Nat. Volatiles & Essent. Oils, 2021; 8(4): 16740-16750

NVEO
Natural Volatiles &
Essential Oils

Formulation And In Vivo Evaluation Of Sonidegib Loaded Poly Ethyl Methacrylate Nanoparticles For Effective Treatment Of Cancer

Aduri Prakash Reddy* , P. Shanmugasundaram , Gaddam Suvarsha

School of Pharmaceutical Science, Vels Institute of Science, Technology & Advanced studies (VISTAS), Velan nagar, Pallavaram, Chennai-600117, Tamil Nadu, Inida.

Abstract

The present research is aimed to develop and evaluate sonidegib loaded poly (ethyl methacrylate) nanoparticles (PEM-NPs) to improve its resistance towards pH and chemical conditions in exposed cancerous lesions. 17 formulations of sonidegib loaded PEM-NPs prepared using 3-factor Box–Behnken design analyzed at 3-levels. Three batches of sonidegib loaded PEM-NPs F1, F2, F3 were prepared based on predicted dependent variables and characterized for least particle size and maximum percentage conversion. The in vitro release study indicated an improvement in drug release of formulation F3 (95.878 %) than pure drug (2.86%). In vivo pharmacokinetic studies were conducted for optimized sonidegib PEM nanoparticles on rats, indicates that C_{max} of the nanoparticles (98.43±4.21ng/ml)was significant ($p<0.05$), T_{max} of both nanoparticle formulation and pure drug suspension was 4.00±0.03 and 6.00±0.01h, respectively. $AUC_{0-\infty}$ for nanoparticles formulation was higher (519.1±5.14ng.h/ml) than the pure drug suspension formulation (93.7±6.22 ng.h/ml), the bioavailability was more than 5 folds increased. These results marked that the proposed SLNs were effective in improving the bioavailability of sonidegib.

Keywords: Sonidegib, poly (ethyl methacrylate) nanoparticles, basal cell carcinoma, Box–Behnken design

Introduction

Cancer treatment using nanomaterial have made many advancement in treatment of squamous cell carcinoma (SCC), such as non-melanoma skin cancer, esophageal cancer and non-small cell lung cancer (Pramanik, 2014; Bansal et al., 2011) a combination of radiation therapy and chemotherapy are used in treating the serious threats of malignancy. The drug-entrapped nanoparticles can aid this process by controlling drug availability in these nanoparticle delivery system, the cytotoxic drugs is either absorbed on surface or encapsulated within the particle to reduce their interaction with non-cancerous cells hence lower the effects. Most of the known anticancer drugs are hydrophobic in nature; hence exhibit low water solubility (Guo et al., 2008; Mukherjee and Vishwanatha, 2009).

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

Sonidegib is a drug used for advanced basal cell carcinoma (BCC) post recovery from surgical cancer therapy (Dreier et al., 2014; Pramanik, 2014). Sonidegib, chemically known as N-[6-(cis-2,6-dimethylmorpholin-4-yl)pyridine-3-yl]-2-methyl-4'-(trifluoromethoxy) [l,-biphenyl]-3-carboxamide. The total absorption of Sonidegib is fewer (roughly 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 (Heidi et al., 2017). The solubility of sonidegib is pH-dependent and is further reduced as pH increases (Zhou et al., 2016).

Materials and Methods

Materials

Pure standard drug of sonidegib (purity>98%) was a kind gift sample from Sun Pharmaceuticals Ltd., India. Commercially available monomer ethyl methacrylate (EMA, containing ≤30ppm MEHQ as inhibitor, 99%, Sigma-Aldrich) was used without any further treatment. The analytical-grade initiators are potassium persulfate (PPS or KPS, Water-Ssoluble, ≥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 sodium dodecyl sulfate (SDS, 99%, Sigma-Aldrich).

Preparation of sonidegib loaded poly (ethyl methacrylate)

A mixture of sonidegib (200mg), sodium dodecyl sulfate (surfactant), potassium persulphate (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, ethyl methacrylate (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 (He et al., 2003).

Characterization of sonidegib loaded poly (ethyl methacrylate)

Particle size measurement (Y2)

The mean particle size and the polydispersity (PDI) were determined using dynamic Light scattering device (Brookhaven Instruments Corporation) at the angle of 90°, 20℃.The values obtained by this instrument is the hydrodynamic diameter (z-average diameter, effective diameter).

