RESEARCH ARTICLE

Preparation and *In-vitro* Evaluation of Pemigatinib Nanosponges Tablets by Box-Behnken Design

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ABSTRACT

Objective: Pemigatinib depicted antitumor action in complex and metastatic tumors and it's a hydrophobic compound having strong pH-reliant solubility. The current work was designed to improve oral solubility of pemigatinib by incorporating into nanosponges (NSs).

Methods: Box-Behnken Design optimized the independent parameters of polystyrene NSs formation. Polystyrene NSs were prepared by ultrasound-assisted method using diaryl carbonate as cross-linking agent, which were later characterized and formulated into tablets. Tablets got screened for the respective quality attributes.

Results: A number of tests were carried out from the test runs generated by a three-factor, three-level BBD. The range of mean PS was 153 to 316 nm, the range for encapsulation efficiency% was 68.2 to 91.4%, and the value for PDI was 0.273 to 0.445. ZP for the finalized formula was determined to be -29.1 mV. The drug and excipients were compatible as confirmed by FTIR studies. SEM study confirmed that pemigatinib has successfully entrapped in interior of the polymer. *In-vitro* examination of the pemigatinib loaded NSs tablets were compared with a marked product and satisfactory results were obtained (98.74 ± 2.65% vs. 93.73 ± 1.06%). The prepared formulations were stable during 6 month stability study period.

Conclusion: The study findings for pemigatinib NS tablets demonstrated quick dissolution since the changed solubility qualities of the drug, satisfying the intended objective of increased absorption. Formulated pemigatinib-loaded NSs can be beneficial in the treatment of cancers

Keywords: Nanosponges, Pemigatinib, Cancer, Box–Behnken Design, Tablets

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INTRODUCTION

Biliary tract cancers (BTC)/cholangiocarcinoma are a type of epithelial cancer that is distinguished by aggressive and chemoresistant tumors with poor long-term survival.[1] Surgery is the sole curable therapy. Still, only 35% of individuals are eligible for it.[2]The existence of vascular involvement and cancer spreading to regional lymph nodes, which are frequently visible at the time of diagnosis given the asymptomatic nature of early disease, are frequently limitations of surgical resection.

Advances in gene sequencing have emphasized BTC's inherited landscape in recent years, demonstrating the molecular patterns partition with anatomical location. Numerous medicines are being produced in this therapeutic setting to target FGFR inhibition. Pemigatinib was among the first agents to receive FDA clearance in April 2020. Pemigatinib belongs to the BCS class-II molecule with BCS class I characteristics in acidic medium. It is a highly strong and specific FGFR1, FGFR2, and FGFR3 antagonist. Water solubilization is around 0.144 mg/mL, log P of 2.26. Diprotic basic molecule having pKa readings of 3.1 & 5.7, respectively. This has pH-sensitive soluble (1.2 – 7.4), which declines as pH increases.[3] The main fundamental factor influencing medicine oral absorption is the drug substance's poor solubility.[4] It is critical to develop an alternate pemigatinib formulation with enhanced properties for enhancing inherent solubilization & lower substantial pharmacokinetic inconsistency found in current tablet formula.

Various traditional techniques, like complexation, co-solvency, salt production, micronization, and the application of permeation boosters, would increase bioavailability.[5] But these techniques showed little success for medication liberation. Nano-based delivery systems for drugs (NBDDS) show significant potential among the various ways for boosting the absorption and uptake of insoluble medicines.[6] Because of the possible benefits such as boosting solubility of substances, strengthening permeability, promoting drug

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stability, regulating the distribution of drugs and elimination, and tailoring drug delivery to particular areas, NBDDS have inspired a lot of studies in recent years. Numerous NBDDS have been produced, including nanocrystals, nanoemulsions, nanosponges (NSs), nanobubbles, liposomes, micelles, nanoparticles, & inorganic nanocarriers.[7,8]

Many studies have shown that NS dosage forms can amplify solubility and thus oral availability of weakly soluble medicines.[9] They shield molecular compounds from degradation and these have high selectivity, biocompatibility, degradability, and extended release behavior which are used in cancer therapy.[10] Polymer is created by combining it in situ with cross-linkers. The ultimate product is circularly formed particles having cavities that can store medicinal molecules.[11] Polyester dissolves slowly in body since it is biodegradable. As it degrades, its drug payload is delivered in a predictable manner. These may be scattered in a framework of ingredients, appropriate for oral intake, and the primary benefits of using such capsules/tablets are a lower dose, retention of form, minimised toxicity, and greater compliance among patients through extended release.[12]

Utilizing Design of Experiments is an innovative advance in optimizing and transmitting experimental factors. Simple experimental plans and statistical tools for information analysis can offer a huge advantage regarding the system under examination after a small number of experiments.[13] A statistical technique called response surface methodology is utilized for DoE and the construction of experimental models that link several interacting components.[14] Box-Behnken designs (BBD) most often utilized in response surface modeling.[15]

The research aimed to improve oral solubilization of pemigatinib during incorporation into NSs. The study included optimization of the parameters for preparation of pemigatinib loaded NSs, characterization and evaluation of pemigatinib loaded NSs tablets.

