

Development of Polymeric Nanoparticles of Sunitinib malate in Eudragit S 100 for anti-colon cancer targeted drug delivery

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Abstract

Sunitinib malate (SM) is a multi-targeted tyrosine kinase (TK) inhibitor. It is proposed as a potent anticancer drug intended for colon cancer. The current work aimed at developing Sunitinib malate -loaded nanoparticles for colon- targeting. SM loaded ES100-nanoparticles were developed by nano-precipitation method using poly vinyl alcohol (PVA) as stabilizer. Different formulation of ESNP (F1-F3) were prepared and evaluated for particle size, poly-dispersity index, and drug entrapment, using Fourier transform infrared spectroscopy (FTIR), x-ray diffraction (XRD), and scanning electron microscopy (SEM). The optimized formulation was further studied by in-vitro drug release and anticancer cell line study. The mean particle size, PDI, %EE of optimized SM loaded ESNPF2 was found to be 384.8 ± 5.53 nm, 0.58, and 60.31% respectively. In vitro release of the optimized ESNPF2 showed 10.2 % drug release till 2 h, at 4h 29.6% and 12 h and 24 h 66.45%, 82.2% respectively at pH 7.4. SEM images and surface morphology confirmed that nanoparticles were spherical and had smooth surfaces. An MTT assay was conducted in the colon cancer cell lines (CACO2) to evaluate anti-cancerous activity of SM. SM loaded ESNPF2 showed a high potential against colon cancer cells. The overall results suggest that SM loaded ES100-nanoparticles could be a potential option for colon targeting.

Keywords: Sunitinib, Eudragit S 100, nanoparticles, particle size, MTT assay, colon cancer.

1. Introduction

With about 1.9 million new instances of colorectal cancer (CRC) recorded in 2018 and 900,000 fatalities, CRC is one of the most prevalent malignancies in the world and is the third most frequently diagnosed cancer overall(1) Around 147,950 cases of CRC are anticipated in 2020, and 53,200 people may pass away from these conditions(2) Despite the fact that the prognosis for CRC patients has improved over the past several decades in many affluent nations, primarily as a result of better prevention and treatment (3-6), the incidence and mortality of CRC are rising quickly in low- and middle-income countries, in part because of the adoption of western lifestyles(7).

Sunitinib malate (SM) is a multi-targeted tyrosine kinase (TK) inhibitor which mainly targets “vascular endothelial growth factor receptors (VEGFRs), platelet-derived growth factor receptors (PDGFRs), FMS-related tyrosine kinase-3 (FLT-3), stem cell factor receptor (c-KIT) and rearranged during transfection (RET) TK” (8, 9). It has been approved for the treatment of advanced renal carcinoma and imatinib-resistant gastrointestinal stromal (GIS) carcinoma (9, 10).

SM shown promising action against colorectal cancer, advanced non-small cell lung cancer (NSCLC) (11), hepatic cancer (12), pancreatic neuroendocrine tumors (pNET) (13), and breast cancer treatment alone (14-16), as well as in combinations with curcumin (17).

SM has been reported as a poorly water-soluble drug (6.328 mg/L at 25 °C, (18)), It is soluble >25 mg/mL over pH of 1.2 to 6.8, (drug bank) i.es. Solubility at 22 °C (µg/mL): 2582 in 20 mM KCl/HCl buffer (pH 2); 364 in 20 mM phosphate buffer (pH 6) (19). Due to their limited aqueous solubility, it lead to poor oral bioavailability, permeability, cell uptake, drug resistance, and systemic toxicity (20). Enhancement of oral bioavailability of drugs chiefly centered on particle size reduction. One promising approach to improve the efficacy and to reduce the systematic side effects of anti-cancer agents for colon cancer is colon targeting nanoparticle drug delivery systems.

