ORIGINAL ARTICLE

Formulation and Evaluation of Sonidegib Loaded Poly (Ethyl Methacrylate) Nanoparticles for Effective Treatment of Cancer

Aduri Prakash Reddy, Ramaiyan Velmurugan, Gaddam Suvarsha

Department of Pharmaceutics, School of Pharmaceutical Science, Vels Institute of Science, Technology and Advanced Studies, Chennai, Tamil Nadu, India

Abstract

Aim: The present research is aimed to develop and evaluate sonidegib loaded poly(ethyl methacrylate) nanoparticles (PEM-NPs) to improve its resistance toward pH and chemical conditions in exposed cancerous lesions. **Materials and Methods:** The polymer PEM is prepared from ethyl methacrylate (monomer) followed by designing 17 formulations of sonidegib loaded PEM-NPs using 3-factor, 3-level Box–Behnken design, and the results analyzed using Stat-Ease Design Expert® software V8.0.1. Three optimal batches (F1, F2, and F3) with comparable values of observed and predicted values are characterized for particle size, polydispersity index (PDI), zeta potential (ZP), entrapment efficiency, and percentage drug loading. The formulation (F3) with minimum particle size and maximum percentage conversion is further subjected to powder X-ray diffraction (PXRD), Fourier-transform infrared (FTIR), scanning electron microscopy (SEM) studies, drug release, and stability study. **Results and Discussion:** The particle size of sonidegib PEM-NPs (F1, F2, and F3) ranges between 191.5 ± 42.9 nm to 355 ± 39.7 nm and PDI 0.454 to 0.626. The ZPs are within the acceptable limits of −22.9 ± 2.48 mV–−24.7 ± 1.89 mV. The entrapment efficiency of the NPs ranges between 68.46 ± 0.37% and 70.24 ± 0.18% and percent drug loading between 20.62 ± 2.12% and 21.24 ± 1.72. The *in vitro* release study indicated an improvement in drug release of formulation F3 (95.878%) in comparison with the pure drug (2.86%). The optimized formulation F3 characterized for FTIR, PXRD, and SEM studies indicated molecular state dispersion of the drug with the polymers. The stability studies conducted for 90 days indicated that the developed formulation is stable. **Conclusion:** Sonidegib loaded PEM-NPs prepared using 3-factor, 3-level Box–Behnken design with increased solubility and stability.

Key words: Basal cell carcinoma, Box–Behnken design, poly(ethyl methacrylate) nanoparticles, sonidegib

INTRODUCTION

Cancer treatment using nanomaterials has made many advancements in the treatment of squamous cell carcinomas, such as non-melanoma skin cancer, esophageal cancer, and non-small cell lung cancer.[1] A combination of radiation therapy and chemotherapy are used in treating the serious threats of malignancy. The drug-entrapped nanoparticles (NP) can aid this process by controlling drug availability. In this NP delivery system, the cytotoxic drugs are either absorbed on the surface or encapsulated within the particle to reduce their interaction with non-cancerous cells hence lowering the effects. Most of the known anticancer drugs are hydrophobic in nature; hence, they exhibit low water solubility.[2-4]

Polymeric nanocarriers possessing hydrophobic shell dissolve the hydrophobic drugs for effective and safe formulations. Among various hydrophobic polymers, the biocompatible polyester poly(ethyl methacrylate) (PEM) is widely used for drug delivery due to its resistance towards chemical hydrolysis, achiral nature, and high permeability.[5]

Address for correspondence:
Aduri Prakash Reddy, School of Pharmaceutical Science, Vels Institute of Science, Technology and Advanced Studies, Chennai, Tamil Nadu, India. Mobile: +91-9705065656.
E-mail: aduri.prakash14@gmail.com

Received: 28-03-2020
Revised: 21-04-2020
Accepted: 26-04-2020

Sonidegib is used for the treatment of advanced basal cell carcinoma after recovering from surgery or radiation therapy.[6,7] Sonidegib, chemically known as N-[6-(cis-2,6-dimethylmorpholin-4-yl)pyridine-3-yl]-2-methyl-4'-(trifluoromethoxy) [l,-biphenyl]-3-carboxamide, possess low absorption rate of 6–7%. The low solubility of sonidegib is due to low and dose-dependent absorption. It is a weak base with a measured pKa value of 4.20 and exhibits relatively poor aqueous solubility.[8] The solubility of sonidegib is pH-dependent and is further reduced as pH increases.[9] Sonidegib has been challenging to formulate due to poor water solubility and wettability.

The current investigation is aimed to develop and evaluate sonidegib loaded PEM-NPs to improve its resistance toward pH and chemical conditions in exposed cancerous lesions. The polymer PEM is prepared from ethyl methacrylate (EMA) using sodium dodecyl sulfate (SDS) (surfactant) and potassium persulfate. The formulations of sonidegib loaded PEM-NPs prepared using 3-factor, 3-level Box–Behnken design.