Percent conversion measurement (Y1)

The percentage conversion of ethyl methacrylate was determined with the following equation:

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

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 ethyl methacrylate.

Design of experiments

The Box-Behnken design (BBD) was used to optimize the formulation variables of sonidegib PEM-NPs containing 3 factors and evaluated at 3 levels. The study indicates that amount of surfactant (A), reaction temperature (B), aging time (C), had a substantial effect on responses percent conversion (Y1) and size of the particle (Y2) of polymer nanoparticles (Table 1).The experimentation designed by using DOE software (Stat-Ease Design Expert ® software V8.0.1) by employing one-way ANOVA test at 0.05 levels (Myers and Montgomery, 2002; Kakodka et al., 2015; Roy, 1990). The responses tabulated in Table 2.based on preliminary study data, amount of surfactant (0.5 – 1.5 gm), reaction temperature (65 - 85°C) and aging time (30 - 90 min) were identified as the process variables.

Table 1. The independent (factors) and dependent variables (responses)

	Factors		**Levels**		
		Units	Low	Middle	High
A	Amount of surfactant	gm	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	

Preparation of sonidegib loaded PEM nanoparticles

The dispersion of PEM diluted 10-fold, 2 mL of which transferred into a dialysis membrane (12 kD) to which sonidegib was gradually added till free drug precipitated. The percentage drug content was estimated by UVspectroscopy at 276 nm

Characterization of sonidegib loaded PEM-NPs

Zeta potential (ZP), particle size (PS), polydispersity index (PDI) of the formulated nanoparticles wereanalyzed by Nano ZS90Zetasizer (Malvern, Worcestershire, UK) (Baloch et al., 2019).

In vitro drug dissolution study

1.5 mL of sonidegib-PEM-nanoparticle 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 3,000 rpm, the supernatant was discarded and the sonidegib pellet dissolved in ethanol (3 mL) and analyzed by UV visible absorption measurement at 276 nm.

Pharmacokinetic study of sonidegib

Healthy wistar rats weighing between 150-180 g picked to perform experiment at temperature of 25^0C, 45% RH and 12h alternate cycle of light - dark .The animal room is aided with fresh air exchange and uninterrupted source of electricity and water (Venkateswarlu et al., 2004). Rats fed with standard diet and waterad libitum. The protocol was approved from the institutional animal ethics committee.

Study Design

The wistar rats were categorized into two groups that were offered with food post four hours of dosing. Group I administered orally with pure sonidegib and group II with formulated sonidegib PEM-NPs by oral route at a dose of 3.125mg as per animal body weight. 500 µL sample of blood collected from the femoral artery of the animals at varying time intervals of 0, 0.50, 1, 1.50, 2, 2.50, 3, 4, 5, 6, 8, 12, 16, 20, 24h post dose and mixed with heparin to check clotting. The plasma separated by centrifugation of samples at 5000 rpm and stored frozen at −20°C (Vijaykumar et al., 2016).

Pharmacokinetic analysis

The pharmacokinetic parameters of C_{max} (maximum plasma concentration), T_{max} (time to attain C_{max}), AUC_{0-t} (area under plasma concentration–time curve from zero to the last sampling time), $AUC_{0-\infty}$ (area under plasma concentration–time curve from zero to ∞) and $t_{1/2}$ were evaluated.

Results and Discussion

Design of experiments (DOE)

Seventeen experiments were performed based on the Box–Behnken design. The combination of factors resulted in varying responses as tabulated in Table 2. The percent conversion of the polymer was ranging between 79.54 to 97.34. The mathematical model of particle conversion (Y1) was significant with Model F-value of 1296.02 implying that significance of the model. The collaborating effect of A and B onthe percent conversion (Y1) at static level of C is shown in Figure1. At lower C levels (aging time), Y1 surges from82.94 % to 95.74 %. Similarly, at higher C, Y1 increases from 84.72 % to 97.34 %.

Particle size of sonidegib PEM-NP lies within 184.82 nm to 252.62 nm (Table 2). The interface of A and B on EE at a static level of C is represented in Fig. 3A. The interface of B and C on EE at a static level of A is represented in Fig. 3B. At lower A levels, Y2 a bridged from 252.62 nm to 225.32 nm, at higher levels, Y2 reduced from 208.42 nm to 184.82 nm. At lower values of B, Y2 reduced from 246.32 nm to 184.82 nm while at higher levels, Y2 reduced from 226.72 nm to 196.24 nm. At lower levels of C, Y2 abridged from 247.82 nm to 202.46 nm while at higher levels, Y2 abridged from 252.62 nm to 208.42 nm.

