Original article

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Development and characterization of ethyl cellulose nanosponges for sustained release of brigatinib for the treatment of non-small cell lung cancer

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Abstract: Non-small cell lung cancer (NSCLC) contributes to about 85% of lung cancer. By 2040, lung cancer cases estimated to rise to 3.6 million globally. Brigatinib (BG) acts as tyrosine kinase inhibitors that target the epidermal growth factor receptor of the epithelial lung cancer cells. BG loaded nanosponges (NSs) were prepared by the emulsion solvent evaporation technique using ethyl-cellulose (EC) and polyvinyl alcohol (PVA) as a stabilizer. Eight formulations were developed by varying the concentration of the drug (BG), EC and PVA followed by optimization through particle characterization; size, polydispersity index (PDI), zeta potential (ZP), drug entrapment and loading efficiency. The optimized formulation BGNS5 showed particles size (261.0 ± 3.5 nm), PDI (0.301) and ZP (-19.83 ± 0.06 Mv) together with entrapment efficiency ($85.69 \pm 0.04\%$) and drug loading ($17.69 \pm 0.01\%$). FTIR, DSC, XRD, and SEM showed drug-polymer compatibility, entrapment of drug in EC core, non-crystallinity of BG in NS and confirm spherical porous nature of the NS. BGNS5 reflects drug release in a sustained manner, $86.91 \pm 2.12\%$ for about 12 h. BGNS5 significantly decreased the cell viability of A549 human lung cancer cell

lines with less hemolytic ratio compared to pure drug BG and EC. Based on the aforementioned results BGNS5 could be used in the effective treatment of NSCLC.

Keywords: A549; brigatinib; nanosponges; NSCLC; sustained release.

1 Introduction

Cancer involves an independent growth of cells leading to malignancy, possess invading activity surrounding the tissues of its origin and other body parts. Cancer cell has the district property of non-specialized in nature without any function and independent cell division [1]. Cancer is considered to be the second largest reason for global mortality, accounting for about 10 million deaths annually with a projection equivalent to 13 million deaths by 2030 [2]. Fundamental theories of cancer claim the causes include: chronic irritation, displaced embryonal tissue and infectious agent [3].

As per reports in 2014, 16 million people suffered and about half-million died due to cancer in the USA alone. Prevalence of cancer found to be in the order of; prostate, lung, colon in male and breast, lung, colon, uterus in the female, respectively [4]. Statistical data in Saudi Arabia as per the health day 2017 fact sheet represents [5], among the various types of cancer, breast cancer ranked first with (16.1%) followed by colorectal cancer (11.9%) and thyroid cancer (7.6%).

Disease management of the cancer is very critical and challenging, due to strenuous in early-stage diagnosis and specific treatment at a cellular level. Treatment of cancer include(s) surgery, nuclear medicine, stem cell-targeted therapy and chemotherapy [6]. Targeted drug delivery systems considered to be the greatest choice to deliver the chemotherapeutics for effective treatment by the

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selectively localized drug at the pre-identified site thereby restricting access to the non-target cellular lining and thus reducing the toxicity with increased effectiveness [7]. There were many new chemical entities (NCE) for cancer treatment but if cancer cell doesn't respond or resistant to an anticancer moiety, then the new drug will be the next choice, therefore anticancer research institutes/centers continuously produce chemo-drugs [8]. Second generation anaplastic lymphoma kinase (ALK) inhibitor brigatinib (BG) recently approved by United States Food and Drug Administration (USFDA) as an anticancer drug and specifically used for non-small cell lung cancer (NSCLC). BG precisely used to treat metastasis conditions where cancer tends to brain and bone marrow [9]. Whereas, the first-generation oral ALK inhibitors failed to act as selective apoptosis due to the passage of cancer cells through the blood-brain barrier (BBB) that causes severe CNS toxicity, ceritinib a second-generation ALK inhibitor though effective in clinical trials but subsequently failed to cure NSCLCs [10]. In NSCLC conditions, rearrangement of the ALK gene takes place leading to modification in the ALK protein cell signaling pathway followed by abnormal growth of cells and metastasis. Clinical trial data reflects brigatinib used as a palliative treatment by prolonging life-span by a year [11]. BG also reported to act as a safe intracranial antitumor activity for crizotinib-resistant patient with ALK-positive, it could be considered as an efficient choice because radiotherapy with ionized radiation and as chemotherapy cannot be an effective at the cerebral site [11–13].