MATERIALS AND METHODS

Materials

Pure standard drug of sonidegib (purity >98%) was a kind gift sample from Sun Pharmaceuticals Ltd., India. Commercially available monomer EMA (EMA, containing ≤30 ppm monomethyl ether hydroquinone as an inhibitor, 99%, Sigma-Aldrich) was used without any further treatment. The analytical-grade initiators are potassium persulfate (PPS or KPS, Water-Soluble, ≥99%, Sigma-Aldrich) and 2, 2- azobisisobutyronitrile (AIBN, Oil-Soluble, 98%, Sigma-Aldrich) and were used as received. The emulsifier (or surfactant) was reagent-grade SDS (SDS, 99%, Sigma-Aldrich).

Instruments

Chemical analysis conducted using the Fourier-transform infrared (FTIR) spectrophotometer (Shimadzu FTIR 8400S, Japan). Powder X-ray diffraction (PXRD) patterns performed on X-ray diffractometer (Bruker D8 Advance). The morphology of the finely ground particles was observed under scanning electron microscopy (JOEL SEM, Model 6400F, JEOL, Tokyo, Japan).

Preliminary solubility studies of sonidegib

Solubility of sonidegib is determined at 28±1°C. 100 mg of pure drug was dissolved in various aqueous systems like distilled water, phosphate buffer (pH 7.4) phosphate buffer (pH 6.8), acetate buffer of pH 4.5, 0.01N HCl, pH 2.0 and 0.1 N HCl, pH 1.0 solution and agitated for 12 hours in rotary shaker. These solutions were allowed to equilibrate for the next 24 h and then centrifuged for 5 min at 2000 rpm. The supernatant liquid of each vial was filtered through Whatman filter paper no. 41. Then, the filtrates were diluted with water to get 30 µg/ml concentrations and absorbance was measured at 276 nm. The content of drug against the solvent blank present in each filtrate was calculated from the standard calibration curve.

Preparation of PEM-NPs

Preparation of sonidegib loaded PEM NP involves two-step processes. The first step involves the preparation and optimization of PEM NP, as reported.[10] The later step involves the preparation of sonidegib loaded PEM NP by freeze-drying technique as reported.[11]

A mixture of SDS (surfactant), potassium persulfate (initiator), 1-pentanol, and deionized water was charged into a three-necked, 250-mL flask equipped with a magnetic stirrer, a reflux condenser, and a thermometer. When the temperature in the system reached a designated level, EMA (monomer) was continuously added in very small drops for about 90 min. After the completion of addition, the reaction system was then maintained at the reaction temperature for a certain aging time.

Characterization of PEM NP

Percent conversion measurement (Y1)

The percentage conversion of EMA was determined with the following equation:

$$\text{Conversion}(\%)=\frac{w_1-w_2}{w_3}\times 100$$

Where w_1 the weight of polymer is, w_2 is the total weight of KPS, SDS, and 1-pentanol, and w_3 is the weight of EMA.

Particle size measurement (Y2)

The mean particle size and the polydispersity (PD) were determined using dynamic light scattering device (Brookhaven Instruments Corporation) at the angle of 90°. The values obtained by this instrument are the hydrodynamic diameter (z-average diameter and effective diameter).

Preliminary experiments

Preliminary experiments conducted to optimize the process variables based on their effect on percent conversion and particle size. The reactions conducted by altering one parameter at a time while keeping other parameters constant.

The effects of SDS and cetyltrimethylammonium bromide (cationic), on the particle size of the resultant polymers, are investigated. The results indicate SDS reduced the particle size of poly ethyl (methacrylate), effectively hence used as the surfactant.

Selection of reaction temperature

The effect of temperature on particle size and percent conversion of the polymer was studied by conducting the experiments at varying temperatures. The results indicated that the particle size decreases with an increase in the reaction temperature from 65 to 85°C. The conversion rate was found to be increased with an increase in the reaction temperature from 67 to 79°C. When the reaction temperature is above 79°C, there is no significant effect of the reaction temperature on the conversion.

The effects of the aging time on the monomer conversion and the particle size were studied. Results indicated that the aging time between 30 and 90 min had a significant impact on particle size and percent conversion.

Table 1: The independent and dependent variables in Box–Behnken design

Independent variables			Levels		
Variable	**Name**	**Units**	**Low**	**Middle**	**High**
A	Amount of surfactant	g	0.5	1	1.5
B	Reaction temperature	°C	65	75	85
C	Aging time	min	30	60	90
Dependent variable				Goal	
Y1	Percent conversion	%		Maximize	
Y2	Particle size	Nm		Minimize	

Design of experiments (DOE)

DOE has been used as a powerful approach to reduce the variation in a process and, ultimately, to produce PEM NP with smaller particle size, and maximum percent conversion. Among various design approaches, the Box–Behnken design was used to optimize and evaluate main effects, interaction effects and quadratic effects of the process variables on the percent conversion and particle size.