MATERIALS AND METHODS

Materials

Pemigatinib from Aelida Pharmaceuticals, Haryana, India. Hyper cross linked Polystyrenes was from Gangwal Chemicals Pvt. Ltd. Mumbai, India. Diaryl carbonate was obtained from Euclid Pharmaceuticals Limited, Mumbai. Dimethyl formamide, ethanol, and methanol were from Qualigens, Thermo Fisher Scientific India Ltd, Mumbai.

Preparation of Polystyrenes NSs

Polystyrenes-based NSs were made using diaryl carbonate for cross linking.[16,17] Five different NSs were generated by different molar ratios of the reactants. In 250 mL flask was used to which the amount of polystyrenes was added in dimethyl formamide. Diaryl carbonate was incorporated into the aforementioned reaction substance and refluxed for 6 hours in a bath of oil at 9°C with stirring. Following the finish of reaction, end result was washed by means of water and purified using Soxhlet extraction using ethanol for 6 hours, resulting powder (white) got dried nightly by a 60°C oven before being pulverised using a mortar. Obtained powder then re-dispersed with water. Lyophilization was used to recover the colloidal component which remained embedded in water (Lark Innovative Fine Technologies Lyophiliser, India). The obtained NSs were termed as PNS1, PNS2, PNS3, PNS4, PNS5 and PNS6 with respective to the molar concentrations.

Fabrication of Pemigatinib-loaded Polystyrenes NSs

The (Ultrasound aided Synthesis) lyophilization procedure was used to prepare pemigatinib-loaded NSs.[18] Employing an automated stirrer (RQ 121-D, Remi India), NSs (400 mg) were dissolved using Milli Q water (100 mL). To prevent aggregation, 13.5 mg of pemigatinib has been added to the aforesaid mixture and sonicated (ENUP 750, Remi India) for 20 minutes. The mixture was then continuously stirred for the required time period. The suspensions were spun in a centrifuge (Micro III, Remi India) at 2000 rpm for 20 minutes to remove the uncomplexed medication. The colloidal effluent was filtered and freeze-dried in a lyophilizer at -20°C at 13.33 mbar pressures. Following lyophilization, dry powder got kept in a desiccator.[19]

Experimental Design

BBD

The main impacts, combination impacts and quadratic impact of formulation constituents on effectiveness of NSs were investigated and optimized using a three-factor, three-level BBD.[20] This method is appropriate for investigating response surfaces (quadratic) and building 2nd-order models.[21] Table 2 displays 15 randomized tests for independently chosen attributes, containing three separate trials at the center point produced by a 3-attributes, 3-level BBD, and related response. This study used three different replicates at its center to obtain a more consistent approximation of the projection variance across the whole design space.[22,23] The medication concentration was maintained steady. A total of three categories of uncorrelated formulation-related factors (molar ratio; polymer:cross-linker, stirring-speed, and stirring-time) were determined based on border of the NSs domain: low (coded 1), intermediate (coded 0), and high (coded + 1).[24] For NSs, series for the attribute was chosen as follows molar ratio (polymer:cross-linker; X1) was 0.4 to 0.8, the stirring speed (X2) was 2000 to 4000 rpm, and

Table 1: Dependent & independent parameters in BBD

Independent Parameters			*Level*		
Parameter	*Name*	*Unit*	*Low (-1)*	*Middle (0)*	*High (+1)*
X1	Molar ratio (polymer:cross-linker)		0.4	0.6	0.8
X2	Stirring Speed	rpm	2000	3000	4000
X3	Stirring time	mins	200	400	600
Dependent Parameters			Goal		
Y1	Average Particle size	nm	Decrease		
Y2	Entrapment Efficiency	%	Increase		
Y3	Polydispersity Index		Increase		

the stirring time (X3) was 200 to 600 minutes. The important responses were found (Table 1). These criteria included average particle size (PS; Y1), entrapment efficiency (EE; Y2), and polydispersity index (PDI; Y3).

The BBD NSs were constructed with Design Expert® software (Version7.0, Stat-Ease Inc., Silicon Valley, CA, USA), and data acquired with same software got assessed. Design Expert software fitted all responses to a 2nd order nonlinear model. The following mathematical framework can approach 2nd order nonlinear or polynomial equation.

Y is calculated response level, 0 is intercept, 1–9 were regression coefficients, X1, X2, and X3 are key effects, X1X2, X2X3, & X1X3 are interactions among key effects, and X12, X22, & X32 are quadratic values of the attributes which are independently applied for modelling curving of designed space. Data was fitted to the quadratic model using a backward elimination approach. ANOVA, lack-of-fit, and multivariate correlation coefficient (R2) checks offered were used to validate the model's appropriateness. The coefficient values highlighted effect of factors that were independent and their interface with dependent attributes. + ve coefficient denotes an additive impact, whereas a -ve coefficient denotes an opposite effect. ANOVA examined the impact of individual coefficients, and dimensional graphs were created using quadratic models derived through the regression study, with the response factor Y portrayed as an angled curve as a function of X. Perturbation plots and 3D contour graphs visualized the impact of independent attributes on response parameters. A desirability function was used to perform additional optimization.