In order for a drug to be delivered specifically to the colon, it must first reach the intended location and exhibit therapeutic activity without releasing any medication into the stomach acid before it is ready. Colon cancer develops when ulcerative colitis is left untreated. It took so long because there were so few malignant colonic polyps and so many current instances of ulcerative colorectal cancer (21, 22). Nanoparticles for colon-targeted oral drug delivery systems must, however, get beyond pH sensitivity and transit time in the stomach. The formulation must be safeguarded for oral administration in order to stop degradation, early drug release, and absorption before the colon (23, 24). Enteric coating polymer can solve these issues. The enteric coating serves as a barrier, defending the loaded drug from the acidic environment of the stomach and regulating its release to reach places in the lower gastrointestinal tract (25 -29)

One of the drug delivery methods that can increase intracellular penetration and retention time, control the release of encapsulated drugs, (27 , 30-32) and target delivery in particular regions of the gastrointestinal tract, such as in the treatment of colorectal cancer, is the use of polymeric nanoparticles of enteric-coated polymer(34) Size, shape, and surface chemistry are three variables that have an impact on the cellular absorption and effectiveness of nanoparticle drug delivery systems for colorectal cancer therapy(34–36) Treatment for colorectal cancer is significantly influenced by the drug delivery system's nano size. Due to the epithelial-enhanced permeability and retention effect, this may lead to an increase in the rate of selective accumulation in the colon tissue.

Typically, enteric coating polymers are either pH-sensitive or resistant to water. A pH-dependent enteric-coated polymer with the highest entrapment capacity (37, 38) among other polymers, Eudragit dissolves in a pH>5.5 solution (39). It enhances medication efficiency, enables targeting of particular gut regions, and shields the active ingredient from gastric juice. Eudragit polymers are adaptable polyacrylate polymers with varying levels of solubility, making them useful for formulations with prolonged release. Because

Eudragit S100 possesses solubility properties above pH 7, it can be used to target colonic release.

Literature revealed the very few method for polymeric nanoparticles drug delivery system of Sunitinib malate was studied for enhancement of oral bio-availability with anticancer activity. (40,41) While other methods are used such as self-nanoemulsifying drug delivery system (14,16). However, a varieties of nanotechnology based formulations have been investigated utilizing various polymers such as chitosan (42), PLGA (43-45), and Eudragit (46-48) for the preparation of nanoparticles of anticancer drugs.

The current work aimed at developing Sunitinib malate -loaded nanoparticles for colon- targeting by Nano-precipitation method (49-52) using enteric coating polymer Eudragit S100. Enteric coated Polymeric nanoparticle formulation that directly lead to an increase in oral bioavailability and colon targeting of anticancer drug.

In the present study, SM-loaded Eudragit S 100 encapsulated nanoparticles were successfully developed. From preliminary characterization, the optimized SM-loaded ESNP with desirable properties was further evaluated for its in vitro anticancer activity against colon cancer cell lines (CACO2).

2. Materials and method

2.1 Materials

Sunitinib malate was obtained from Mesochem Technology (Beijing, China). Eudargit S 100 and Poly vinyl alcohol were procured from “Sigma-Aldrich, St. Louis, USA”. Human colon cancer cell line (CACO2) purchased from American Type Culture Collection (ATCC). Ultrapure water was obtained from Milli Q water purifier unit from research, college of pharmacy.

2.2 Preparation of SM loaded ES100-NPs

SM loaded Eudragit nanoparticles were prepared by Nanoprecipitation method (52). The solubility of SM and polymer in several organic solvents, including dichlorometane, dimethyl sulfoxide, acetone, ethyl alcohol, and ethyl acetate, was tested. However, SM with polymer were easily soluble in dimethyl sulfoxide, the organic phase of choice. In order to solubilize the drug, the Eudragit S-100 polymer was first dissolved in DMSO (10 ml). Eudragit S100 polymer was used in

various proportions to create various SM formulations. Water that included PVA as a stabilizer was gently infused with the organic phase. Under reduced pressure and 45 °C, organic solvent was evaporated. After centrifuging at 5000 rpm for 60 min to separate the SM-loaded Eudragit nanoparticles from the bulk aqueous phase (2015, Centurion Scientific, UK), the nanoparticles were then washed with cold distilled water and dried using a freeze-drier (Martin Christ Alpha-1-4LD freeze-drier, Osterode, Germany). Composition of prepared SM loaded ES100NPs were documented (Table.1)

Table 1: Composition of developed SM loaded Eudargit- NP

Formulae	Suniti nib Malate (mg)	Eudragit S-100 (mg)	PVA (mg)
ESNPF1	10	100	100
ESNPF2	10	150	100
ESNPF3	10	200	100

2.3 Particles characterization

Mean particle size and PDI of developed ESNP (F1-F3) were measured by dynamic light scattering technique. Each developed NPs formulae were evaluated for mean particle size and PDI by “photon correlation spectroscopy using Zetasizer Nano ZSP, Particle Size Analyzer, Malvern, UK”. The SM loaded ESNPs dispersion were diluted (200 times) with deionized water and analyzed at room temperature (25°C) with an angle of detection of 90°. Each samples were analyzed three times.