According to Box–Behnken design, a total number of 17 experiments, including 12 factorial points at the midpoints of the edges of the process space and five replicates at the center point for estimation of pure error sum of squares, were performed to choose the best model among the linear, two-factor interaction model, and quadratic model due to the analysis of variance (ANOVA) F-value. The obtained $P < 0.05$ is considered statistically significant.

The study indicates that the amount of surfactant (A), reaction temperature (B), aging time (C) had a significant effect on the percent conversion (Y1) and particle size (Y2) of polymer NP. Therefore, by fixing the initiator amount and monomer amount and selected variables (A, B, C) studied at three different levels as low (−1), medium (0), and high (+1). The independent factors and the dependent variables used in the design [Table 1].

The obtained responses for the dependent variables were given in Table 2. On the basis of preliminary studies, factors such as amount of surfactant (0.5–1.5 g), reaction temperature (65–85°C), and aging time (30–90 min) were identified

Table 2: Box–Behnken experimental design and observed responses

Run	Factor A amount of surfactant	Factor B reaction temperature	Factor C aging time	Response Y1 percent conversion	Response Y2 particle size
1	1	65	30	82.94	226.18
2	1	65	90	84.72	234.24
3	1	75	60	93.86	212.62
4	1.5	75	30	89.18	202.46
5	1	75	60	94.12	213.23
6	1	75	60	93.78	212.94
7	1	85	30	95.74	223.32
8	0.5	85	60	92.46	225.32
9	1.5	65	60	83.48	184.82
10	1	85	90	97.34	226.72
11	0.5	65	60	79.54	246.32
12	1	75	60	94.28	214.12
13	1.5	75	90	90.42	208.42
14	0.5	75	90	87.16	252.62
15	1.5	85	60	96.13	196.24
16	1	75	60	93.62	213.46
17	0.5	75	30	85.98	247.82

as the process variables. The results analyzed using Stat-Ease Design Expert® software V8.0.1. Subsequently, three experiments were conducted for verifying the validity of the statistical experimental strategies.

The effect of the independent variables on each response parameters was visualized from the perturbation plots. In addition, two-dimensional contour plots were constructed using the output files generated by the Design-Expert software.[12-14]

Preparation of sonidegib loaded PEM NP

Accurately weighed quantity of PEM NP was suspended in 50 ml of Milli-Q water using a magnetic stirrer, then the excess amount of sonidegib was added until the free drug is precipitated, and the mixture was sonicated for 10 min and was kept for 24 h under stirring. The suspensions were centrifuged at 2000 rpm for 10 min to separate the free drug as a residue below the colloidal supernatant. The supernatant was freeze dried on a lyophilizer (LARK INDIA) at −20°C temperature and operating pressure 13.33 mbar. The dried powder was stored in a desiccator.

Characterization of sonidegib loaded PEM-NPs

Determination of sonidegib loading in NPs

A weighed amount of sonidegib loaded nanoformulation was diluted suitably and sonicated for 10 min, analyzed by ultraviolet (UV) spectrophotometer at 276 nm to determine the drug loading and entrapment efficiency using the following equations:

$$\text{Loading efficiency (\%)} = \frac{\text{Wt. of sonidegib in NPs (mg)}}{\text{Total wt. of NPs}} \times 100$$

$$\text{Entrapment efficiency} = \frac{\text{Wt. of sonidegib in NPs (mg)}}{\text{Wt. of total sonidegib (mg)}} \times 100$$

FTIR spectroscopy

FT-IR spectroscopy of sonidegib and its optimized formulations was performed using FTIR spectrophotometer (Shimadzu FTIR 8400S, Japan).

X-ray powder diffraction (XRPD) studies

XRPD patterns of sonidegib and optimized formulations were conducted on an X-ray diffractometer (Bruker D8 Advance) at a scan rate of 5 min in the 2θ range.

Measurement of particle size, PD index PDI, and zeta potential (ZP) of sonidegib loaded PEM-NPs

The particle size, PDI, and ZP of the PEM–NPs are measured using a Zetasizer (NanoZS90, Malvern, Worcestershire, UK). From the prepared nanodispersion, 100 mL was diluted to 5 mL with double-distilled water to get optimum kilo counts per second (Kcps) of 50–200 for measurements.

Scanning electron microscopy (SEM) studies

The morphology of PEM NP studied by scanning electron microscope (SEM, Hitachi, Tokyo, Japan). The drug-loaded PEM-NPs were suitably diluted with double distilled water (1 in 100) and a drop of NP formulation was placed on the sample holder and air dried. Then, the sample was observed at an accelerating voltage of 15,000 volts at various magnifications. Imaging was carried out in high vacuum.

In vitro drug release studies

1.5 mL of sonidegib PEM-NP dispersion was added to 1.5 mL of each of buffer (pH 7.4), buffered saline, and bovine fetal serum in triplicate and incubated at 37°C up to 10 days. After periodic intervals, the samples were centrifuged at 3000 rpm, the supernatant was discarded, and the sonidegib pellet dissolved in ethanol (3 mL) and analyzed by UV visible absorption measurement at 276 nm.