Sonidegib PEM nanoparticle prepared basedon predicted levels of factors. The predicted and observed values are shown in Table 3.Obtained response values were similar to that of the predicted values. All these batches characterized further.

Table 2. Box–Behnken experimental 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

Table 3. Values obtained by the constraints applies on Y1andY2.

Factors	Nominal values	Predicted			Observed	
		Percent conversion (Y1)	PS(Y2)	Batch	Percent conversion (Y1)	PS (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					

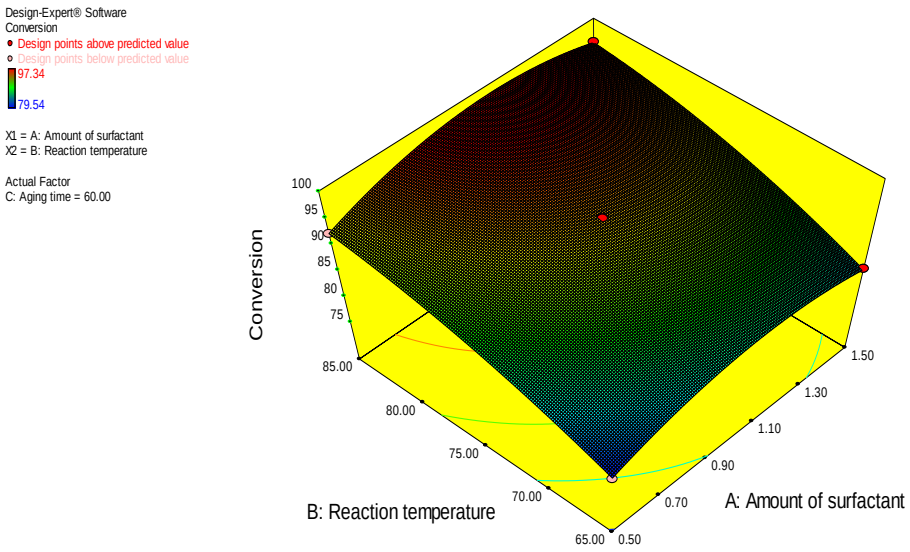

Figure 1. Response surface plot indicating the influence of amount of surfactant and reaction temperature on percent conversion

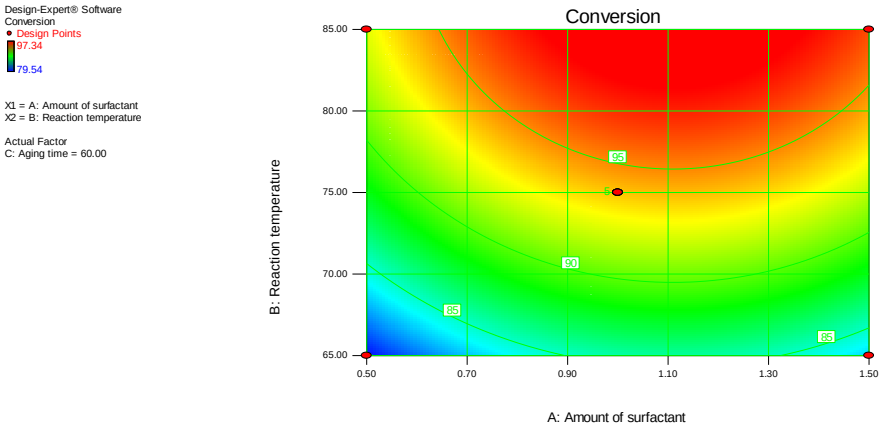

Figure 2: Contour surface plot demonstrating the influence of surfactant amount and temperature on percent conversion