2.4 Determination of drug encapsulation efficiency

Weighed amount of dried SM loaded ESNPs were dissolved in organic solvent and shaken lightly followed by sonication for 6 minutes. Thereafter, methanol will be added to precipitate the polymer that was present in NPs. The difference of drug content

between entrapped SM within ESNPs and free SM in organic phase of supernatant was used to calculate the percent drug entrapment efficiency (%EE) (53). The free SM in the supernatant obtained after ultracentrifugation of colloidal NPs was measured by HPLC method (54-55). The drug entrapment efficiency was then determined by using following equation

Where; W= amount of SM added initially in NPs;
w= amount of SM found in supernatant

2.5 Scanning Electron Microscopy (SEM)

The images of the optimized NPs were examined under the SEM “JSM-6360LV Scanning Microscope, Jeol, Tokyo, Japan”. The optimized ESNPF2 were vortexed for 2 minutes, and a drop of suspended NPs was spreaded on a glass slide and put up in a desiccators for drying. The dried NPs sample was put on carbon tape and sputter-covered utilizing a thin gold palladium layer under an argon climate utilizing a gold sputter module in a high-vacuum evaporator and coated sample was then scanned for imaging.

2.6 Fourier Transforms Infrared spectroscopy (FT-IR)

FT-IR spectra of pure SM and SM loaded ESNPs (F1-F3) were recorded with the help of “ALPHA-FTIR Spectrometer OPTIK, USA” The disc of each sample matrices was prepared using the potassium bromide (KBr) transparent pellets by applying pressure. The spectra was recorded in the wavelength range of “4000 to 400 cm^{-1} ” and spectra were interpreted with the help of IR software.

2.7 X-ray diffraction (XRD) studies

XRD studies of pure SM and optimized SM loaded ESNPs (F1-F3) were recorded by using X-ray diffractometer “Altima-IV, Japan”. The voltage/current used was 30 kV/25 mA and the target/filter (monochromator) was copper. Each spectrum was scanned at a rate of 4 $^{\circ}$ /min.

2.8 In vitro drug release

Using dialysis bags and a dissolution medium with a hydrochloric acid buffer (0.1 M) with a pH of 1.2 for

the first two hours, 6.8 for the next two hours, and 7.4 for the final end of the study till 24hr, the release profile of the medication that was entrapped in ES100 encapsulated NPs and Pure SM was investigated. To fill the dialysis bags, 0.1N HCl (10 mL, pH 1.2) was used to disseminate the exact weight of the nanoparticles (each holding the equivalent of 10 mg of drug) and pure drug. On a biological shaker (LBS-030S-Lab Tech, Kyonggi, Korea) set to 100 rpm, the dialysis bags were submerged in a beaker containing 40 mL of dissolution fluid. A 1.0 mL aliquot was taken at intervals of 0.5, 1, 2, 4, 6, 8, 12, 16, and 24 hours. To keep the overall volume constant, the removed volume was substituted with an equal volume of fresh medium. After an appropriate dilution, the samples were centrifuged for 10 min. at 500 rpm, and the supernatant was then subjected to HPLC analysis.

2.9 MTT Cell Proliferation Assay

The cytotoxicity of optimized ESNPF2 on colon cancer cell lines (CACO2) was determined via the reduction of “3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide, MTT, Sigma” to (dark purple) Formosan. Briefly, CACO2 cells were seeded in flat-bottom 96-well culture plate and incubated at 37 $^{\circ}$ C and 5% CO₂ in DMEM medium with various concentrations from 1.9-2000 μgM of NPs and pure SM for 72h. Immediately after removing of DMEM media, the CACO2 cells were incubated with MTT solution (5mg.ml⁻¹ in phosphate buffer) at 37 $^{\circ}$ C for 3h, and the control group was called as the untreated cells. Then, the clear supernatant medium was thrown away after centrifugation, and precipitated Formosan was solubilized with the addition of DMSO solvent. Finally, converted dye was analyzed in an ELISA reader at wavelength of 430 nm.