Drug release kinetics

The data obtained from the drug release study were fitted into various kinetic models such as zero order, first order, and Higuchi and Korsmeyer–Peppas model. The release data from the nanoformulation were determined by curve fitting method. Data obtained from *in vitro* release studies were fitted to various kinetic equations.

Table 3: Regression equations for the responses – percent conversion and particle size

Response	Regression equation
Y1	93.93+1.76 A+6.37 B+0.72 C–4.01 A^2–2.01 B^2–1.73 C^2
Y2	213.23–22.52 A–2.49 B+2.78 C+8.10 AB–1.16 BC+14.49 C^2

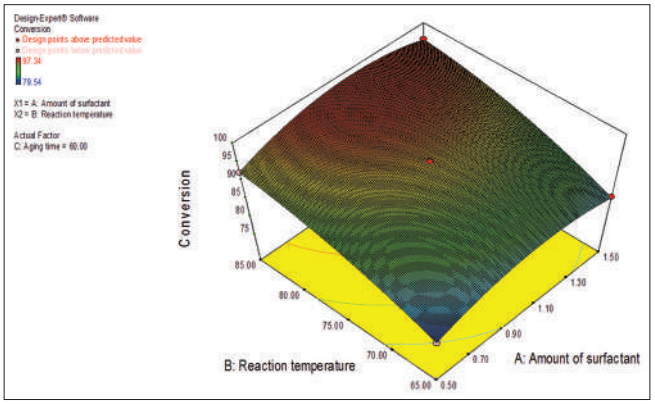

Figure 1: Response surface plot indicating the influence of the amount of surfactant and reaction temperature on percent conversion

Stability studies

Stability of sonidegib PEM-NPs suspension in screw-capped glass vials was evaluated over a time period of 90 days. Six samples were divided into two groups and stored at 25°C and 4°C. Drug leakage from NP and mean particle size was determined at the end of 1, 7, 15, 30, 45, 60, and 90 days.

RESULTS AND DISCUSSION

DOE

Seventeen experiments were performed based on the Box–Behnken design. The factor combinations yielded different responses as presented in Table 2. Data analyzed using Stat-Ease Design Expert® software V8.0.1 to obtain ANOVA, regression coefficients, and regression equation. Mathematical relationships were generated through multiple linear regression analysis for the mentioned variables [Table 3]. These equations represent the effect of drug quantity to the amount of surfactant (A), reaction temperature (B), and aging time (C) and their effect on percent conversion (Y1) and particle size (Y2). The coefficients of A, B, and C are related to the effect of these variables on the responses Y1 and Y2.

The percent conversion of the polymer was found to be in the range of 79.54–97.34. The mathematical model generated for particle conversion (Y1) was found to be significant with model F-value of 1296.02 implies the model is significant. There is only a 0.01% chance that a "Model F-value" this large could occur due to noise. Values of "Prob >F" <0.0500 indicate that model terms are significant. In this case, A, B, C, A^2, B^2, and C^2 are significant model terms. Values >0.1000 indicate that the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model. The "Pred R-Squared" of 0.9964 is in reasonable agreement with the "Adj R-Squared" of 0.9979. "Adeq Precision" measures the signal to noise ratio. A ratio >4 is desirable. The ratio of 112.115 indicates an adequate signal. This model can be used to navigate the design space. The "Lack of Fit F-value" of 0.73 implies the Lack of Fit is not significant relative to the pure error. There is a

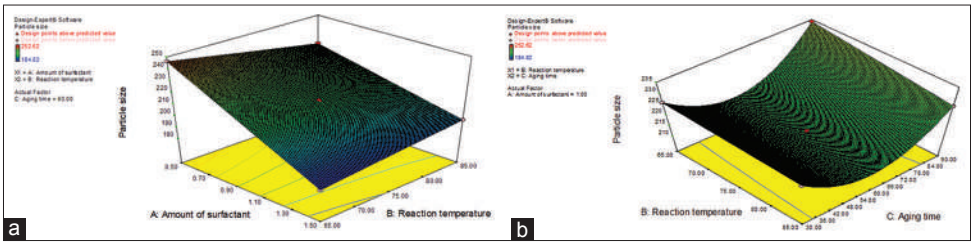

Figure 2: a. Response surface plot showing the influence of the amount of surfactant and reaction temperature on particle size at constant level of C b.Response surface plot showing the influence of reaction temperature and aging time on particle size at constant level of A

Table 4: Optimized values obtained by the constraints applies on Y1 and Y2

Independent variable	Nominal values	Predicted values		Observed values		
		Percent conversion (Y1)	Particle size (Y2)	Batch	Percent conversion (Y1)	Particle size (Y2)
Amount of surfactant (A)	1.4	97.09	199.11	1	96.82	204.2
				2	97.11	199.8
Reaction temperature (B)	85			3	96.23	208.3
Aging time (C)	60.5					