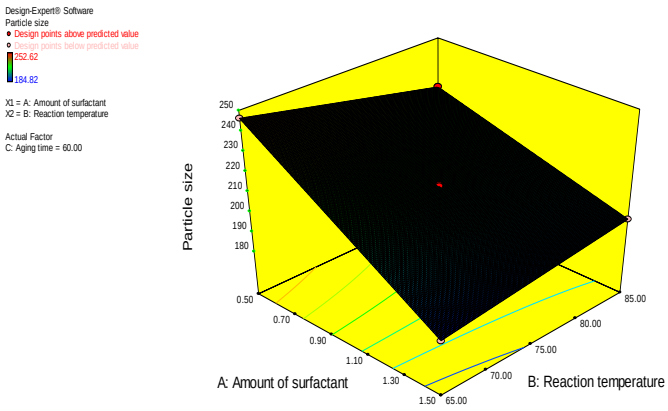

Figure 3A: Response surface plot demonstrating the influence of amount of surfactant and reaction temperature on PS at constant level of C

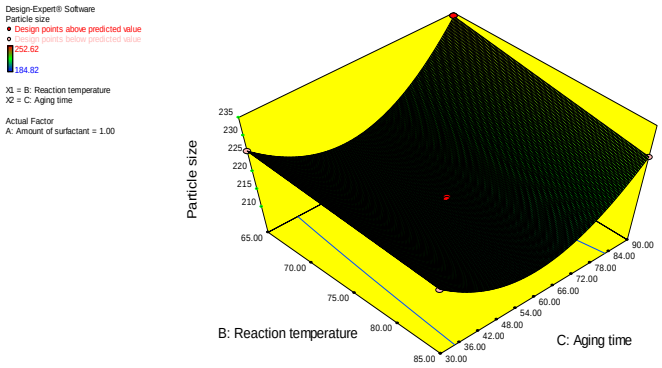

Figure 3 B. Response surface plot showing the influence of temperature and aging time on PS at constant level of A

Measurement of PS, PDI&ZP

The PS of sonidegib-PEM-NPs ranges between 191.5 ± 42.9 nm to 355 ± 39.7 nm (Table 4) indicating that drug loaded nanoparticleshave increasedparticle size compared to the plain nanoparticles. The PDI ranges between 0.454 to 0.626, indicating the wide range of size distribution. The nanoformulations exhibited –ve surface charge on inclusion of sonidegib suggesting the orientation of sonidegib in the lipid matrix. In current case, the ZP values of sonidegib-PEM-nanoparticles were within -22.9 ± 2.48 mV to -24.7 ± 1.89 mV. Therefore, it seems that the sonidegib nanoparticles may have a short-term stability. Total EE of the nanoparticles formulations range between 68.46 ± 0.37 % to 70.24 ± 0.18 %. The percent drug loading were in the range from 20.62 ± 2.12 % to 21.24 ± 1.72.

Table 4: The mean particle size, PDI, ZP, EE and % drug loading of optimized formulations

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

Drug release study

The dissolution profiles of plain sonidegib and sonidegib PME nanoformulation in simulated gastric medium. 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. The dissolution of pure sonidegib is not even 2% in 120 minutes, whereas the drug encapsulated in nanoparticles showed faster release. An average of 25–30 % sonidegib was released within 60 minutes showing rapid burst release. The maximum release of Sonidegib after 120 minutes 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 diffusion of the sonidegib entrapped within the nanoparticles. (Figure 4)

