnken design with a small number of trial runs.[17] The lyophilized optimized formulation was inspected by FTIR spectroscopy, DSC, transmission electron microscopy, X-ray diffraction, and cytotoxicity

MATERIAL AND METHOD

Material

Sorafenib (SFB) was procured from MSN Lab in Hyderabad, India. Dichloromethane, PVA, and PLGA(Poly) were purchased from S.D. Fine Chemicals (Mumbai, India). All of the other materials and analytic reagents were of analytical grade and were employed precisely as prescribed.

Preparation of 'Sorafenib' loaded polymeric nanoparticle (SFB-NP)

A slightly modified single-emulsion solvent-evaporation technique was used to create 'sorafenib'-loaded PLGA nanoparticles.[18] To put it briefly, 100 mg of 'PLGA' polymer was dissolved in 3 ml of dichloromethane to generate a primary emulsion. Next, an ice bath was used to create the emulsion using a microtip probe sonicator (VC 505, Vibracell Sonics, NewtonUSA) set to 55W for two minutes. The organic solvent was then evaporated by stirring the oil-in-water emulsion for a whole night after it was emulsified into an aq.PVA solution (2% w/v). The next day, three washes with double-distilled water were conducted after the excess PVA was eliminated using a Sorvall Ultraspeed Centrifuge (Kendro, USA) set at 50,602×g for 20 minutes at 4°C. The resulting nanoparticulate dispersion was freeze-dried for two days at -80°C and 10 µm mercury pressure in order to obtain the lyophilized powder for use in the future.

Optimization of sorafenib loaded PLGA nanoparticle using the Box-Behnken Design

When designing drug delivery systems, the goal of using a nonlinear quadratic polynomial model is to get insight into how various components interact and affect the system's overall performance. It is made simple to identify the ideal conditions or factor configurations that maximize or minimize the dependent variable by estimating the coefficients and performing a model analysis. This approach simplifies the development of more operative and efficient medication delivery systems. In the context of response surface methodology (RSM) and experimental design in pharmaceutical research and development, applying this modeling technique is essential. Particle size, entrapment efficiency, and zeta potential were the dependent variables. The effects of the independent components, PLGA (mg), PVA (mg), and sonication duration (min), were examined using the Box-Behnken design (BBD) (Table 1 and 2). This architecture is well suited for the analysis of quadratic response surfaces and the construction of second-order polynomial models. To precisely assess the influence of the independent factors on the dependent variables, a nonlinear quadratic polynomial model was built with an emphasis on both linear and quadratic components using Design Expert Software (version 13.0, Stat-EaseInc., Minneapolis, MN).

Characterization of sorafenib-loaded PLGA nanoparticle (Shown in Table 3)

Particle Size, PDI and zeta potential

After an appropriate dilution with deionized water, the PS and PDI values of all freshly generated formulations were ascertained using photon-correlation spectroscopy (Malvern Instruments Ltd.Malvern, Worcestershire, UK). Every sample was examined three times.

Entrapment efficiency (EE%)

The previously described indirect method was used to calculate the EE%. Using this procedure, the amount of free or unentrapped SFB in the supernatant of each formulation following centrifugation was assessed. The amount of free SFB was measured at 261 nm using a spectrophotometric technique (Spectro UV-VIS double beam, Lab India 3200).

Table 1: Variables used in experiment

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) (sec)	5	7.5	10
Responses(Y)	Constraint		
ParticleSize (Y1) EE% (Y2) ZetaPotential (Y3)	Min. Max. 20mV to -30mV		

Table 2: Optimization Design with Coded value using 'Box–Behnken Design'

Batch code	*PLGA Qua(X1)*	*PVA(X2)*	*Sonication time(X3)*
SFB1	+1	0	+1
SFB2	-1	-1	0
SFB3	0	0	0
SFB4	0	0	0
SFB5	0	0	0
SFB6	+1	-1	0
SFB7	-1	0	-1
SFB8	+1	+1	0
SFB9	-1	0	+1
SFB10	0	0	0
SFB11	0	+1	-1
SFB12	0	+1	+1
SFB13	0	-1	+1
SFB14	+1	0	-1
SFB15	0	0	0
SFB16	0	-1	-1
SFB17	-1	+1	0

Zeta potential (ZP)

Using a Zeta- sizer nano series (MalvernInstruments Limited, UK) to evaluate electrophoretic mobility in an electric field at 25°C, the zeta potential values of SFB-PLGA-NPs were determined.