Optimization

By imposing constraints on response characteristics and influencing factors, the best locations for variables that are independent were found using a numerical optimization technique. NSs composition was synthesized in triplicate under ideal conditions to validate the validity optimization technique.

Characterization of Prepared Pemigatinib NSs

PS, PDI and ZP

The dynamic light scattering technology was applied to examine PS arrangement of pemigatinib NSs. For all samples, readings were taken at an angle of 90°. Before measuring, the samples got diluted using Milli Q water. Particles' average hydrodynamic diameter (Dh) & PDI were estimated employing culminated analysis after averaging three data. Using an additional electrode, ZP measurements were also performed with the same equipment (Mastersizer 2000, Malvern Instruments Ltd, Worcestershire, UK). All experiments were carried out in triple at 25 ± 2°C.[25]

EE%

EE is the quantity of drug captured in a carrier system divided by the entire drug added. Ratio of drug to total carrier system weight is known as drug loading. A weighed quantities of pemigatinib NSs intricate was then immersed (methanol), then sonicated about 10 minutes to rupture complex, diluted appropriately, and then tested at 290 nm using a UV spectrophotometer (Labindia UV- 3000 +, Labindia instruments Pvt. Ltd.) to calculate concentration of Pemigatinib available in the formulation.[26] Equation used to compute the EE was.

Scanning Electron Microscopy (SEM)

SEM used for examining microstructure of conventional NSs and pemigatinib-loaded NSs. A single drop of NSs mixture

$$Encapsulation\ efficiency(\%) = \frac{Amount\ of drug\ after\ filtration}{Total\ amount\ of drug\ in\ the\ sample} X\ 100$$

Table 2: Composition of pemigatinib NSs formulation by BBD

	Variables 1	*Variables 2*	*Variables 3*	*Response 1*	*Response 2*	*Response 3*
F.No	*X1:Molar Ratio (polymer: cross linker) mg*	*X2: Stirring- Speed (RPM)*	*X3: Stirring- Time (Min)*	*Y1: Mean Particle size (PS)*	*Y2: Encapsulation efficiency (%)*	*Y3: PDI*
PF1	0.4	2000	200	316	68.2	0.273
PF2	0.8	2000	200	205	84.7	0.389
PF3	0.2	4000	400	282	75.6	0.275
PF4	0.6	4000	600	256	81.9	0.417
PF5	0.4	3000	200	305	74.6	0.324
PF6	0.8	3000	200	212	88.3	0.422
PF7	0.4	3000	600	279	72.9	0.335
PF8	0.8	3000	600	203	87.7	0.432
PF9	0.6	2000	200	255	79.5	0.418
PF10	0.6	4000	200	261	80.3	0.405
PF11	0.4	2000	600	272	76.5	0.281
PF12	0.8	4000	400	153	91.4	0.445
PF13	0.6	3000	400	289	83.2	0.399
PF14	0.6	3000	400	278	79.9	0.412
PF15	0.4	3000	400	294	75.1	0.285

was applied over a film-embedded copper grid, pigmented using 2% (w/v) phosphotungstic acid solution, and permitted to air-dry for contrast enhancement.[27] Transmission electron microscopy (TEM; JEM-2000 EXII; JEOL, Tokyo, Japan) investigated samples at a magnification of 45000.

Fourier Transformed Infrared (FTIR) Spectroscopy

FTIR spectra of polystyrene, simple NSs, pemigatinib, physical blend and Pemigatinib-NSs were depicted by potassium bromide disc process using Tensor 27 FTIR Spectrophotometer in region of 4000–600 cm^{-1} (Tensor 27, Bruker Optics, Germany).[28]

Preparation of Pemigatinib Loaded NSs Tablets

The oral formulation of pemigatinib loaded NSs were prepared by wet granulation method. The binding agents gelatin and the polymer hydroxypropyl methyl cellulose (HPMC K100M; 100 mg) were used to prolong the drug release up to 24 hours. An exactly quantity of Pemigatinib NSs analogous to 13.5 mg pemigatinib was mixed with required quantity of lactose monohydrate for 200 mg weight. Then granules were prepared using different binder solutions. After granulation and drying, magnesium stearate was mixed for more 2 minutes. Then 25 mm diameter round tablets were made by a mono punch tablet equipment (Tablet Compression Machine - Single Punch, Harrisons Pharma Machinery Private Limited) with plane faced mono punch.[29]

Evaluation

Uniformity of weight

Tables 2 got chosen at hit and miss, weighed separately in an electronic balance, and middling weight determined. Weight uniformity got determined using British Pharmacopoeia requirements (BP 2013).

Drug content

Pemigatinib percentage of the produced tablets was determined using the previously described method. To extract the medication from the NSs, processed tablets got treated using ethanol. Diluted sample got used & a UV spectrophotometer30 determined content.