Brigatinib available in the dose strength of 30, 90 and 180 mg, the optimum dose was found to be 180 mg once a day for sustained apoptosis of NSCLC [10]. Brigatinib considered being a novel potent tyrosine kinase inhibitor used in lung cancer treatment for the patients reflecting the resistance to osimertinib. Due to this reason BG was approved by USFDA with a fast track [9].

Commercially brigatinib available in conventional tablet forms as Alunbrig® tablets licensed under Takeda Pharmaceutical Company Limited, with claimed backup by ARIAD Pharmaceuticals [10]. BG exposed to exhibit pulmonary toxicity in some patients which could be life threatening [9]. Chemotherapeutics developed in conventional dosage forms (tablets, capsules, solution, and emulsion) encompassed certain demerits includes; hepatic first-pass, plasma drug fluctuations, instability, toxicity and doesn't have sustained drug release and apoptosis efficacy for long durations [14].

To circumvent the aforementioned drawbacks, technologies with nano-size (10–1000 nm) drug carriers can be used for enhancement of dissolution rate, absorption, improved bioavailability, increase half-life of the drug in

biological systems with site-specificity and sustained drug release [15]. Nanosponges are emerging technologies for cancer treatment. Nanosponges are porous polymeric delivery systems that are microscopic spherical particles with few nanometers wide cavities and large porous surface, into which both lipophilic and hydrophilic drug substances can be encapsulated [16]. Among the various types of polymers used in the fabrication of nano matrix, ethyl cellulose (EC) was reported being non-biodegradable, non-toxicity, biocompatible and tolerable with reduced toxicity. Habashy et al. described nanocarriers prepared by EC with the particle size of >200 nm to 5 µm anticipated to be removed by the reticuloendothelial system (RES) and mechanically filtered by the spleen and glomerular filtration [17, 18].

Reports on EC reflects its wide implications in drug delivery systems; for sustaining the effects of anticancer, anti-inflammatory, anti-infective, film-forming agent, the binding agent in controlled release solid dosage units [19].

The objective of the current study was to develop and characterize brigatinib loaded ethyl cellulose nanosponges for sustained drug release to prolong anti-cancer efficacy. Henceforth, nanosponges prepared by EC, rate retarding polymer could prolong the half-life of brigatinib due to uptake by RES and hydrophobic inheriting property of EC reducing water penetration into polymer matrix reduces the drug release.

2 Materials and methods

2.1 Materials

Brigatinib (BG) was purchased from Mesou Chemical Technology (Beijing, China). Ethylcellulose (EC), polyvinyl alcohol (PVA) and dichloromethane (DCM) were procured from Sigma Aldrich, Germany. All the other chemicals and solvents used were of analytical grades.

Human lung adenocarcinoma A549 (ATCC® CCL 185™) cell lines were received from the American Type Culture Collection (ATCC, Manassas, VA, USA).

2.2 Development of brigatinib loaded nanosponges

Brigatinib loaded nanosponges (BGNS) were prepared by ultra-sonication assisted-emulsion solvent evaporation technique [19, 20], by using different proportions of polymers (EC and PVA) and the drug was taken in two concentrations (90 and 180 mg). BG was dissolved in EC polymeric solution in DCM with the help of sonication for 1 min. The prepared drug solution was then emulsified dropwise into 100 ml of aqueous phase (100 ml) containing a different proportion of PVA by probe sonication (probe # 423, model CL-18; Fisher Scientific, USA) for 5 min with power 60% voltage efficiency. The developed emulsion was kept on a magnetic stirrer (Fisher Isotemp Hot Plate and Stirrer; Fisher

Scientific, USA) and stirred at (1000 rpm) for about 24 h at atmospheric conditions [17]. The prepared BGNS were then collected by ultracentrifugation followed by lyophilization overnight, collected samples then packed in a well-closed container (Vial) and used for further characterization. The composition of nanosponges containing BG, EC, and PVA concentrations were given in (Table 1).

2.3 Characterization of brigatinib loaded nanosponges

2.3.1 Particle characterization: size, polydispersity index (PDI), and zeta potential (ZP): Particle size analysis of the BGNSs was performed by using dynamic light scattering (Zetasizer Nano ZS instrument, Malvern Instruments, UK) at room temperature ($25 \pm 2^\circ\text{C}$). Measurement of average particle size, ZP and PDI values for each formulation was taken by dispersing the samples in Milli-Q water (1:200) till transparency was achieved [21]. All the samples were run in triplicates ($n = 3$).