Fourier-Transform IR spectroscopy (FTIR)

Using the potassium bromide pellet method, the FTIR spectrophotometer (Shimadzu, Singapore) was used to produce the FTIR spectra of the physical mixture, sorafenib, and PLGA. Samples were pressed into disks after being uniformly mixed with KBr. The 400 to 4000 cm^{-1} scanning range was used.[19]

Differential scanning calorimetry (DSC)

DSC tests were performed on PLGA, the improved formulation, and sorafenib. The materials were subjected to thermal examination with a Shimadzu DSC 50 device (Tokyo, Japan). Under a steady, dry nitrogen environment, the samples were heated from 35 to 300°C at a rate of 10°C per minute, with a purge flow rate of 20 mL/min.[20]

Powder X-ray diffractometry (PXRD)

All components' variations in crystallinity throughout the formulation process are examined using PXRD analysis.[20]

An X-ray diffractometer (Diano, Woburn, MA, USA) operating at a current of 9 mA anda voltage of 45 kV at a 2 ϴ angle was used to acquire X-ray diffractograms of Sorafenib, PLGA, and Optimized Formulation.[21]

Morphological study by transmission electron microscopy (TEM)

Using TEM (JEOL 100CX; JEOL Inc., Peabody, MA, USA) at an accelerating voltage of 80 kV, the morphology of the SFB-NPs was examined. The SFB-NPs formulation was diluted from 1 to 0.01% w/w and treated in an ultrasonic bath (Model 3510, BransonMS) in order to reduce particle aggregation on the copper grid. For TEM analysis, a single drop of the modified mixture was dried and put on a copper grid coated with carbon.[21]

Determination of in-vitro drugrelease

The dialysis method was used to measure the invitro release of sorafenib from the produced PLGA nanoformulation in 0.1N HCl (pH 1.2) and phosphate buffer (pH6.8). To put it briefly, a cellulose dialysis bag with a capacity of about 1.61 mL/cm, an average size of 14.3 mm, and a flat width of 24.26 mm was filled with pure drug dispersion. After sealing the bag on both ends, it was submerged in 900 mL of dissolution medium in a USP dissolution apparatus-II (Lab India, DS 8000). A temperature of 37 ± 0.5°C was maintained and the device was run at 75 rpm. Five ml samples were taken out at prearranged breaks over the course of a day and replaced with an equivalent volume of new medium.[22] The amount of drug released over time was measured at 261 nm using UV spectroscopy (Lab India 3200).

Ex-vivo intestinal permeation Study

The mentioned method was used to conduct a comparative *ex-vivo* intestinal permeation analysis of the optimised sorafenib loaded PLGA NPs. Excess ether inhalation was used to sacrifice the rat. The abdomen was opened, and the ileum section was cut. One end of the intestinal segment was knotted, and then both formulations each carrying 2 mg of sorafenib were closely ligated in the mucosal side of the intestinal sac. Beakers were set on a hot plate magnetic stirrer set at 37°C after the sac was submerged in 100 mL of Krebs solution. An aerator was used to continuously add 95% oxygen to the Krebs solution. After that, 1-mL of the sample was extracted, and at predefined intervals, 1-mL of new Krebs solution was added to maintain the sink state. The amount of material that had penetrated the samples was measured using the UV technique after they had been collected and passed through a 0.2 µm membrane filter.

Cytotoxicity Studies

Cell culture preparation

The ATCC VR HB8065 HepG2 cells were utilized for cytotoxicity studies. In brief, after being taken out of liquid nitrogen storage, HepG2cells were rapidly thawed to ambient temperature. After adding 9 mL of full culture media to the contents of the vial, the mixture was centrifuged at 125 g for 5 minutes. The pellets were centrifuged again and again in 10 mL of full DMEM medium. They were then put into a T25 flask, and the supernatant was thrown away. The cells were cultured with 5% CO_2 at37°C. After that, they were divided using trypsin-EDTA and put into two T25 flasks. When cell confluency was between 80 and 90 percent, the experiment was performed.

MTT assay

Without using a test agent, 200 mL of the cell solution was placed on a 96-well plate, and it was left 24 hours to develop.

The media was changed to one with the proper concentration of test chemicals after a day. It was incubated in an environment with 5% CO2 for 48 hours at 37°C. The MTT reagent was added through a sterilized 0.2 mm filter to achieve a final concentration of 0.5 mg/mL after the media was removed from the incubation chamber. To protect the plates from light, they were covered with aluminum foil and incubated for three hours. After the incubation, 100 mL of DMSO was added, and the MTT reagent was removed. Absorbance at 570 nm was then measured using a Lab India 3200 spectrophotometer with UV spectroscopy.

RESULTS AND DISCUSSION

Particle Size, PDI and Zeta Potential Analysis

The sorafenib-loaded PLGA nanoparticles in this study showed particle sizes, PDIs, and zeta potentials ranging from 162.9 to 182.6 nm, 0.143 to 0.157, and −21.5 to −26.9 mV, respectively.

Entrapment Efficiency

The entrapment efficiency of sorafenib-loaded PLGA nanoparticles ranged from 75.1% to 90%. The polymer concentration has been reported to increase drug entrapment efficiency.[23]

Fourier Transforms Infrared Spectroscopy (FTIR)

FT-IR spectra of Sorafenib, PLGA, and their physical mixing. The distinctive peaks representing the drug-excipient interaction were examined. Because of the N-H, the medication showed maxima at 3281.02 and 3250 cm^{-1}. The amide C=O group's distinctive peaks are located at 1691 and 1714 cm^{-1}. The O–H stretch at 3390 cm^{-1} and the C–H alkane stretch at 2925 cm^{-1} are the more intense peaks of the PLGA. Similar peaks show up in the drug and PLGA physical combination. Every important peak of the medication is present in the physical mixture's infrared spectra. The absence of drug-polymer interaction is confirmed by the drug peak's appearance in the physical mixture.