Table 5: The mean particle size, PDI, zeta potential, entrapment efficiency and % drug loading of sonidegib PEM-NPs

Batch	MPS±SD (nm)	PDI	ZP±SD (mV)	EE±SD (%)	DR±SD (%)
F1	355±39.7	0.626	−24.2±1.68	70.24±0.18	21.24±1.72
F2	344.9±41.6	0.475	−22.9±2.48	68.46±0.37	20.62±2.12
F3	191.5±42.9	0.454	−24.7±1.89	69.72±0.82	20.84±0.94

n=3 (*P*<0.05)

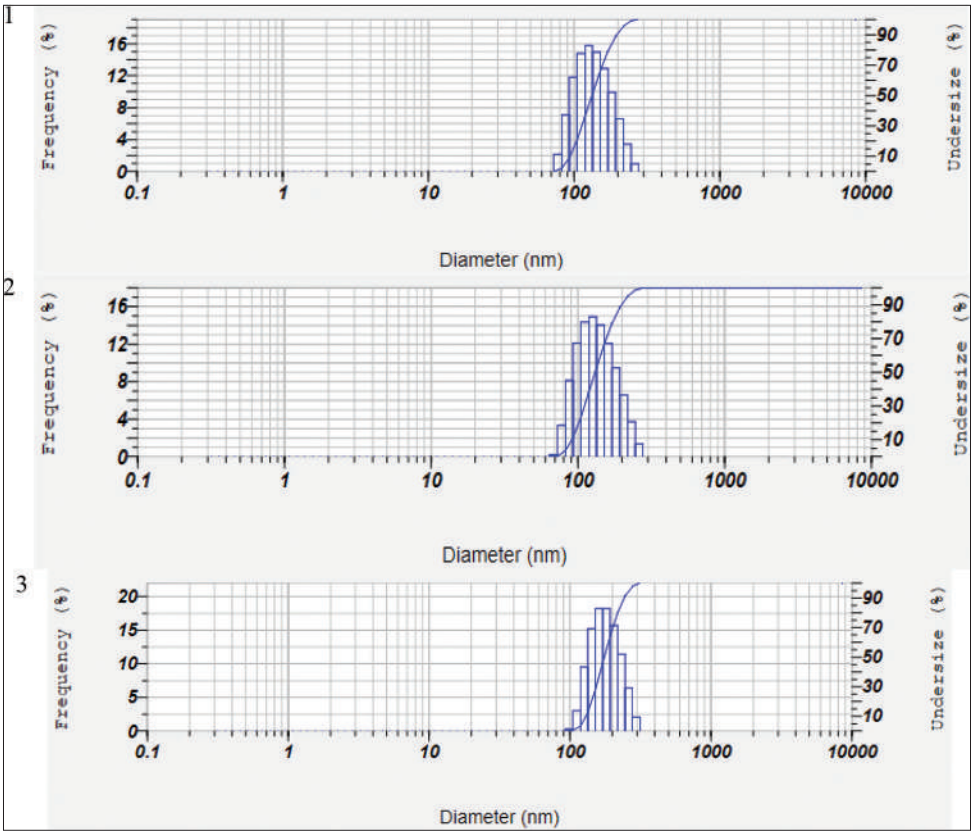

Figure 3: Particle size distribution of sonidegib poly(ethyl methacrylate) nanoparticles

65.06% chance that a "Lack of Fit F-value" this large could occur due to noise. Non-significant lack of fit is good – we want the model to fit. Results of the equation indicate that the effect of B is more significant than B and C. The influence of the main and interactive effects of independent variables on the particle size was further elucidated using the perturbation, three dimensional (3D) response surface plots, and the perturbation plot showing the main effect of A, B, and C on the percent conversion (Y1). Figure 1 clearly shows that B has the main and the major effect on Y1 followed by A and C which have a moderate effect on Y1. The relationship between the dependent and independent variables was further elucidated using 3D response surface plots and corresponding contour plots. At low levels of C (aging time), Y1 increases from 82.94% to 95.74%. Similarly, at high levels of C, Y1 increases from 84.72% to 97.34%.

The particle size of sonidegib PEM-NP lies in the range of 184.82 nm to 252.62 nm as shown in Table 2. The interaction between A and B on particle size at a fixed level of C is shown in Figure 2a. The interaction between B and C on particle size at a fixed level of A is shown in Figure 2b. At low levels of A, Y2 reduced from 252.62 nm to 225.32 nm. Similarly, at high levels of A, Y2 reduced from 208.42 nm to 184.82 nm. At low levels of B, Y2 reduced from 246.32 nm to 184.82 nm. Similarly at high levels of B, Y2 reduced from 226.72 nm to 196.24 nm. At low levels of C, Y2 reduced from 247.82 nm to 202.46 nm. Similarly at high levels of C, Y2 reduced from 252.62 nm to 208.42 nm.