2.3.2 Entrapment efficiency and drug loading calculation: The percent entrapment efficiency (EE%) and drug loading (DL%) of the NS nanocarriers was measured by indirect method of analysis taking the sample from the dispersion medium followed by centrifugation at 12,000 rpm for 25 min, then the collected supernatant was analyzed for free drug content by using UV spectrophotometer (Jasco UV/Visible Spectrophotometer V-630 Japan) at 284 nm (λ_{max}) [22]. The percentage of EE and DL were calculated by applying the following equations [23]:

$$\text{EE}(\%) = \frac{\text{Total Drug} - \text{Free drug}}{\text{Total Drug}} \times 100 \quad (1)$$

$$\text{DL}(\%) = \frac{\text{Entrapped drug}}{\text{Weight of nanosponge}} \times 100 \quad (2)$$

2.3.3 Fourier transform infrared (FTIR) analysis: FTIR analysis was performed to evaluate the compatibility of BG with the excipients used for the fabrication of nanosponges. BG (drug), and optimized BGNS5, were mixed individually with potassium bromide (KBr) followed by compression to form a disc followed by scanning from 400 to 4000 cm^{-1} to detect fingerprint region of BG in BGNS5 [24] (Jasco 4600 Mid-IR FTIR spectrometer, Japan).

2.3.4 Differential scanning calorimetry (DSC) analysis: Physico-chemical drug-excipient interaction was performed by differential scanning calorimetry (DSC), BG and BGNS5 (5 mg) were sealed in the

aluminum and subjected to a heating rate of $10^\circ\text{C}/\text{min}$ for temperature ranged from 30 – 250°C (DSC N-650; Scinco, Italy).

2.3.5 X-ray diffraction (XRD) analysis: X-ray diffraction (XRD) study was performed to evaluate the crystalline behavior of pure BG and optimized BGNS5. The samples were analyzed using Ni-filtered CuK α radiation ($\lambda = 1.5418 \text{ \AA}$) at voltage 40 kV, current 40 mA, receiving slit 0.2 q inches, 2θ range of 5 – 75°C with a scan rate $0.040^\circ/\text{s}$ [25] (Siemens D5000 Diffractometer).

2.3.6 Scanning electron microscopy (SEM): The surface morphology of the optimized nanosponge BGNS5 was assessed by spreading the sample suspension over a thin glass slab then dried under vacuum. Further, the sample was shadowed in a cathodic evaporator having a gold layer thickness of 20 nm, the machine was operated at 15 kV acceleration voltage. The image processing program was utilized for the estimation of surface morphology and the mean particle size of samples [23] (JEOL JSM 5200 SEM, Tokyo, Japan).

2.3.7 In-vitro drug release and mathematical model fitting: The *in-vitro* drug release behavior of the optimized BGNS5 and BG suspension was assessed by using dialysis bag method in phosphate-buffered saline (PBS) pH 7.4. The samples (optimized BGNS5 and BG suspension) dispersed in (PBS pH 7.4; 5.0 ml), filled in dialysis bag (Mol. Wt.:14 kDa) closed from both the ends, then suspended into a beaker containing 150 ml of PBS (pH 7.4), maintained at $37 \pm 2^\circ\text{C}$, under magnetic stirring (100 rpm). At the pre-determined time interval, a 1.0 ml sample was withdrawn with replenishment to maintain the sink condition of the medium. The aliquots were analyzed for drug concentration at 284 (λ_{max}) by UV/Visible Spectrophotometer (Jasco V-630 Japan) [22, 26].

The results of *in-vitro* drug release were obtained by plotting cumulative percent drug release (% Cu DR) versus (time, h). Each experiment was performed in triplicate ($n = 3$).