Optimization and Statistical Analysis

Effect on particle size

The polymer and surfactant concentrations determine the sorafenib-loaded PLGA nanoparticle's particle size. The program recommended a quadratic model, and a statistical examination of the model yields a model that fits reduced polynomial equations for particle size.

Table 3: Characterization data

Formulation	*Particle size (nm)*	*EE%*	*Zeta potential (mV)*	*PDI*
SFB1	182	90	-24.1	0.143
SFB2	166	76.5	-22.9	0.145
SFB3	168.2	82	-23.5	0.146
SFB4	170	82.1	-23	0.147
SFB5	168.5	81.8	-23.9	0.150
SFB6	181.1	89	-21.5	0.155
SFB7	164	76.2	-23.9	0.149
SFB8	181.9	88	-26.9	0.154
SFB9	163	75.6	-24.2	0.157
SFB10	167.9	82.4	-23.6	0.143
SFB11	170.2	83	-26.5	0.152
SFB12	169	83.9	-25.9	0.146
SFB13	175	85.1	-23.8	0.147
SFB14	182.6	89.5	-23.1	0.157
SFB15	168.1	84	-23.2	0.148
SFB16	169.5	84.3	-21.9	0.144
SFB17	162.9	75.1	-24.9	0.151

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

Particle size increases with higher PLGA concentration, suggesting that PLGA (X1) positively affects particle size. The interaction between PLGA and PVA also shows a beneficial influence on nanoparticle size. The increase in particle size could be due to more particle aggregation as PLGA concentration rises. Larger nanoparticles are also formed because the increased polymer content increases the viscosity of the solution, slowing the pace at which nanoparticles diffuse into the aqueous phase. With just a 0.01% chance that the high F-value is the consequence of noise, the model's F-value of 57.24 shows substantial results. Less than 0.0500, or a *p-value*

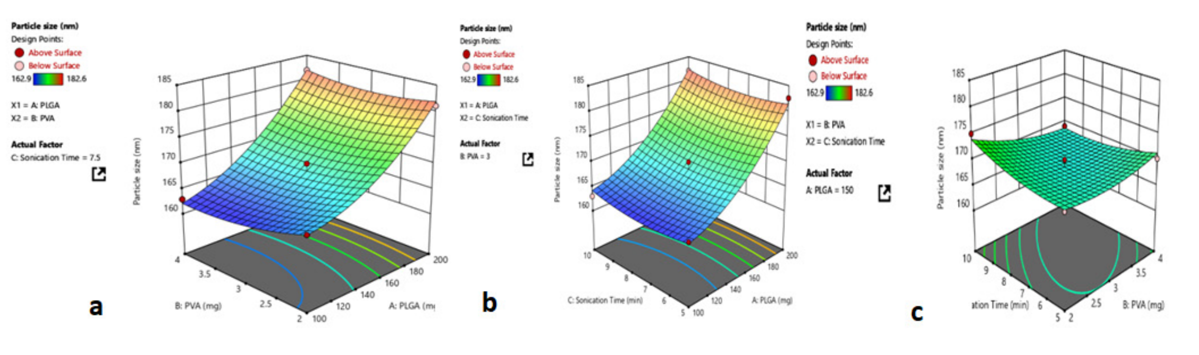

Figure 1: Three dimension surface plot (a, b, c) representing the effect ofPLGA (A) , PVA (B) and Sonication Time(C) on particle size

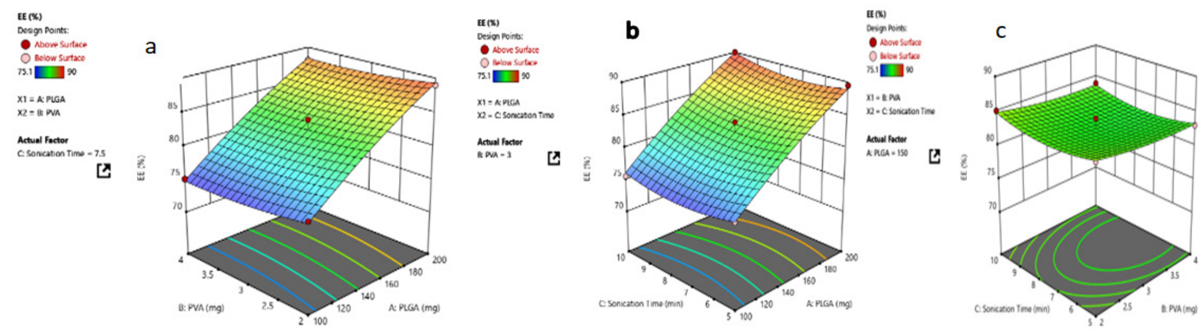

Figure 2: Three dimension surface plot (a, b, c) representing the effect of PLGA (A) , PVA (B) and Sonication Time (C) on Entrapment Efficiency

of 0.0001, indicates that the model terms are significant. X1 and X2 are important model terms in this context. There is substantial agreement between the modified R2 of 0.9881 and the projected R2 of 0.9986. A three-dimensional surface map of the impacts of the independent variables (PLGA, PVA, and sonication time) on particle size is presented in Figure 1 (a, b, and c) (Seham IElsayed *et al*., 2023).