3. Results and discussion

3.1 Particles characterization and entrapment efficiency

The size of the resulting nano-particles was measured by dynamic light scattering (DLS) technique, the averaged particle size and PDI of ESNP (F1-F3) ranged from 129.5 \pm 4.85 to 462.5 \pm 7.03 nm and 0.32 to 0.65, respectively given in Table 2. The PDI is an

indicator of dispersion of particles. As per Malvern guide line mid value PDI ranged from 0.08 to 0.7 considered general purpose. All the formulae ESNP (F1–F3) could be considered average PDI come within the limit. In order to optimize NPs, three different amount of Eudragit S100 polymer concentrations (100, 150 and 200mg) were evaluated with constant amount of SM (10 mg) and Poly vinyl alcohol as surfactant (100 mg). It was observed that at minimum concentration of polymer, particle size decrease with minimum entrapment efficiency but at optimum concentration the increase in the concentration of Eudragit polymer in nanoparticles formulation could slight increase in particle size but decrease in entrapment efficiency. The result revealed that an increment in Eudragit S100 concentration also enhances the drug entrapment at optimum level after that it was decreasing. The higher content of Eudragit accommodated maximum amount of drug, it may be due to more space provided by Eudragit and would also minimize the leakage of drug into outer phase, thus securing maximum drug loading.

Table 2: Particle size, Poly-dispersity index and entrapment efficiency of prepared formulae of Sunitinib Malate

3.2 Surface morphology

Scanning electron microscopy (SEM) images of SM

Formulae	Particle size (nm)± SD*	PDI	% EE
ESNPF1	129.5 ± 4.85	0.327	30.70 ± 4.7
ESNPF2	384.8±5.53	0.58	60.31± 6.8
ESNPF3	462.5 ± 7.03	0.65	40.71 ± 3.7

loaded optimized ESNPF2 formulation are shown in Figure. 1. The shape and size of the nanoparticles were also confirmed by the SEM studies. The particle size observed from SEM image strongly supports the result of the particle size analyzer.

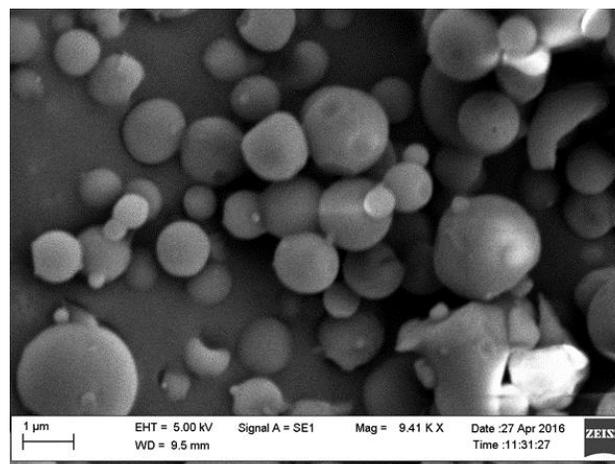


Figure. 1: SEM image of optimized ESNP formulation

3.3 FTIR analysis

Compatibility study of selected polymer Eudragit S100 and drug SM in nanoparticles formulation was carried out by the FTIR. The absorption peaks in the spectra were observed for SM, and SM loaded ESNPs in the region of 700–3600 cm⁻¹ (Figure.2). The major peaks assigned to the drug SM confirmed the presence of different functional groups in finger print region. The peak at 3224cm⁻¹and 3299 cm⁻¹ due to presence of acidic O-H, 2967cm⁻¹due to presence of H C=C H (aryl) str, 2867cm⁻¹, due to presence of C-H (alkyl)str, 1635 cm⁻¹ due to -NHCO str, and 1073 cm⁻¹ bands correspond to the (C-F stretching) (56) It was revealed from the spectra that there are no significant shifting /reduction in intensity of peaks between the drug and NPs. But reductions in the intensity of FTIR peaks were observed in the absorption spectra of SM loaded ESNPs corresponding to the drug in the region of 700–1650 cm⁻¹, which resulted from the overlapping of the SM and Eudragit S100.