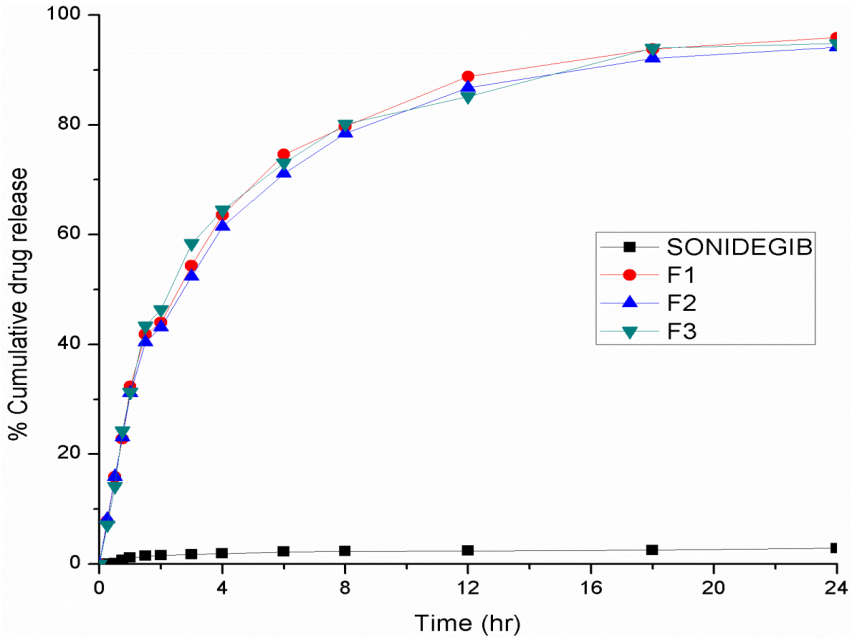

Figure 4. Dissolution profile of original sonidegib and sonidegib PEM-NP

In vivo drug bioavailability data

Pharmacokinetic parameters

Figure 5 demonstrate the plasma concentration–time graph in rats post first oral dose of sonidegib nanoparticles formulation. At any time points, the sonidegib concentrations in rat plasma treated with PMS-NPs formulation was significantly higher than pure drug (Table 5).

C_{max} of the nanoparticles 98.43±4.21ng/ml was significant ($p<0.05$) as compared to the pure drug suspension formulation 16.15±1.56ng/ml. T_{max} of both nanoparticles formulation and pure drug suspension was 4.00±0.03and 6.00±0.01h, respectively. $AUC_{0-\infty}$ infinity for nanoparticles formulation was higher (519.1±5.14ng.h/ml) than the pure drug suspension formulation 93.7±6.22ng.h/ml. The AUC_{0-t} of the nanoparticles formulation was significantly higher ($p<0.05$) than pure drug suspension formulation. The sonidegib PEM-NPs formulation bioavailability was higher and faster than that of the pure drug.

Table 5. Pharmacokinetic Parameters

Parameters	Sonidegib Pure drug	Sonidegib PEM-NPs
C_{max} (ng/ml)	16.15±1.56	98.43±4.21
AUC_{0-t} (ng. h/ml)	82.2±3.62	434.4±4.61
AUC_{0-inf} (ng. h/ml)	93.7±6.22	519.1±5.14
T_{max}(h)	6.00±0.01	4.00±0.03
$t_{1/2}$ (h)	8.02±0.02	6.02±0.02

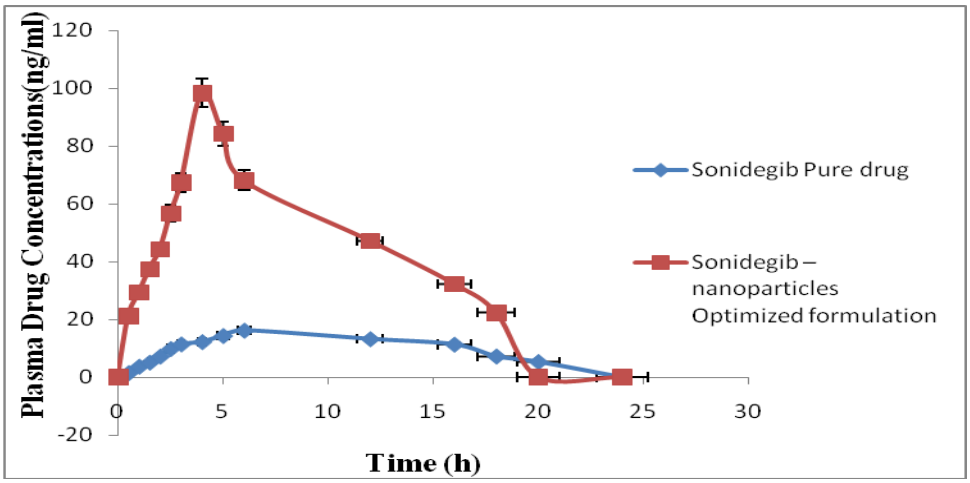

Figure 5. Plasma concentration profile sonidegib pure drug and optimized Formulations.