Effect on entrapment efficiency

Figure 2(a, b, c) shows the effect of independent parameters (PLGA, PVA and sonication time) on Entrapment efficiency with the help of three dimension surface plot. The Entrapment efficiency of sorafenib in polymeric nanoparticles is expressed by following the quadratic equation

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

"The polynomial equation shows that the polymer concentration has an effect on entrapment efficiency, with coefficients X1 and X2 having a significant impact. As polymer concentration increases, drug entrapment also rises. This can be attributed to higher PLGA concentrations producing smaller nanoparticles, which hold less medication and release more into the aqueous phase, resulting in less PVA being encapsulated in PLGA nanoparticles. Drug molecules traveling from the organic to the aqueous phase will face more diffusional resistance due to the organic phase's increased viscosity caused by a higher initial concentration of PLGA.[24] Furthermore, larger particle sizes produced by a greater initial concentration of PLGA have been shown in the literature to improve drug encapsulation effectiveness (Serap Derman, 2015). The model's F-value of 67.25 indicates a high degree of reliability because the likelihood that the high F-value is the product of random noise is only 0.01%. *p-values* less than 0.0001, which is considerably less than 0.0500, confirm the significance of the model terms X1 and X2. The predicted R2 of 0.9404, which is quite similar to the Adjusted R2 of 0.9739, indicates a good model fit".

Effect on zeta potential

ZP values provide information about the colloidal dispersion's storage stability. The colloidal dispersion frequently needs higher ZP values of the nanoparticulate system in order to be stable. The following quadratic equation can be used to illustrate the effect on zeta potential.

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

The negative coefficients for X1 and X2 in the polynomial equation imply that the concentration of PLGA (X1) correlates with a decrease in zeta potential values. We noticed that the zeta potential of all formulations consistently showed negativity. After analyzing the equation more closely, we came to the conclusion that a rise in PLGA content causes a larger zeta potential. The negatively charged PLGA adhering to the surface can be used to explain this phenomenon. When PLGA is in its aqueous solution, carboxyl groups (COOH)

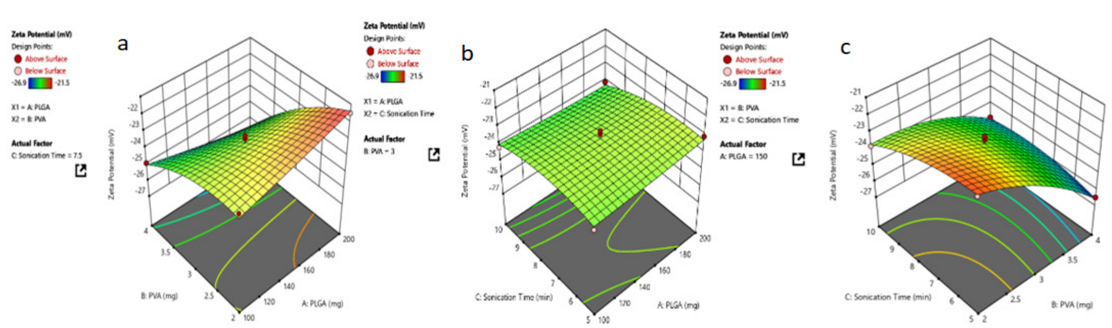

Figure 3: Three dimension surface plot (a, b, c) representing the effect ofPLGA (A) , PVA (B) and Sonication Time(C) on Zeta Potential

are present on its surface, which causes a negative charge. Three-dimensional surface plots in Figure 3(a, b, c) show how independent parameters (PLGA, PVA, and Sonication Time) affect zeta potential. The F-value of 30.76, which shows that there is only a 0.01% chance that such a huge F-value would occur by chance, supports the importance of the model. Model terms, especially X1 and X2, are significant when *p-values* are less than 0.0001, which is below the significance criterion of 0.0500. The expected R^2 value of 0.8153 closely matches the adjusted R^2 of 0.9436, indicating a strong fit of the model.