Further, the drug release data were fitted into zero-order, first-order, Higuchi and Korsmeyer–Peppas kinetics models, followed by regression analysis [27]. The respective equation for each model is depicted as:

$$\text{Zero-order; } Q_t = Q_0 + k_0 t \quad (3)$$

$$\text{First-order; } \log Q_t = \log Q_0 - k_1 t / 2.303 \quad (4)$$

$$\text{Higuchi; } Q_t = k_H t^{1/2} \quad (5)$$

$$\text{Korsmeyer-Peppas; } M_t / M_\infty = k t^n \quad (6)$$

where, Q_t (drug dissolved over time t), Q_0 (initial amount of drug dissolved in diffusion medium i.e., equal to zero), k_0 (zero-order kinetics constant), k_1 (first-order rate constant), $k_H t^{1/2}$ (Higuchi model constant), M_t and M_∞ are cumulative drug release at time t and infinite time, respectively; k is rate constant of BGNS5 structural and geometric characteristics feature, t is the release time and n denotes diffusional exponent indicating release mechanism. Furthermore, in all of the aforementioned models, suitable parameters were plotted and from the r^2 value (coefficient of multiple determination; $0 \leq r^2 \leq 1$), the release kinetic behavior of BG was evaluated. Also, from the values of $n = 0.45$ (Case I or Fickian diffusion), $0.45 < n < 0.89$ (anomalous behavior or non-Fickian transport), $n = 0.89$ (Case II transport) and $n > 0.89$ (Super Case II), the release mechanisms were described.

2.3.8 MTT cell proliferation assay: MTT (3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide) assay was performed to assess

Table 1: Development of brigatinib loaded nanosponges.

Formulation code	Brigatinib (mg)	Ethyl cellulose (mg)	PVA (mg)
BGNS1	90	400	40
BGNS2	90	500	30
BGNS3	90	500	50
BGNS4	90	600	40
BGNS5	180	400	40
BGNS6	180	500	30
BGNS7	180	500	50
BGNS8	180	600	40

the cytotoxicity and anticancer activity of BG, BG suspension and optimized BGNS5 against A549 cells (human lung carcinoma cells). Before the evaluation, the A549 cell lines were passaged in culture media Dulbecco's Modified Eagle's Medium (DMEM) supplemented with the 10% fetal bovine serum (FBS) and incubated at 37 °C overnight. 1 ml of the freshly cultured cell suspension (5×10^4 cells/ml) was seeded in a 24-well plate, incubated at 37 °C for overnight. All the samples with varying concentrations (0, 5, 12.5, 25 and 50 µg/ml) were added individually to the pre-treated well plates. DCM was considered to be as control. Subsequently, 100 µl of MTT solution (5% w/v) was added to treated wells and further the plates were incubated at 37 °C for 5 h [28]. The absorbance of each sample was measured at 540 nm using an ELISA microplate reader (Thermo Fisher Scientific, USA) and the % cell viability was calculated by using the following formula:

$$\% \text{ Cell viability} = \frac{\text{Mean absorbance of treated sample}}{\text{Mean absorbance of control}} \times 100 \quad (7)$$

2.3.9 Biocompatibility studies: A biocompatibility study was performed by testing the compatibility between BGNS and erythrocytes. The fabricated nanosponges need to be evaluated for their biocompatibility as they enter the body and contact with tissues and cells directly, thus this enhances its biomedical applications. A biocompatibility study was performed by testing the compatibility between BGNS5 and rat blood erythrocytes. Hemolytic activity of BG, EC, PVA, and BGNS5 was assessed by incubation samples with erythrocyte isotonic suspension at 37 °C for 1 h, followed by centrifugation and measuring the absorbance of the supernatant at 540 nm using a spectrophotometer. Erythrocyte with sodium dodecyl sulfate and dimethyl sulfoxide served as positive and negative control respectively [29].

$$\text{Hemolysis (\%)} = \frac{\text{ABS Sample} - \text{ABS Negative control}}{\text{ABS Positive control} - \text{ABS Negative control}} \times 100 \quad (8)$$

where ABS is the absorbance.

2.3.10 Stability studies: The optimized formulation BGNS5 was kept for stability studies at room temperature (30 ± 2 °C), at refrigerator temperature (4 ± 2 °C) and at accelerated condition (40 ± 2 °C, 75%RH) in programmable environmental test chamber following the ICH guidelines. Evaluation of cumulative % drug release, particle size, calculation of (EE%) and (DL%) was performed for 3 months (12 weeks) with intervals of 4 weeks i.e., 0,1,2 and 3rd month.