Hardness test

Produced tablets' hardness got tested by a hardness analyzer (Monsanto). Tablets (triplicate) were chosen and the results got reported in kg/cm^2.

Friability test

Tablets got exposed to effect of attrition and distress in electronic tablet friability analyzer equipment in a plastic compartment that circles at 25 rpm by lowering tablets at an elevation of 6 inch with single turn. Early quantified 20 samples be set in friability compartment and turned 100 times. At the end of the movement, tablets were gently brushed to remove any clinging powder and reweighed. Pills would lose ≤0.5 to 1.0% of original weight, which was acceptable in most cases.[31] Friability% is specified by the formula:

$$\%\ Friability = \frac{W1 - W2}{W1} \times 100$$

W0 is weight prior test and W is weight post test.

In-vitro release study of pemigatinib NSs

The dialysis method was used to determine drug release; 2 ml of every formula (test and control) was kept into dialysis bags and kept in 25 mL phosphate buffer (pH 7.4) & swirled (100 rpm at room temperature). 2 mL buffer got obtained at predefined intervals and replaced using new buffer. Finally, Pemigatinib released in phosphate buffer was calculated using a spectrophotometer set to 290 nm. Using a UV-visible spectrophotometer, aliquots were analyzed at an equal period for the drug released at a maximum of 290 nm, retaining buffer pH 7.4 as a blank, and quantity of released drug got estimated.[32]

In-vitro release of pemigatinib loaded tablets

The type II USP dissolution device was used to perform drug release of pemigatinib tablets and drug. The medium used for dissolution was 900 mL 0.1 N HCl for initial 2 hours, then replacement using phosphate buffer pH 6.8 at 50 rpm and 370.5°C. The samples were collected at 0, 1, 2, 4, 8, 12, 16, 20, and 24 hours intervals. An equal amount of fresh dissolving medium was immediately replaced and kept at similar temperature. Samples were appropriately dilated before being examined with a UV-spectrophotometer at 290 nm. Dissolution studies were carried out and the findings were compared to the commercial product.[33]

Drug release kinetics

Confirmation of method of drug liberation and its approach from *in-vitro* release test got conducted by integrating into kinetic models (zero order, 1st order, Higuchi's & Korsemeyer Peppas model) (Table 3). Pemigatinib release through NSs formulation was understood with curve fitting technique.[34] Results through *in-vitro* release test were verified with different kinetic formulas.[35]

Stability studies

Batches of Pemigatinib-NSs underwent immutability investigations in harmony with the ICH stability protocol standards. 40°C ± 2°C and 75 ± 5% RH were tested for 6

Table 3: Kinetic equations

Zero order Model	*First order Model*	*Higuchi model*	*Korsmeyer–Peppas Model*
$Q_t = Q_0 + K_0t$	$l\ n(Q_\infty - Q_t) = lnQ_0 + Kt$	$Q_t = k_H\ t(1/2)$	$Q_t/Q_\infty = K_K\ t^n$
Qt - Quantity of drug dissolved in time t, Q0- Originalquantity of drug K0- Zero order release constant.	Qt - Quantity of drug dissolved in time t, Q0 - Original quantity of drug Q∞- Amount released in time ∞ (100 % drug release) K - First order release constant.	Qt- Quantity of drug dissolved in time t, kH- Higuchi dissolution constant.	Qt- Amount of drug dissolved in time t, Q∞ - Amount released in time ∞, Kk - Rate constant n - Diffusional exponent

month at a humidity chamber (REMI, Mumbai) are the test criteria. PS, EE, *in-vitro* drug released, and drug composition are among the specifications to be assessed during the stability research period.[36]

RESULTS AND DISCUSSION

Preparation of Pemigatinib-loaded NSs

Over the last decade, abundant efforts were directed to recognize system of preparation and analysis of NSs. Among numerous types of NSs, polystyrene-based NSs have gotten the most attention and are being investigated the most.[37]

Polystyrene NSs can combine with various lipophilic or hydrophilic compounds to create complexes. Release of enclosed molecules is altered by modifying the structure for obtaining either longer or quick release kinetics. They have distinct benefit of regulated release and are medically safe and biodegradable.[38] NSs were generated by shifting hydrogen from the polystyrene's outer cavity's main hydroxyl groups. The drug may get inserted inside nano cavities, anr further associations of the visiting molecules with additional polystyrene components might be anticipated as a result of the cross-linking. The subsistence of cross-linked system would consequence in formation of nano channels at NSs framework. Unusual structural association may be accountable for NSs improved solubilization and protective capacities.[39] The produced NSs were loaded using the freeze drying procedure since lyophilization effectively enhances NS stability. A good lyophilizate, in general, preserves physical and chemical qualities of the primary product.[40]

Experimental Design

Molar ratio (polymer:cross linker), stirring-speed, and stirring-time have been determined as significant factors affecting the performance parameters such mean PS% EE, and PDI of pemigatinib NSs during preliminary screening. These separate variables were initially tested to identify the variable range by altering their value of one at a time. Molar ratio (polymer:cross linker; 0.4-0.8), stirring-speed (2000–4000 rpm), & stirring-time (200–600) got determined by the trials. Based on the preliminary findings, a BBD was used to optimize the impacting variables. On the basis of the experimental results generated by a three-factor, three-level BBD, a number of tests were carried out. The mean PS (Y1) range for every batch was 153 to 316 nm, the% EE (Y2) range was 68.2 to 91.4%, and the PDI (Y3) range was 0.273 to 0.445. Every response was adapted to 2nd quadratic representation, and the model's appropriateness got validated using ANOVA, tests supplied by Design-Expert software.