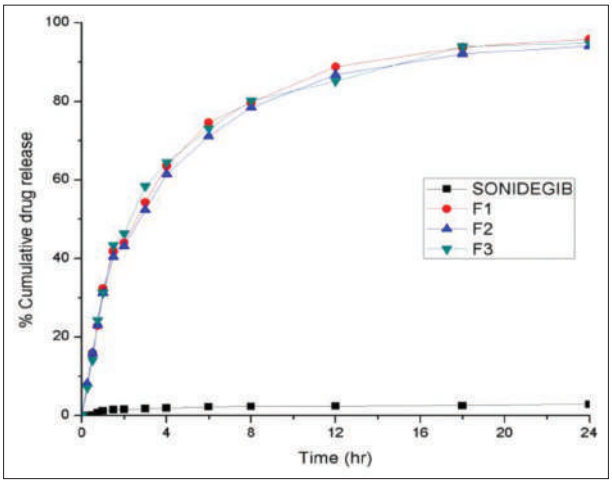

Figure 4: Dissolution profile of original sonidegib and sonidegib poly(ethyl methacrylate) nanoparticles

Optimization and confirmation experiments

The optimized levels and predicted values of Y1 and Y2 are shown in Table 4. Three batches of sonidegib PEM-NP prepared based to the predicted levels of A, B, and C. The predicted and observed values are shown in Table 4. Obtained

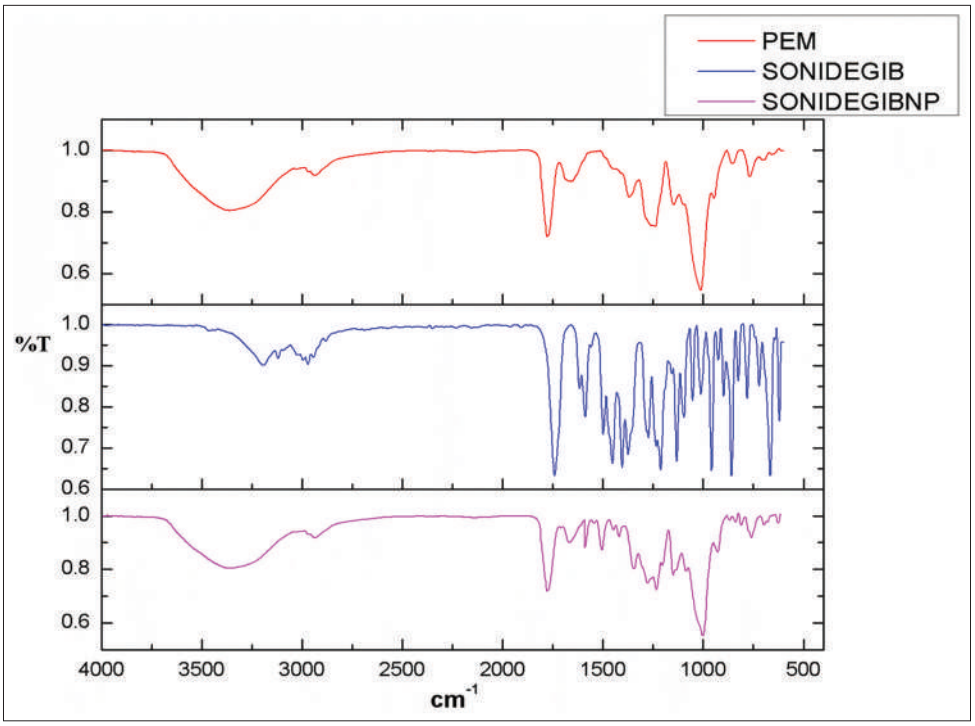

Figure 5: Fourier-transform infrared spectra of poly(ethyl methacrylate) nanoparticles, sonidegib and sonidegib poly(ethyl methacrylate) nanoparticles (F3)

Y1 and Y2 values were similar to the predicted values. All these batches characterized further.

Measurement of particle size, size distribution, and ZP

The particle size of sonidegib PEM-NPs was found to be in the range of 191.5 ± 42.9 nm–355 ± 39.7 nm [Figure 3 and Table 5]. The particle size of the drug-loaded NP was increased compared to the plain NP. The PDI was ranging from 0.454 to 0.626, indicating the wide range of size distribution. The nanoformulations exhibited negative surface charge with the inclusion of sonidegib which clearly suggested the orientation of sonidegib in the lipid matrix. The surface charge is a key factor for the stability of colloidal dispersion. In our case, the ZP values sonidegib PEM-NPs were within −22.9 ± 2.48 mV–−24.7 ± 1.89 mV. Therefore, it seems that the sonidegib NP may have short-term stability. The total encapsulation efficiency of the NP formulations was determined and found to be ranging from 68.46 ± 0.37% to 70.24 ± 0.18%. The percent drug loading was in the range from 20.62 ± 2.12% to 21.24 ± 1.72.