3 Results and discussion

Formulation were optimized by varying the concentrations of ingredients. Drug (BG), rate retarding polymer EC, stabilizer PVA were used in different concentrations. Drug amount was selected based on the dose (90 and 180 mg), polymer (EC) and stabilizer (PVA) were taken in three different quantities (400; 500; 600 mg) and (30; 40; 50 mg), respectively. The ratio of dispersing to aqueous phase was set to 1:5 (20 ml DCM: 100 ml PVA aqueous phase). Emulsification solvent evaporation is most widely applied technique with reproducibility and ease of the process. The concentration of EC influences the size of NS whereas PVA modified the flocculation [30]. It was observed that size of the particles influenced by combined factors like drug, EC and PVA content [18, 31, 32].

3.1 Particle characterization: size, polydispersity index (PDI), and zeta potential (ZP)

Particle size and PDI of BGNSs were found to be in the range of (261.0–697.5 nm), (0.3–0.6) respectively, whereas ZP found in between (–14 to –26 mV). All the developed NSs were in nano-size ranged, narrow in particle size distribution as per PDI. Zeta potential for all NSs was negatively charged with ≥ 14 . ZP indicates non agglomeration and a stable dispersion system (Table 2). EC pertaining the negative charge over the particles could have resulted in inter-particle repulsion [30]. Based on the aforementioned criteria BGNS5 formulation was considered to be optimum with particle size (261.0 nm); PDI (0.3) and ZP (–19.8), respectively (Figure 1), and selected for further characterizations studies.

Table 2: Particle size, polydispersity index (PDI), zeta potential (ZP), drug loading and entrapment efficiency of PCL formulations.

Formulation code	Particle size (nm)	Polydispersity index (PDI)	Zeta potential (mV)	Entrapment efficiency (%)	Drug loading (%)
BGNS1	380.5 ± 2.4	0.351	–14.32 ± 0.02	21.67 ± 0.07	2.17 ± 0.05
BGNS2	484.6 ± 3.1	0.442	–20.12 ± 0.01	28.45 ± 0.08	5.38 ± 0.03
BGNS3	423.2 ± 4.2	0.355	–26.42 ± 0.07	35.61 ± 0.04	6.06 ± 0.06
BGNS4	484.6 ± 3.6	0.428	–20.54 ± 0.08	47.32 ± 0.06	8.57 ± 0.02
BGNS5	261.0 ± 3.5	0.301	–19.83 ± 0.06	85.69 ± 0.04	17.69 ± 0.01
BGNS6	697.5 ± 4.6	0.613	–18.87 ± 0.03	52.36 ± 0.07	9.87 ± 0.04
BGNS7	517.4 ± 7.5	0.437	–18.55 ± 0.02	35.61 ± 0.02	6.06 ± 0.03
BGNS8	556.2 ± 3.2	0.548	–22.74 ± 0.07	64.28 ± 0.05	11.12 ± 0.02

All values expressed are mean ± SD where $n = 3$.

Figure 1: (A) Particle size (histogram) and (B) zeta potential for optimized BGNS5.

3.2 Entrapment efficiency (EE) and drug loading (DL) calculation

Calculation of EE (%) and DL (%) of the formulations indicates drug entrapment and drug content in NS after separation from the aqueous media of PVA respectively (Table 2). EE was found to be in the range of (21.67–85.69%), whereas DL of brigatinib ranged in (2.17–17.69%). From the results it was observed that, BGNS1 showed the lowest % EE ($21.67 \pm 2.12\%$) and % DL ($2.17 \pm 0.53\%$), whereas the optimized formulation (BGNS5) showed the highest % EE ($85.69 \pm 0.04\%$) and % DL ($17.69 \pm 0.01\%$). Based on size (261.0 ± 3.5 nm), *PDI* (0.301), *ZP* (-19.83 ± 0.06 mV), %EE ($85.69 \pm 0.04\%$) and %DL ($17.69 \pm 0.01\%$), the BGNS5 was found optimum with content of BG (180 mg), EC (400 mg) and PVA (40 mg).