PS

PS determination is a critical quality control measure for determining the capability of any NSs composition. PS is an important parameter for NSs because it gives drug uptake a greater contact surface area. Furthermore, a lower PS may allow for an increased release rate.[37] The average PS of NSs was determined to be between 153 and 316 nm. According to polynomial make, all variables (X1, X2, & X3) showed substantial impact. As seen in figure. 1, the actual values are very close to the expected values.

Mathematical representation developed due to average PS (Y1) being determined as pivotal, F-value of 0.0483 indicating that model was substantial. "Model F-Value" would arise due to noise 0.03% of time. Because scores ≤ 0.05 imply pivotal model terms, quadratic term considerably affects the PS. The perturbation, contour, and 3D response surface graphs were in use to investigate primary and interacting impacts of self-governing factors on PS. Using 3D response surface graphs and accompanying contour graphs, link among reliant and self-governing parameters was examined further. Figure 2 (a-c) depicts an association among A and B on average PS at a preset value of C. Figures 2(a) and (b) illustrate the relevant contour plots. The increase in mean PS accompanied by a rise in molar ratio (polymer:cross linker), stirring-speed, and stirring-time. This effect can be attributed to the fact that a higher molar ratio of polymer:cross-linker results in optimized PS. PS reduced as stirring speed increased. Similarly, when the stirring duration increases, the PS decreases. As molar ratio (polymer:cross-linker) increased, particle size dropped.

Entrapment Efficiency (%)

Drug encapsulation is significant for improving solubilization and bioavailability of drug.[38] The EE of the NSs was established to be 68.2 to 91.4%. Polynomial model depicted that every attribute (X_1, X_2 and, X_3) has a noteworthy consequence. Observed results are in conformity with the observed results as depicted in Figure 3.

The F-value for the mathematical model created for EE (Y2) was 0.0362, indicating that model is substantial. "Lack of Fit F-value" of 0.0172 suggests the Lack of Fit is considerable in comparison to error. "Lack of Fit F-value" this big has a 0.75% probability of taking place owing to sound. Equations show that impact of X1 is larger than effects of X2 and X3. The correlation coefficient for the factorial equation for EE was 0.9991. Perturbation and contour plots were used to investigate 1^{o} and mixed impacts of self-governing attributes on EE. Using 3D response surface graphs and accompanying contor graphs, link among dependent and independent factors was investigated further. Figure 4(a) depicts the interaction of X1 and X2 on EE at a preset value of C. Figure 4(b) depicts the respective contour plots. The EE rose as stirring speed increased, but the amount of solvent had an opposing influence on EE.

PDI

The PDI is an indication of the size distribution range. Values larger than one suggest a polydispersed distribution. The PDI values were discovered as being 0.273 and 0.445. PDI has a considerable impact on cancer tissue and organ stability, solubility, dissolution, and permeation.[39] According to the polynomial model, every variable has a substantial outcome on PDI of drug embedded NSs. As seen in Figure. 5, the observed values closely match the expected values.

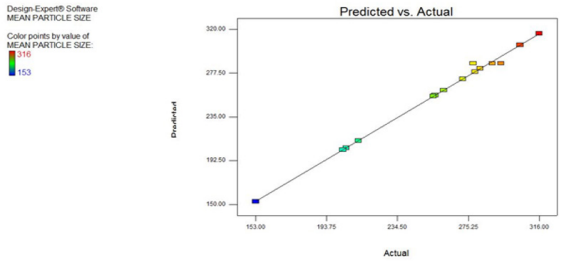

Figure 1: Association between predicted & actual values of Mean PS

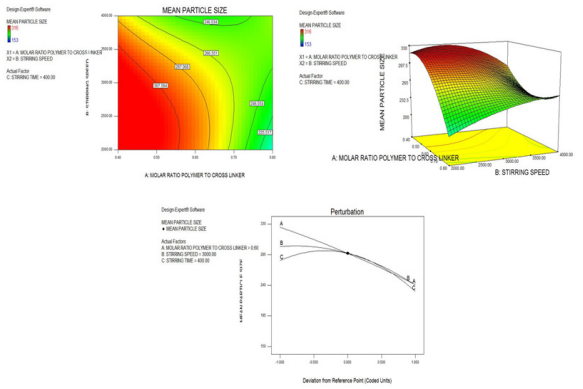

Figure 2(a): Contour graph depicting influence of molar ratio (polymer:cross-linker), stirring-time, and stirring speed on average PS fixed point of C; 2 (b): 3D-Contour graph depicting impact of molar ratio (polymer:cross-linker), stirring-time, and stirring-speed on average PS preset point of C; 2 (c): 2-D Perturbation graph depicting influence of molar ratio (polymer:cross-linker), stirring-time, and stirring-speed on average PS fixed level of C.