3.4 X-ray diffraction (XRD) studies

XRD studies are important tools to identify the nature of powder, i.e., crystal or amorphous. X-ray diffraction patterns of SM, and SM loaded ESNPs (F1-F3) formulations are presented in Figure. 3. X-ray diffraction pattern of SM revealed several characteristic intense peaks at 13.3.1° 2-Theta, 19.5 ° 2-Theta, 22.4° 2-Theta, and 25.6 ° 2-Theta which represents the

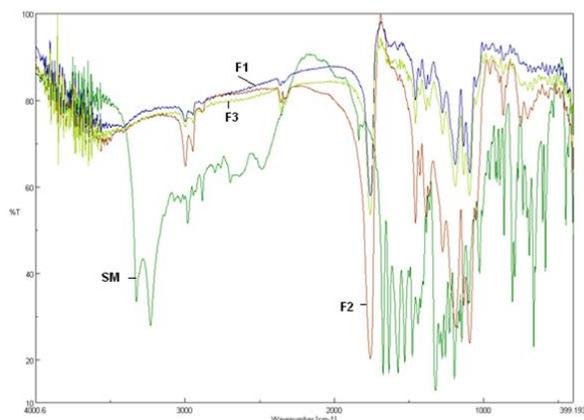


Figure. 2: Fourier transforms infrared (FTIR) spectra of SM and SM loaded ESNP (F1-F3)

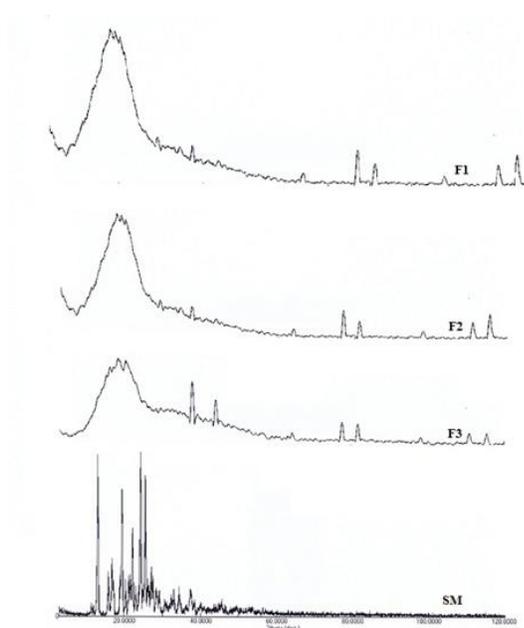


Figure. 3: X-ray diffraction (XRD) spectra of SM and SM loaded ESNP (F1-F3)

crystalline state of the drug [57]. However, these peaks were absent or reduced in peak intensity in the SM loaded ESNPs. This clearly indicates the reduction in the crystallinity of the precipitated ESNPs that SM was successfully encapsulated in the polymer.

3.5 In-vitro release studies

The release profile of pure SM and from optimized SM loaded ESNPF2 were evaluated at different pH 1.2, 2h pH 6.8, 4h and pH 7.4 end of study, their results was

showed in Figure 4. It was observed that pure drug showed rapid drug release (97.9 %) at the end of 6 h as compare to SM loaded ES100 nanoparticles. The ESNPF2 showed retarded drug release profiles, it showed 10.2 % drug release till 2 h, at 4h 29.6% and 12 h and 24 h 66.45%, 82.2% respectively at pH 7.4. The result showed that SM loaded ESNP has slow release at acidic pH and at the colonic pH it showed the maximum drug release.