3.3 Fourier transform infrared analysis

FTIR spectra of pure drug BG and optimized nanosponge (BGNS5) were showed in (Figure 2) Pure BG showed major peaks at 1012 cm^{-1} (C|O, stretching), 1620 cm^{-1} (N|H, bending), 1157 cm^{-1} (P|O, stretching), 2784 cm^{-1} (C|H, stretching) and 3286 cm^{-1} (N|H, stretching). BGNS5 peaks represent the presence of functional group peaks of EC, (3100 cm^{-1}) due to Hydroxyl stretching and hydrogen bonds between hydroxyl groups (2950 cm^{-1}) was for alkyl group stretching and ($1600\text{--}1750\text{ cm}^{-1}$) identical to bending of alkyl in pyrane ring. It also showed the PVA peaks, ($2750\text{--}2900\text{ cm}^{-1}$) and ($3650\text{--}3700\text{ cm}^{-1}$) for alkyl

band stretching hydroxyl bond. Further, in BGNS5 spectrum shifting of identical peaks of BG was observed with the reduction in intensity in the fingerprint region of the drug. FTIR analysis determines no physicochemical drug interaction only physical bonding of drug with the polymeric matrix. These results were in accordance with the previously reported literature.

3.4 Differential scanning calorimetry analysis

DSC thermograms of pure BG and BGNS5 were plotted in (Figure 3). The thermogram of the pure BG showed a sharp endothermic peak at 215.81°C , while the thermogram of BGNS5 does not show the respective thermal peak of pure BG. DSC analysis supports the interpretation of possible drug-polymer interaction with the significance of drug encapsulation in porous cavities of BGNS5.

3.5 X-ray diffraction analysis

XRD diffraction patterns of pure a drug (BG) demonstrated numerous characteristic sharp peaks at different angles, however, BGNS5 revealed broad and diffused diffraction peaks, indicative of lost crystallinity nature of pure drug due to entangled of BG inside the polymer matrix. Also, drug encapsulation in the nanosponges could be assured due to the broadening or weak diffraction pattern of drug [23]. The encapsulated drug may be in the non-crystalline

Figure 2: Fourier transform infrared (FTIR) spectrum of pure brigatinib (BG) and optimized BGNS5.

Figure 3: Differential scanning calorimetry (DSC) plots of pure BG and optimized BGNS5.

form trapped in the porous BGNS5 nanosponges. X-ray diffraction patterns of pure BG and BGNS5 were represented in (Figure 4).

3.6 Scanning electron microscopy

The Scanning electron microscopy (SEM) micrograph(s) of the BGNS5 represents the spherical shape of NS with nanosize range. It was predicted that the in-ward diffusion of DCM on the EC polymeric surface leads to the porous

nature of the nanosponges. Also, the micrographs (Figure 5) revealed that the EC matrix was properly coated over the BG and spherical also glazed by PVA responsible for anti-adhesiveness between the particles and smooth surface of BGNS5.

3.7 *In-vitro* drug release and mathematical model fitting

In-vitro drug release study was performed to determine the release behavior and kinetics mechanism of the drug release from the polymeric matrix in PBS (pH 7.4) diffusion medium. As shown in (Figure 6) within the first five hours of study, the release of BG from BG suspension and BGNS5 was 86.91 and 62.35 % respectively. EC based nanosponge loaded with BG showed a two-phase release from optimized BGNS5, an initial burst release essential for rapid onset of action followed by sustained drug release.[33] The initial burst effects within 1 h was possibly due to the desorption of BG particles layered over the surface of NSs and also due to the surface attrition. EC showed significant effects and was specifically responsible for the sustained and progressive release of BG from NSs. The sustained -release of BG from BGNSs could be due to the slow dispersion of aqueous media inside the hydrophobic EC.

Also, the release data of BGNSs was fitted with various kinetic models and calculated the regression coefficient (R^2) and rate constant (K). The results of (R^2 & K) values were found (zero-order; R^2 : 0.853, K_0 :15.554, First-order; R^2 :

Figure 4: X-ray diffraction (XRD) analysis of pure BG and BGNS5.

Figure 5: Scanning electron microscopy (SEM) image of BGNS5 (A) spherical image NS at 512x magnification (B) surface image of NS at 1.07 Kx magnification.

0.954, K_1 :1.955, Higuchi; R^2 : 0.998, K_H :5.386, Korsmeyer–Peppas; R^2 : 0.929, K_c :1.282), respectively. The best fit was found to be Higuchi model for BGNS5, showing a higher correlation ($r^2 > 0.99$) as compared to other models, ensuring that the slow release of BG from the polymer bilayer in the presence of PVA were due to process of

particle diffusion through the membrane. Moreover, the Korsmeyer–Peppas model with exponent ($0.45 < n < 0.89$) denoted the anomalous non-Fickian release kinetics. All nanosponges represented non swellable matrix diffusion drug release mechanism attributed to porosity in these nanosponges formulations [34].