Drug release study

The dissolution profiles of plain sonidegib and sonidegib PME nanoformulation in the simulated gastric medium are as shown in Figure 4. The results indicate rapid and complete release of sonidegib from nanoformulation. From *in vitro* release, it was found that the nanoformulation showed an increase in the rate of release as compared with the pure drug.

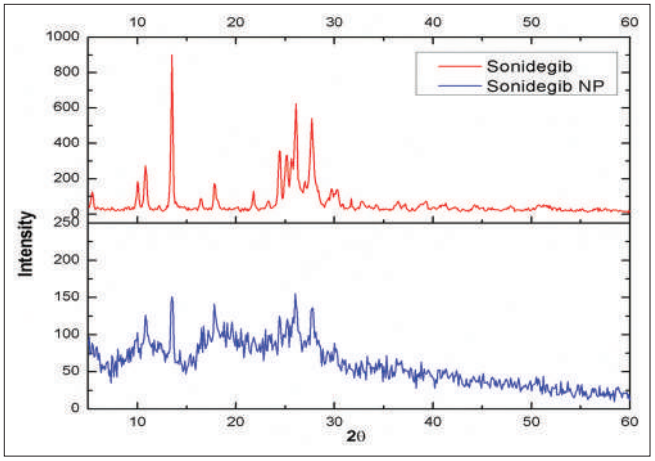

Figure 6: X-ray diffractogram of sonidegib pure drug and sonidegib poly(ethyl methacrylate) nanoparticles (F3)

The dissolution of pure sonidegib is <2% in 120 min, while the drug encapsulated in NP exhibited faster release. An average of 25–30% sonidegib was released within 60 min showing rapid burst release. The maximum release of sonidegib after 120 min from F3 was 46.334%. After the initial effect, the release rate was found to be slower from the nanoformulation. The slower and sustained release of sonidegib can be attributed to the diffusion of the sonidegib entrapped within the NP.

Drug release kinetics

Drug release data for the optimized PEM nanoformulation (F3) were fitted into various kinetic equations. These indicate

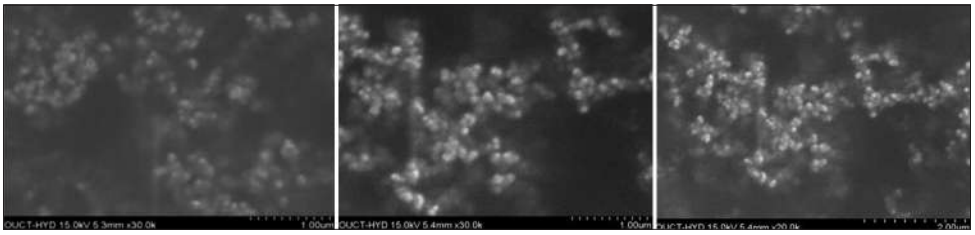

Figure 7: Scanning electron microscopy of sonidegib poly(ethyl methacrylate) nanoparticle (F3)

Table 6: Particle size and entrapment efficiency of sonidegib PEM-NPs after 90 days of storage at refrigerated and room temperature

Temperature (°C)	Particle size (nm)		Entrapment efficiency (%)		Release data (% CDR)			
	0 months	3 months	0 months	3 months	0 months		3 months	
					2 h	4 h	2 h	4 h
4±1	191.5±42.9	198.5±33.2	69.72±0.82	66.83±2.12	7.13±0.56	13.26±1.45	7.56±1.34	14.12±2.15
25±2	191.5±42.9	194.27±21.86	69.72±0.82	67.18±1.56	7.26±1.16	13.6±0.92	8.12±1.16	13.82±1.13

n=3 (P<0.05)

that sonidegib follows a non-Fickian (anomalous) with a strong correlation coefficient (R^2 = 0.94572) of Korsmeyer–Peppas model.

FTIR spectroscopy

The spectrum of sonidegib PEM-NPs revealed characteristic peaks of PEM at 1046 cm^{-1} and 840 cm^{-1}, 1723 cm^{-1} for acrylate carboxyl group, similarly bands were found at 2920 cm^{-1}, 1461 cm^{-1}, and 1024 cm^{-1} due to aliphatic C-H stretches. The mixed vibrations of CH3, aromatic C-C, and C-H of sonidegib were present in the loaded NP. The bands at 1383 cm^{-1}, 1233 cm^{-1}, and 962 cm^{-1} for bending of –OH of the two phenolics and an enolic group, respectively, indicate the presence of intact sonidegib in sonidegib loaded NP [Figure 5].

PXRD pattern

The PXRD of pure sonidegib indicated crystalline structure due to peaks observed at 10, 10.8, 13.5, 17.8, 24.4, 25.2, 26.1, and 27.7°. A reduction in crystallinity observed in the optimized formulation [Figure 6]. The diffraction spectrum of the nanoformulation vis-à-vis pure drug indicates the changes produced in the drug crystal structure. The sharp diffraction peaks associated with pure sonidegib are characteristic of its crystalline form. The absence of diffraction peaks in drug-loaded NP confirms that the sonidegib is present in amorphous form.