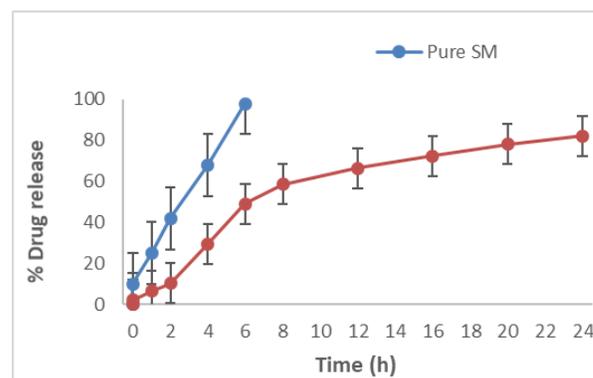


Figure. 4: In-vitro release studies of pure SM and SM loaded ESNP (F2) at pH 1.2, pH 6.8 and 7.4.

3.6 MTT Cell Proliferation Assay

The Figure 5 shows the in-vitro cytotoxic effects of pure SM and SM-encapsulated ES100NPs on (CACO2) cells. According to the results, the drug synthesized in ES100 NPs exhibits more cytotoxicity than the free drug. The free SM IC₅₀ dosage in 72 hours was approximately 9.825 μM. Encapsulated ESNP's IC₅₀ dose, however, was 5.916 μM during a 72-hour period. No (CACO2) cells were cytotoxic to the control cells.

In comparison to pure drug SM (76.28%, 69.08%, 55.049 %, 40.16.49%, 34.7%, 29.14.94%, 24.88%, and 16.6 % and 7.30% at 1.950, 3.910, 7.820, 15.6, 31.2, 62.5, 125, 250, and 500 μM, respectively), the formulation ESNPF2 significantly reduced cell viability (71.21%, 54.79%, 44.26%, 37.91%, 15.3%, 20.44%, 11.31%, 4.74% and 2.92 %). According to the results of the MTT assay, it was discovered that ESNPF2 showed potential anticancer action against colon cancer cell lines. This was most likely caused by the formulation's improved SM release. To treat colon

cancer, SM-loaded ES100NPs (ESNPF2) may be used as a powerful carrier. With increasing concentration cell viability will reduced.

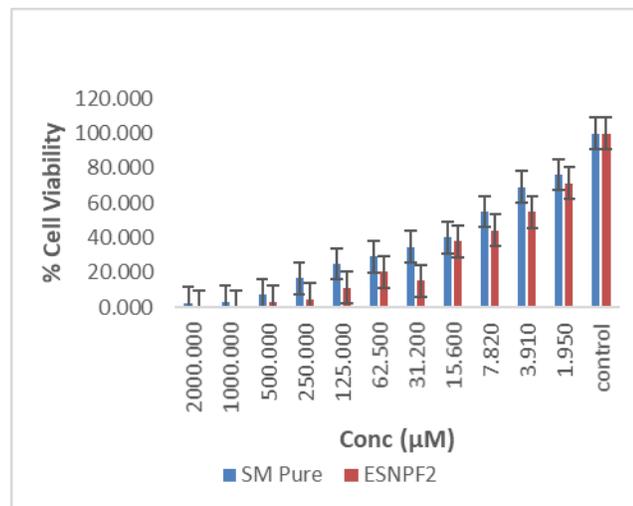


Figure. 5: (a) Cytotoxicity of pure SM and ESNPF2 after 72 h incubation with (CACO2) cells

4. Conclusion

ES100 nanoparticles of a novel anticancer drug Sunitinib malate were prepared by nano-precipitation method using PVA as a stabilizer so as to enhance release of drug and its efficacy. The optimized ESNPF2 of Sunitinib were characterized physicochemically and evaluated for in vitro drug release studies and cytotoxicity studies. The results of physicochemical evaluation suggested successful formation of ES100 nanoparticles. In vitro drug release studies indicated significant release profile of Sunitinib Malate from optimized ESNPF2 in comparison with pure Sunitinib. The result of cytotoxicity studies against CACO2 cells suggested significant cytotoxic effects of optimized SM loaded ESNPF2 in comparison with free Sunitinib. The outcomes of present study suggested the potential of ES-100 nanoparticles in the enhancement of in vitro dissolution rate and therapeutic efficacy of Sunitinib.

Conflict of interest: No conflict of interest is associated with this manuscript.

Contribution of authors: We declare that this work was done by the authors named in this article, and all

liabilities relating to the contents of this manuscript will be borne by the authors.

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