Figure 6: Comparative *in-vitro* cumulative drug release (%) versus time for BG suspension and BGNS5.

3.8 MTT cell proliferation assay

The MTT assay was performed for pure blank NSs, pure drug BG and optimized formulation BGNS5. MTT assay results showed that the percent cell viability of all the samples followed a dose-dependent pattern. Sample with lower concentration showed more % cell viability and so were less toxic against the A549 cells. *In-vitro* anticancer activity was performed in different concentration of samples (0, 5, 12.5, 25 and 50 $\mu\text{g/ml}$). BGNS5 showed (100, 18.48, 18.18, 20.28, 22.98% viability) for respective concentrations. Furthermore, the % cell viability results for all the samples have been summarized in (Figure 7). Henceforth, based on the results of the MTT assay, it was observed that BGNS5 formulation exhibited potential anticancer activity against A549 lung cancer cell lines, thus could be used as a potent anticancer agent for the treatment of non-small cell lung cancer.

3.9 Biocompatibility studies

Biocompatibility study was performed by hemolysis study using rat blood (erythrocytes), from the results it was found that the percentage of rupture of rat RBC was in the order of BG > BGNS5 > EC > PVA with hemolysis of (48.87, 32.76, 16.88, and 3.43%), respectively. Based on Equation (8) the hemolytic ratio was calculated by substituting the absorbance values; 3.19 nm for sodium dodecyl sulfate (positive control), 0.18 nm for dimethyl sulfoxide (negative control) and 0.32 nm for test BGNS5. The hemolytic ratio calculated was 4.87%, which was found to be less than 5% as per the standards for assessment of hemolytic features of matters or materials (F756-93 standards) [35, 36]. Results

Figure 7: *In-vitro* concentration-dependent cell viability testing of blank NSs, free drug suspension (BG) and BGNS5 on A549 human lung cancer cell lines.

plotted in (Figure 8) confirmed; polymer used EC was biocompatible, optimized BGNS5 most suitable for administration as its less toxic (hemolytic) and could be safely used in the treatment of NSCLCs [37].

3.10 Stability studies

After 3 months (12 weeks) with interval of 4 weeks, i.e., 0, 1, 2 and 3rd month of study, the sample was assessed for *in-vitro* drug release. BGNS5 doesn't have significant difference in cumulative drug released (%) at freeze and accelerated condition as compared to the data of BGNS5 at normal conditions ($p\text{-value} \leq 0.05$). Particle size, EE (%) and DL (%) were also evaluated for BGNS5 in different conditions before and after stability studies, results revealed no significant change in the results as per the null hypothesis ($p\text{-value} \leq 0.05$).

Figure 8: Percent hemolysis of optimized NSs (BGNS5) in comparison to polyvinyl alcohol (PVA), ethylcellulose (EC) and pure drug (BG).

4 Conclusion

Brigatinib loaded nanosponges were prepared by ultrasonic-assisted emulsion solvent evaporation technique. From eight formulations BGNS5 nanocarriers composed of BG (180 mg), EC (400 mg) and PVA (40 mg) was optimized based on the characterization results; particle size (261.0 nm), PDI (0.301), ZP (−19.83), EE (85.69%), DL (17.69%) which direct the investigator for further evaluations. FTIR, DSC peaks reflect of drug-polymer doesn't have any physico-chemical interactions, the drug was integrated and encapsulated in the polymer matrix, XRD diffractograms represents drug was completely dissolved with polymer and nanosponge available in amorphous form, the spherical shape with porosity nature of nanosponges were visible in the SEM images. *In-vitro* release showed 86.91 and 62.35% drug release in the first five hours for BG drug suspension and BGNS5, respectively where, as from optimized nanocarriers drug continues to release in the sustained manner for about 12 h. The mathematical release equations represent drug release was followed Higuchi model with anomalous non-Fickian release kinetics. MTT assay exhibited the dose depended on cell apoptosis activity against A549 cells, with hemolytic testing confirmed biocompatibility of fabricated NS and its suitability for human administrations. The stability study as per ICH guidelines showed BGNS5 formulation was stable for more than 3 months (12 weeks) with no-significant variation in particle size, EE%, DL% characterization. Henceforth, BGNS5 was considered to be an optimized formulation of brigatinib showing anticancer activity against NSCLC with sustained drug release and prolong anticancer activity.

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