By putting limitations on the dependent variables, the optimal Opt-SFB-PLGA-NPs formulation was found. The optimal nanoparticles were found using the Design-Expert software version 12, which used point prediction to aim for a desirability factor near 1. This process yielded projected values for particle size (Y1) of 178.53 nm, entrapment efficiency (Y2) of 86.05%, and zeta potential (Y3) of -23.98 mV. The ideal process parameters were expected to be 150 mg of PLGA (X1), 3 mg of PVA (X2), and a sonication period of 7.5 minutes (X3). "The optimized formulation (Opt-SFB-PLGA-NPs) was then created and tested in terms of zeta potential, entrapment efficiency, and particle size". The improved formulation's experimental values for zeta potential (Y3), particle size (Y1), and entrapment efficiency (Y2) were measured at 175 nm, 85.1%, and -23.8 mV, respectively. These experimental findings validated the validity of the response surface methodology (RSM) model by closely matching the expected values produced by the RSM.

Characterization of Optimized Sorafenib PLGA NPs(Opt-SFB-PLGA-NPs)

The size, distribution, and polydispersity index of nanoparticles are their three main features. These properties control solubility, physical stability, chemical kinetics, release kinetics, and colloidal properties.[25] PS is one of the most important factors in liver absorption and targeting. The small formulation size, which involves particles with a diameter of only 20 to 500 nm, makes it easier for medications to be efficiently absorbed into the intestine.[26] "The optimized Opt-SFB-PLGA-NPs has a mean particle size of 175 nm" (Figure 4a). The uniform size distribution and particle aggregation can both be well-indicated by the PDI. The typical range for PDI is 0.1 to 0.5. Higher PDI values signify a highly polydispersed particle size distribution, while lower values imply samples that are predominantly monodispersed. For the optimized formulation, the PDI value is 0.134.

One useful method for predicting the durability of nanoparticles is the zeta potential, which shows the electrical charge or electrostatic repulsion of the particles.[27,28] Particle aggregation was caused by insufficient stability, as indicated by the lower ZP value. The ZP of the Opt-SFB-PLGA-NPs formulation was determined to be −23.8 mV (Figure 4b).

Differential scanning calorimetry

The DSC thermogram of sorafenib, PLGA, and optimized formulation is shown in Figure 5. The crystalline nature of PLGA and the medication sorafenib was indicated by

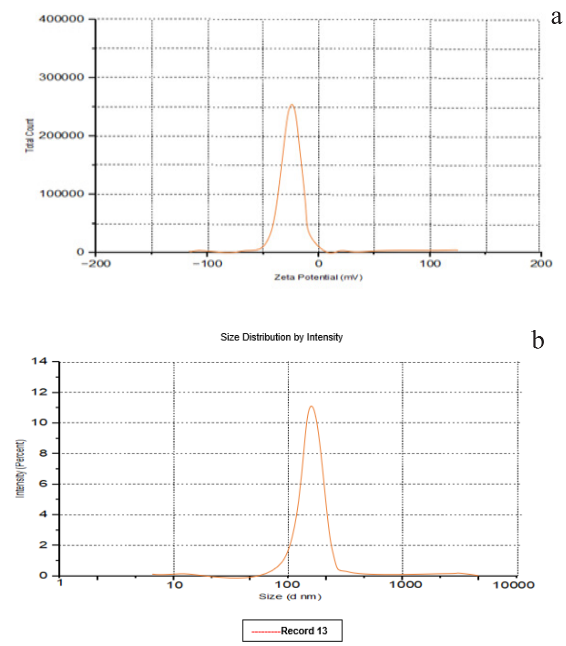

Figure 4: a. Particle size distribution b. Zeta potential

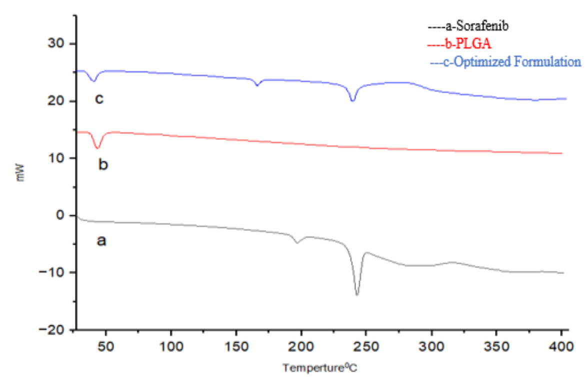

Figure 5: DSC study

their strong endothermic peaks, which occurred at 240 and 31.2°C, respectively. It appears that the crystalline form has transformed into an amorphous form because the Optimized formulation does not have a drug-distinctive peak. The drug's solubility in the PLGA may be the cause of this. It illustrates how a medication becomes stuck in the PLGA nanocarrier.