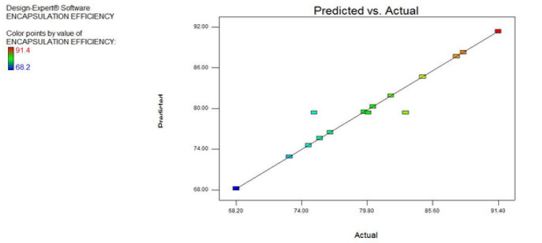

Figure 3: Association between expected & real values of Entrapment Efficiency (%)

PDI mathematical model was determined as considerable; F-value of 0.0267 indicating that model is credible. A "Model F-Value" such big would arise due to noise only (0.02%). "Lack of Fit F-value" of 0.0186 suggests that Lack of Fit is considerable in comparison to pure error. "Lack of Fit F-value" has a 1.22% probability of taking place because of noise. Perturbation and contour charts were used to further investigate the consequences of main and interacting impacts of independent variables. Figure 6(a) depicts the interaction of X1 and X2 on PDI at an established value of C. Figure 6(b, c) shows the respective contour plots. The increase in PDI is accompanied by a rise in molar ratio (polymer:cross-linker), stirring-speed, and stirring time. This phenomenon can be attributed to the fact that a larger molar ratio of (polymer:cross linker) results in optimized PDI. PDI decreased as the stirring

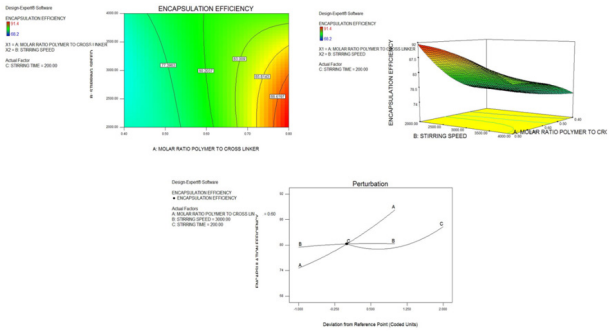

Figure 4 (a): Contour graph depicting influence of molar ratio (polymer:cross-linker), stirring-time, and stirring-speed on EE preset point of C; 4 (b): 3D-Contour graph depicting consequence of molar ratio (polymer:cross-linker), stirring-time, and stirring-speed on EE fixed level of C; 4 (c): Two-dimensional Perturbation plot showing the influence of molar ratio (polymer:cross-linker), stirring-time, and stirring-speed on EE fixed level of C

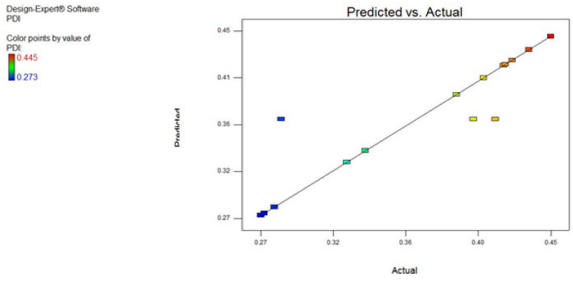

Figure 5: Association among expected & real values of PDI

speed increased. Similarly, when the stirring duration increases, the PDI decreases. As molar ratio (polymer:cross-linker) grew, so did PDI.

Characterization of Prepared Pemigatinib NSs

PS, PDI, ZP, and EE

Average PS of final formulation was determined as 153 and 316 nm (within nano metric range) (Figure 7). PS is vital for NSs; low PS gives a superior interfacial surface area for drug incorporation. Accumulation, a lower PS may allow a quicker release rate.[38] PDI of optimized formulation was found out to be 0.273 and 0.445, indicating uniformity of PS within formulation. PDI is momentous in stipulations of stability, solubilization, dissolution and infiltration through a variety of cancer tissues and organs.[39] The ZP study was done by zetasizer. The ZP for the final formulation was depicted in Figure. 7 and was determined to be -29.1 mV which show that the composition is stable.[40] The EE of the NSs was found to be in the range of 68.2 to 91.4%.

FTIR Spectroscopy

FTIR spectra of pemigatinib, HPMC K100M and physical mixture are observed. FTIR spectrum of final formulation revealed that functional groups of pemigatinib, HPMC K100M and other excipients were seen without deviation.[18] Hence it was concluded that all excipients in the optimized formulation mixture were compatible with each other and did not interact with each other (Figure 8).