SEM studies

SEM studies carried out on F3 formulation indicate that the sonidegib PEM-NPs were spherical with uniform size containing small porous and rough surface [Figure 7]. The rough surface is result of the rapid moisture loss from the wet mass thus resulting in pours.

Stability studies

Table 6 indicates that no significant difference ($P < 0.05$) was found in entrapment efficiency and particle size of optimized formulation (F3) stored at refrigerated conditions and at room temperature.

CONCLUSION

This work demonstrated the use of a 3-factor, 3-level Box–Behnken design for optimizing the process variables in the preparation of sonidegib loaded PEM-NPs. The PEM prepared from SDS (surfactant), potassium persulfate (initiator), and 1-PEM (monomer). Seventeen formulations of sonidegib loaded PEM-NPs prepared as per the DOE by considering the effect of drug quantity to the amount of surfactant (A), reaction temperature (B), and aging time (C) and their effect on percent conversion (Y1) and particle size (Y2). Three batches F1, F2, and F3 sonidegib loaded PEM-NPs prepared under optimized conditions and characterized. The formulation F3n with particle size 191.5 ± 42.9 nm, PDI of 0.454, ZP of −24.7 ± 1.89 mV is chosen for further investigation. From *in vitro* release data, a significant improvement is observed in the rate of release of F3 when compared with the pure drug. The drug delivery followed coupled diffusion and erosion mechanism. The PXRD, FTIR, and SEM studies indicated the uniform stable distribution of the drug in its nanoformulations with spherical and porous structure. The stability study indicated that the formulation is stable for 3 months indicative of a stable sonidegib loaded PEM-NPs with increased solubility.

REFERENCES

1. Pan S, Wu X, Jiang J, Gao W, Wan Y, Cheng D, *et al*. Discovery of NVP-LDE225, a potent and selective smoothened antagonist. ACS Med Chem Lett 2010;1:130-4.
2. Bansal SS, Goel M, Aqil F, Vadhanam MV, Gupta RC. Advanced drug delivery systems of curcumin for cancer chemoprevention. Cancer Prev Res (Phila) 2011;4:1158-71.
3. Guo LY, Cai XF, Lee JJ, Kang SS, Shin EM, Zhou HY, *et al*. Comparison of suppressive effects of demethoxycurcumin and bisdemethoxycurcumin on expressions of inflammatory mediators *in vitro* and *in vivo*. Arch Pharm Res 2008;31:490-6.
4. Mukerjee A, Vishwanatha JK. Formulation, characterization and evaluation of curcumin-loaded PLGA nanospheres for cancer therapy. Anticancer Res 2009;29:3867-75.
5. Yao Y, Saw PE, Nie Y, Wong P, Jiang L, Ye X, *et al*. Multifunctional sharp pH-responsive nanoparticles for targeted drug delivery and effective breast cancer therapy. J Mater Chem B 2019;7:576-85.
6. Dreier J, Dummer R, Felderer L, Nägeli M, Gobbi S, Kunstfeld R. Emerging drugs and combination strategies for basal cell carcinoma. Expert Opin Emerg Drugs 2014;19:353-65.
7. Dipankar P. Development of hedgehog pathway inhibitors (HPI) in treatment of cancer. Curr Chem Biol 2014;8:132-48.
8. Einolf HJ, Zhou J, Won C, Wang L, Rebello S. A physiologically-based pharmacokinetic modeling approach to predict drug-drug interactions of sonidegib (lde225) with perpetrators of CYP3A in Cancer Patients. Drug Metab Dispos 2017;45:361-74.
9. Zhou J, Quinlan M, Glenn K, Boss H, Picard F, Castro H, *et al*. Effect of esomeprazole, a proton pump inhibitor on the pharmacokinetics of sonidegib in healthy volunteers. Br J Clin Pharmacol 2016;82:1022-9.
10. He G, Pan Q, Rempel GL. Synthesis of poly (methyl methacrylate) nanosize particles by differential microemulsion polymerization. Macromol Rapid Commun 2003;24:585-8.
11. Swaminathan S, Vavia P, Trotta F. Structural evidence of differential forms of nanosponges of beta-cyclodextrin and its effect on solubilization of a model drug. J Incl Phenom Macrocycl Chem 2013;76:201-11.
12. Journals H, Article R, Parameters P, Kakodka SS, Gajare P. Pharmaceutical quality-by-design (QbD): Basic principles. Int J Res Method 2015;1:1-19.
13. Roy RA. Primer on the Taguchi Method. New York: Van Nostrand Reinhold; 1990.
14. Myers RH, Montgomery DC. Response Surface Methodology: Product and Process Optimization Using Designed Experiments.2nd Edition, John Wiley & Sons, New York.

Source of Support: Nil. **Conflicts of Interest:** None declared.