Xray diffraction studies

XRD "measurements were conducted to analyze the crystalline structure of the drug and to examine the interactions among different nanocarrier components. Figure 6 illustrates the X-ray diffraction patterns of PLGA, sorafenib, and the optimized formulation. The diffraction patterns of pure sorafenib exhibited several lower-intensity peaks alongside sharp peaks with high intensity at 2θ scattering angles of 5.140, 11.020, 15.790, 17.690, and 24.390, indicating its crystalline nature. In contrast, the XRD pattern for PLGA showed no distinct peaks, suggesting its amorphous properties. Interestingly, in the XRD pattern of the optimized formulation, the distinctive peaks

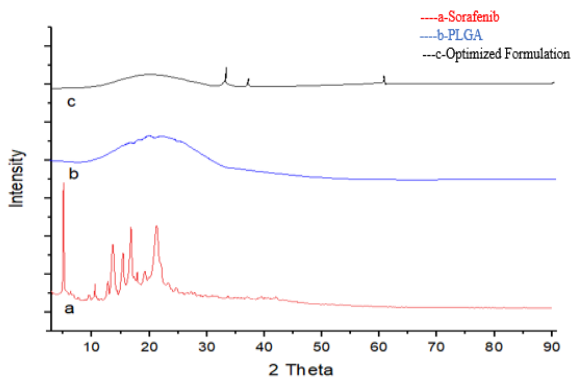

Figure 6: XRD study

of sorafenib either disappeared or became less prominent. This observation suggests that the drug is more soluble in the polymer when its crystallinity decreases, likely due to the formation of amorphous nanoformulations".

Transmission electron microscopy

The size, average diameter, and surface shape of the improved formulation formulations were investigated using TEM. The spherical shape and smooth surface of the nanoformulation are shown in a TEM photomicrograph (Figure 7). The picture demonstrates that the particles were not uniformly distributed and consolidated.

In-vitro drug release of 'Sorafenib' from Opt-SFB-PLGA-NPs

"Using the dialysis bag diffusion method. Figure 8 shows the release patterns of Opt-SFB-PLGA-NP and pure sorafenib dispersion in simulated stomach (0.1N HCl, pH 1.2) and intestinal (phosphate buffer, pH 6.8) fluids. The observed variations in release patterns are probably explained by the inclusion of PLGA and surfactant in the Opt-SFB-PLGA-NP formulation (Figure 8). A sustained release followed a fast release in the biphasic release profile of opt-SFB-PLGA-NP. In particular, it showed a 30% burst release within 5 hours and a 90% steady release over 24 hours. In contrast, pure sorafenib dispersion released 96% of the drug within 6 hours. The initial burst release from Opt-SFB-PLGA-NP may be attributed to drug molecules adhering to nanoparticle surfaces, while the sustained release could be due to drug entrapment within the polymer matrix. These findings highlight the superior efficacy of PLGA nanoparticles as a carrier for prolonged sorafenib administration compared to pure sorafenib dispersion".

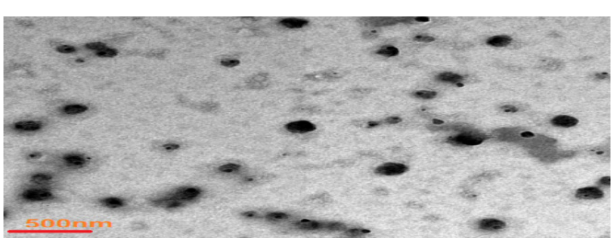

Figure 7: TEM image of optimized sorafenib loaded PLGA nanoparticle

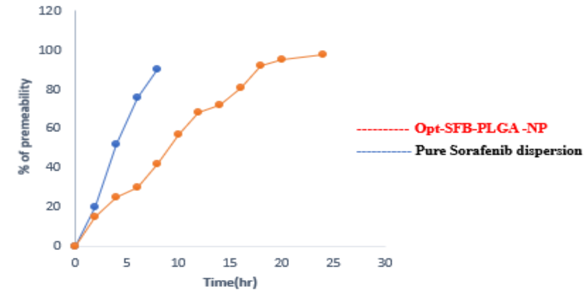

Figure 8: *In-vitro* dissolution study

Ex-vivo intestinal permeation study

The amount of drug absorbed *via* the gastrointestinal tract is determined by the rate of drug penetration. Rat intestinal sections were used in ex-vivo permeation studies to learn more about the potential for improved absorption of Opt-SFB-PLGA-NPs formulations. Since most medications are ingested *in-vivo* by the small intestine, rat tissue is more justified. Henceforward we utilized the ileum section to investigate the potential of Opt-SFB-PLGA-NPs formulations that can improve the penetration of sorafenib throughout the intestinal barrier. The cumulative amount permeated (CAP) was computed and shown throughout the gut. The cumulative amount of sorafenib permeated from the pure drug was 821 µg/mL and was considerably elevated to 1624 µg/mL in Opt-SFB-PLGA-NPs formulation (Figure 9).