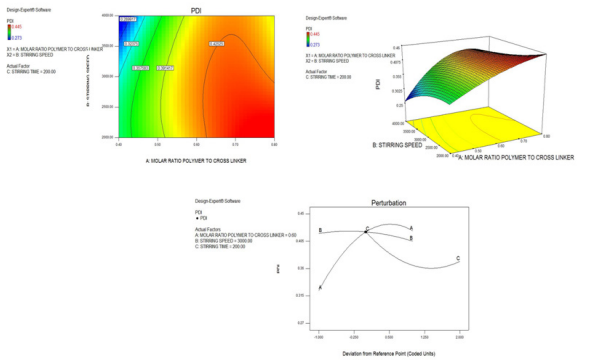

Figure 6 (a): Contour graph depicting the impact of molar ratio (polymer:cross-linker), stirring-time, and stirring-speed on PDI preset point of C; 6 (b): 3D-Contour graph depicting the impact of molar ratio (polymer:cross-linker), stirring-time, and stirring speed on PDI fixed level of C; 6 (c): Two-dimensional Perturbation plot showing the influence of molar ratio (polymer:cross linker), stirring time, and stirring speed on PDI fixed level of C.

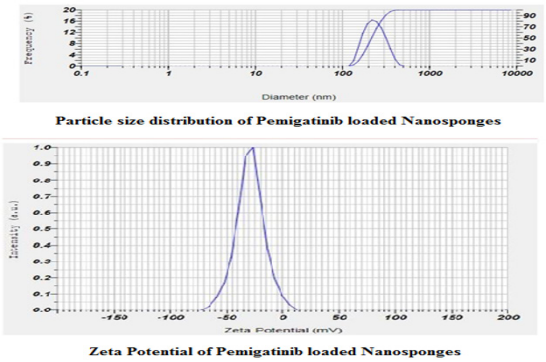

Figure 7: PS distribution and ZP of optimized Pemigatinib NSs

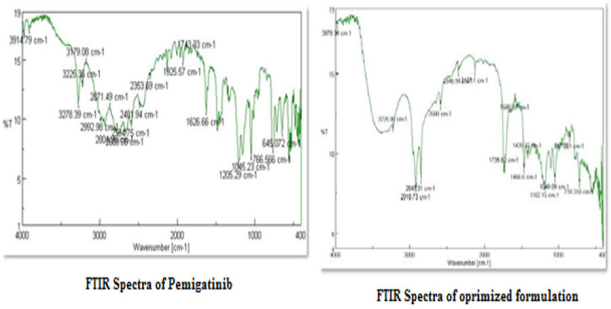

Figure 8: FTIR Spectra of pemigatinib and optimized formulation

SEM

SEM analysis of pemigatinib, polystyrene, diaryl carbonate and physical mixture (Figure. 9) depicted blend of pointed, prismatic, slim and trampled crystal habits with an average PS array of > 30 microns confirm the crystalline nature of pemigatinib whereas, SEM image of polystyrene exhibits aggregate and paracrystalline in nature. Diaryl carbonate image of SEM showed the slug mass, which may be due the presence of some moisture. SEM image of the physical mixture revealed that the formed mixture was dried, prismatic and flaky in nature. SEM image revealed that formulated pemigatinib -NSs formulation was found to be highly porous structure

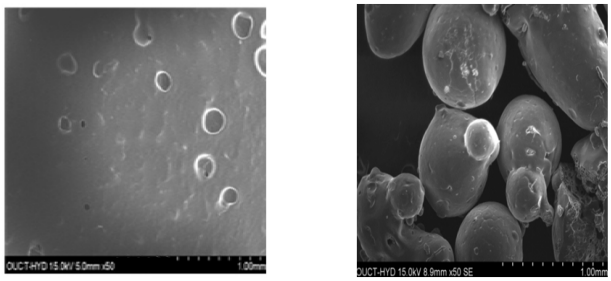

Figure 9: SEM Images of pemigatinib optimized SNEDDS formulation

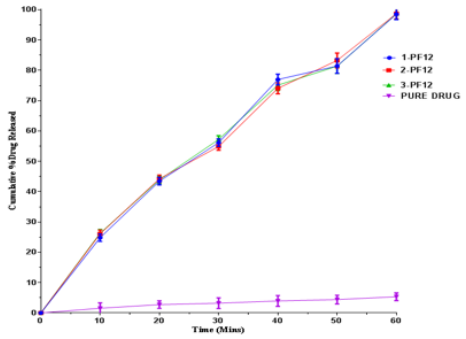

Figure 10: In-vitro release test of pemigatinib NSs (triplicate) in comparison with pure drug

and it revealed that the functional groups of drug, polymer and cross-linker were detected in the sample. Thus, it was confirmed that the Pemigatinib has successfully entrapped in the core of polymer.