Conclusion

This work validated the use of Box–Behnken design, regression analysis and contour plots for optimizing the process variables during formulation of sonidegib loaded PEM-NPs. Seventeen formulations of sonidegib loaded PEM-NPs prepared and characterized. Based on design of experiment three batches F1, F2 and F3 sonidegib loaded PEM-NPs prepared and characterized. The percent conversion of the

polymer ranges between 79.54 to 97.34 with particle size ranges 184.82 nm to 252.62 nm. The particle size of the drug loaded NPs are considerably higherthan unadorned nanoparticles. The PDI ranging from 0.454 to 0.626 is indicative of wide range of size distribution. Total entrapment efficiency found to be ranging from 68.46 ± 0.37 % to 70.24 ± 0.18 % and the precent drug loading range from 20.62 ± 2.12 % to 21.24 ± 1.72%. From in vitro release data an significant improvement is observed in the rate of release of F3 when compared with the pure drug. In vivo pharmacokinetic studies were conducted for optimized sonidegib PEM nanoparticles on rats, indicates that C_{max} of the nanoparticles (98.43±4.21ng/ml) was significant ($p<0.05$), T_{max} of both nanoparticle formulation and pure drug suspension was 4.00±0.03 and 6.00±0.01h, respectively. $AUC_{0-\infty}$ for nanoparticles formulation was higher (519.1±5.14ng.h/ml) than the pure drug suspension formulation (93.7±6.22 ng.h/ml), the bioavailability was more than 5 folds increased. The sonidegib PEM-NPs formulation bioavailability was higher and faster than that of the pure drug. These results marked that the proposed sonidegib loaded PEM-NPs were effective in improving the bioavailability of sonidegib.

References

Baloch, Sohail., Sarwar., Kiani., Khan., Jahan., Shahnaz. (2019). Self-Nanoemulsifying Drug Delivery System (SNEDDS) for Improved Oral Bioavailability of Chlorpromazine: In Vitro and In Vivo Evaluation. Medicina, 55(5), 210.

Bansal, S.S., Goel, M., Aqil, F., Vadhanam, M.V., Gupta, R.C. (2011). Advanced drug delivery systems of curcumin for cancer chemoprevention. Cancer Prev Res (Phila). ;4(8), 1158–1171.

Dreier, J., Dummer, R., Felderer, L., Nägeli, M., Gobbi, S., Kunstfeld, R. (2014). Emerging drugs and combination strategies for basal cell carcinoma. Expert OpinEmerg Drugs, 19, 353–365.

Guo, L.Y., Cai, X.F., Lee, J.J. (2008). Comparison of suppressive effects of demethoxycurcumin and bisdemethoxycurcumin on expressions of inflammatory mediators in vitro and in vivo. Arch Pharm Res, 31(4), 490–496.

He, G., Pan, Q., Rempel, G. L. (2003). Synthesis of Poly(methyl methacrylate) Nanosize Particles by Differential Microemulsion Polymerization. Macromol Rapid Commun, 24(9), 585–588.

Heidi, J., Einolf, Jocelyn, Zhou., Christina, Won., Lai, Wang,, Sam, Rebello. (2017). A Physiologically-Based Pharmacokinetic Modeling Approach To Predict Drug–Drug Interactions of Sonidegib (LDE225) with Perpetrators of CYP3A in Cancer Patients. Drug Metab Dispos, 45, 361–374.

Kakodka, S.S.S., Gajare, P. (2015). Pharmaceutical Quality-by-Design (QbD): basic principles. Int J Res, 1(1), 1–19,24, 25.

Mukherjee, A., Vishwanatha, J.K. (2009). Formulation, characterization and evaluation of curcumin-loaded PLGA nanospheres for cancer therapy. Anticancer Res, 29(10), 3867–3876.

Myers, R.H., Montgomery, D.C. (2002). Response Surface Methodology: Product and Process Optimization Using Designed Experiments. 2nd Edition, New York ,John Wiley & Sons.

Pramanik, D. (2014). Development of hedgehog pathway inhibitors (HPI) in treatment of cancer. Curr Chem Biol, 8, 132–148.

Roy, R.A. (1990). Primer on the Taguchi Method, New York: Van Nostrand Reinhold.

Venkateswarlu, V., Manjunath, K. (2004). Preparation, characterization and in-vitro release kinetics of clozapine solid lipid nanoparticles. J Control Release, 95(3),627–638.

Vijaykumar, N., Zhijun, W., Guru, V.B. (2016). Pharmacokinetic Evaluation of Improved Oral Bioavailability of Valsartan: Proliposomes versus Self-nanoemulsifying drug delivery systems. AAPS Pharm Sci Tech, 17(4), 851-862.

Zhou, J., Quinlan, M., Glenn, K. (2016). Effect of esomeprazole, a proton pump inhibitor on the pharmacokinetics of Sonidegib in healthy volunteers. Br J Clin Pharmacol. 82(4), 1022–1029.