Cytotoxicity studies

HepG2 cells were used in the cytotoxicity research of the pure medication pure SFB, OPT-SFB PLGA NP, and blank PLGA NP (Human Hepatocellular carcinoma cells). OPT-SFB PLGA NP efficiently suppressed HepG2 cells (Figure 10). The size of the particles has a big impact on how well cancer cells absorb medications. The reduced particle size of the improved formulation may have contributed to its significant cytotoxic

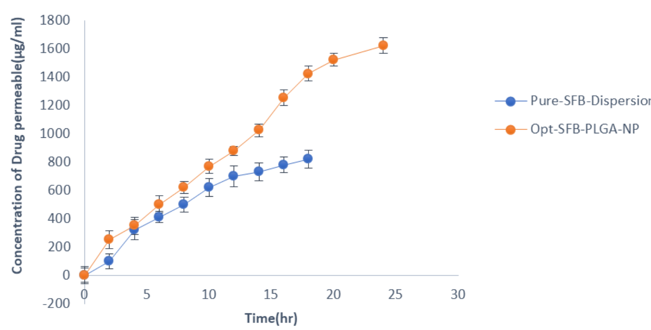

Figure 9: *Ex-vivo* intestinal permeation study

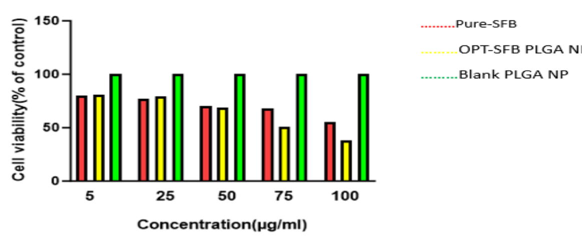

Figure 10: Cell viability study

effect. Cancer cells are more susceptible to cytotoxicity when there is a higher zeta potential value because it causes a more direct engagement with the tumor cell.

CONCLUSION

The single emulsion solvent evaporation method, which has several advantages over other nanoparticle production methods, is used to create sorafenib-loaded PLGA nanoparticles. This technique stands out for its simplicity and versatility as it produces nanoparticles with controlled and narrow particle size distributions. The improved formulation, with an ideal particle size of 175 nm and a PDI of 0.134, showed good entrapment efficiency (85%). Excellent release characteristics were exhibited by the improved sorafenib-loaded nanoformulation (30% in 5 hours and 90% up to 24 hours). Its size and surface features greatly influence the ability of a nanoparticle to reach its intended location. The TEM image verified the presence of nanometer-sized, spherically-shaped particles. The improved formulation's zeta potential readings (-23.8 mV) showed that it was stable. The cytotoxicity study's findings demonstrated that PLGA NP loaded with sorafenib efficiently inhibited HepG2 cells. The study's optimized sorafenib-loaded PLGA nanoparticle was found to be an effective carrier for targeting liver cancer cells and a possible means of delivering sorafenib over an extended period of time.