Evaluation of Tablet Formulation

Physico-chemical parameters evaluation

The weight and the thickness of pemigatinib loaded NSs tablets were within the limits of uniformity. The weight was ranged from 200 ± 5.31 to 201 ± 6.13. Thickness ranged between 3.1 ± 0.26 to 3.4 ± 0.23 mm. The drug content ranged from 97.88 ± 1.37% to 99.37 ± 1.21%. Satisfactory hardness is a compulsory requisite for end user approval & usage. The measured hardness was ranged between 4.4 ± 1.27 to 4.6 ± 1.88 Kg/cm^2. The normal protocol was followed for all friability testing. I.P. the friability test findings. The statistics show that the friability in all formulations was less than 1%, confirming that the medication were mechanically stable.[29-32]

In-vitro release test of pemigatinib NSs

In-vitro release of pemigatinib NSs was related to plain drug, as depicted in Figure 10. Pemigatinib behavior release from NSs recommended a sustained release for 60 minutes. Sustained release from NSs represented the release of 98.53 ± 1.88% vs. 5.36 ± 1.93% by drug suspension for 1 hours. Pure pemigatinib's delayed release may be owing to hydrophobic property and low aqua solubility. Pemigatinib NSs clearly demonstrated better release characteristics than regular pemigatinib. This could be due to the ability of NSs to increase the dissolving of poorly soluble pharmaceuticals by confining them within nanochannels and spaces, concealing their hydrophobic moieties, and increasing their solubility.[41,42]

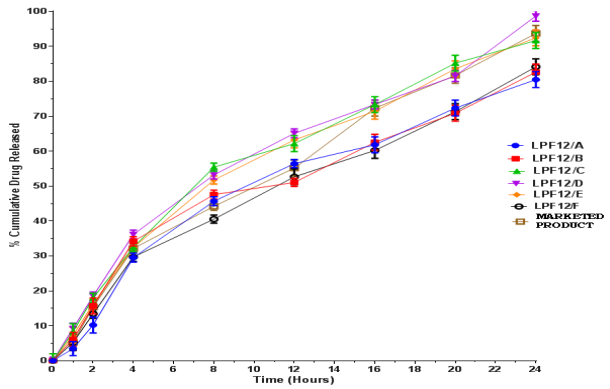

Figure 11: Drug released profile of pemigatinib nanosponges loaded tablets and marketed product

In-vitro release test of pemigatinib loaded NSs tablets

The optimized and prepared pemigatinib loaded NSs were compacted into tablet and further was tested for %collective release. It represented quick dissolubility in 0.1 N hydrochloric acid for initial 2 hours showing 18.53 ± 1.88% of drug released and its near to the comparator (15.42 ± 1.77), 98.74 ± 2.65% of drug release was observed in Pemigatinib loaded NSs and 93.73 ± 1.06 in marketed product. The dissolution profile for the composition in comparison to ROZLYTREK (marketed product) is presented in Figure 11. The fabricated NSs comprised of intricate network of polystyrene with a roughly circular makeup, with channels, pores & abundant interrelated void within explains chemistry behind drug loading and drug release patterns.[41] They 'cross connect' segments of diaryl carbonate to generate a porous structure with multiple nooks where medicines can become entrapped.[24] The medications are comprehensively complexes in these porous cages. Following administration, the natural polystyrene polymer progressively degrades to release the enclosed medicines.[42]

Drug release kinetics

The information gathered for release study got utilized in several mathematical depictions and analyzed using correlation coefficient to explicate method and mechanism of drug release. Mathematical model based on drug release kinetics with a greatest degree of the correlation coefficient is chosen[43]. The drug release is governed by zero-order kinetics (0.951), making it suitable for any drug delivery device. Korsmeyer-Peppas power law formula specifies the category of propagation assessed by value, n, which is >0.978, implying drug release through the system follow Super case II transport. This happens when sorption is completely governed by stress-related relaxations at an abrupt edge separating an outer swelling shell, virtually at optimum penetrates quantity, from an un-penetrated vitreous core.[44]

Stability studies

For six months, the storage stability of optimized Pemigatinib loaded NSs was investigated at assessment condition of 40°C ± 2°C & 75 ± 5% RH. Drug content, EE, *in-vitro* drug release, & PS got measured at the 0th, 30th, 60th, 120th, and 180th day. No note worthy alteration in drug quantity and PS was noted at days of storage. The EE hardly changed, signifying that NSs could shield pemigatinib from deterioration or degradation[45]. Furthermore, as storage time passed the mean *in-vitro* drug release pattern was also no changed.[40] The results indicated that the prepared pemigatinib loaded NSs were stable throughout the storage time and did not shown any leakage or drug degradation.

CONCLUSION

Pemigatinib-loaded NSs can be synthesized employing polymers with hydrophobic qualities such as polystyrene in a low-cost and simple ultrasound-assisted process. According to NS characterization experiments, the medication was entrapped inside the colloid 3D structure of polystyrene, as evidenced by the creation of an inclusion complex. The characteristic of the entrapped pharmaceutical has been changed from crystalline to amorphous, improving drug solubilization. *In-vitro* dissolution experiments of NS tablets revealed quick dissolution due to altered solubility qualities associated with the drug, in contrast to pure drug satisfying the desired goal of increased absorption. Cancer patients may benefit from the formulation of pemigatinib-loaded NSs. This can be directed toward cancer cells, resulting in sustained medication delivery, lowering dose, periodic administration, & adverse effects. In the future, more research will be required to verify the anticancer effects of pemigatinib-loaded NSs.

AUTHOR CONTRIBUTION

Ms P. Mamatha completed the research work and writing part and Dr D V R N Bhikshapathi made the correction submission for publication.

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