REFERENCES

1. Balogh J, Victor III D, Asham EH, Burroughs SG, Boktour M, Saharia A, Li X, Ghobrial RM, Monsour Jr HP. Hepatocellular carcinoma: a review. Journal of hepatocellular carcinoma. 2016 Oct 5:41-53. doi: 10.2147/JHC.S61146
2. Hao X, Fan R, Zeng HM, Hou JL. Hepatocellular carcinoma risk scores from modelling to real clinical practice in areas highly endemic for hepatitis B infection. Journal of Clinical and Translational Hepatology. 2023 Dec 12;11(7):1508.
3. Jafri W, Kamran M. Hepatocellular carcinoma in Asia: a challenging situation. Euroasian journal of hepato-gastroenterology. 2019 Jan;9(1):27.
4. Mazzoccoli G, Miele L, Oben J, Grieco A, Vinciguerra M. Biology, epidemiology, clinical aspects of hepatocellular carcinoma and the role of sorafenib. Current drug targets. 2016 Jun 1;17(7):783-99.
5. Chen F, Fang Y, Chen X, Deng R, Zhang Y, Shao J. Recent advances of sorafenib nano formulations for cancer therapy: Smart nanosystem and combination therapy. Asian journal of pharmaceutical sciences. 2021 May 1;16(3):318-36.
6. Mans JC, Dong X. The Development of Lipid-Based Sorafenib Granules to Enhance the Oral Absorption of Sorafenib. Pharmaceutics. 2023 Nov 28;15(12):2691. doi: 10.3390/pharmaceutics15122691
7. Kabil MF, Nasr M, El-Sherbiny IM. Conventional and hybrid nanoparticulate systems for the treatment of hepatocellular carcinoma: An updated review. European journal of pharmaceutics and biopharmaceutics. 2021 Oct 1;167:9-37.
8. Wang L, Chen M, Ran X, Tang H, Cao D. Sorafenib-Based Drug Delivery Systems: Applications and Perspectives. Polymers. 2023 Jun 9;15(12):2638. doi: 10.3390/polym15122638
9. Kong FH, Ye QF, Miao XY, Liu X, Huang SQ, Xiong L, Wen Y, Zhang ZJ. Current status of sorafenib nanoparticle delivery systems in the treatment of hepatocellular carcinoma. Theranostics. 2021;11(11):5464. doi: 10.7150/thno.54822
10. Sabit H, Abdel-Hakeem M, Shoala T, Abdel-Ghany S, Abdel-Latif MM, Almulhim J, Mansy M. Nanocarriers: a reliable tool for the delivery of anticancer drugs. Pharmaceutics. 2022 Jul 28;14(8):1566.
11. Ye H, Zhou L, Jin H, Chen Y, Cheng D, Jiang Y. Sorafenib-loaded long-circulating nanoliposomes for liver cancer therapy. BioMed Research International. 2020 May 6;2020. doi: 10.1155/2020/1351046
12. Wang H, Wang H, Yang W, Yu M, Sun S, Xie B. Improved oral bioavailability and liver targeting of sorafenib solid lipid nanoparticles in rats. AAPS PharmSciTech. 2018 Feb;19:761-8. doi: 10.1208/s12249-017-0901-3
13. Wang Z, Duan X, Lv Y, Zhao Y. Low density lipoprotein receptor (LDLR)-targeted lipid nanoparticles for the delivery of sorafenib and Dihydroartemisinin in liver cancers. Life sciences. 2019 Dec 15;239:117013.
14. Su Y, Wang K, Li Y, Song W, Xin Y, Zhao W, Tian J, Ren L, Lu L. Sorafenib-loaded polymeric micelles as passive targeting therapeutic agents for hepatocellular carcinoma therapy. Nanomedicine. 2018 May 1;13(9):1009-23.
15. Cai H, Yang Y, Peng F, Liu Y, Fu X, Ji B. Gold nanoparticles-loaded anti-miR221 enhances antitumor effect of sorafenib in hepatocellular carcinoma cells. International journal of medical sciences. 2019;16(12):1541. doi: 10.7150/ijms.37427
16. Walbi IA, Ahmad MZ, Ahmad J, Algahtani MS, Alali AS, Alsudir SA, Aodah AH, Albarqi HA. Development of a curcumin-loaded lecithin/chitosan nanoparticle utilizing a box-behnken design of experiment: formulation design and influence of process parameters. Polymers. 2022 Sep 8;14(18):3758.
17. Mohanty D, Gilani SJ, Zafar A, Imam SS, Kumar LA, Ahmed MM, Jahangir MA, Bakshi V, Ahmad W, Eltayib EM. Formulation and optimization of alogliptin-loaded polymeric nanoparticles: In vitro to in vivo assessment. Molecules. 2022 Jul 13;27(14):4470. doi: 10.3390/molecules27144470
18. Anit J. George Fels Saju1, Bharat Mishra, Development of size optimized bromelain loaded nanocarriers by box behnken design, International journal Drug Delivery Technology 2023;13(1):10-16. doi: 10.25258/ijddt.13.1.02
19. Mohanty D, Alsaidan OA, Zafar A, Dodle T, Gupta JK, Yasir M, Mohanty A, Khalid M. Development of atomoxetine-loaded NLC in situ gel for nose-to-brain delivery: optimization, in vitro, and preclinical evaluation. Pharmaceutics. 2023 Jul 20;15(7):1985. doi: 10.3390/pharmaceutics15071985
20. Chekkilla Bhargavi, Pathuri Raghuveer, Development of piribedil solid lipid nanoparticles incorporated in nasal in-situ gel by DoE, International Journal of Pharmaceutical Quality Assurance. 2024;15(1):199-209. doi: 10.25258/ijpqa.15.1.31
21. Atul Pund , Atishkumar Mundada, Formulation development and evaluation of brivaracetam extended release tablets by QbD approach, International Journal of Pharmaceutical Quality Assurance. 2024;15(1):372-381. doi: 10.25258/ijpqa.15.1.59
22. Chougule PN, Koumaravelou K, Chougule NB, Fabrication, optimization and evaluation of escin enriched emulgel system for treatment of varicose veins, International Journal of Drug Delivery Technology. 2023;13(3):833-841. doi: 10.25258/ijddt.13.3.10
23. Barzegar-Jalali M. An Alternative Approach for Improved

Entrapment Efficiency of Hydrophilic Drug Substance in PLGA Nanoparticles by Interfacial Polymer Deposition Following Solvent Displacement. Jundishapur Journal of Natural Pharmaceutical Products.;13(4).

24. Budhian A, Siegel SJ, Winey KI. Haloperidol-loaded PLGA nanoparticles: systematic study of particle size and drug content. International journal of pharmaceutics. 2007 May 24;336(2):367-75.
25. Herdiana Y, Wathoni N, Shamsuddin S, Muchtaridi M. Drug release study of the chitosan-based nanoparticles. Heliyon. 2022 Jan 1;8(1). doi: 10.1016/j.heliyon.2021.e08674
26. Hua S. Advances in oral drug delivery for regional targeting in the gastrointestinal tract-influence of physiological, pathophysiological and pharmaceutical factors. Frontiers in pharmacology. 2020 Apr 28;11:524.
27. Singh C, Yashwant AK. Formulation and the Study of Finished Products used for Anginal Disease.
28. Faris TM, Harisa GI, Alanazi FK, Samy AM, Nasr FA. Developed simvastatin chitosan nanoparticles co-crosslinked with tripolyphosphate and chondroitin sulfate for ASGPR-mediated targeted HCC delivery with enhanced oral bioavailability. Saudi Pharmaceutical Journal. 2020 Dec 1;28(12):1851-67.