Section A-Research paper
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DEVELOPMENT AND EVALUATION OF SOLUPLUS/TPGS 1000 LOADED MIXED POLYMERIC MICELLES OF SORAFENIB TOSYLATE FOR HEPATOCELLULAR CARCINOMA

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Abstract:
One of the most prevalent malignant tumours is hepatocellular carcinoma (HCC), and the majority of HCC patients receive an advanced-stage diagnosis. [1-2] Sorafenib Tosylate is frequently used for liver cancer treatment. In addition to increasing sorafenib's water solubility, sorafenib Tosylate loaded polymeric mixed micelles of soluplus and Vitamin E TPGS 1000 additionally enhance sorafenib's bioavailability. Thin-film hydration method was used for producing the mixed micelles, which helped to increase the stability and effectiveness of drug solubilization. To determine the ideal polymeric ratio, we generated several batches of sorafenib micelles using different ratios. Polymers like Soluplus, TPGS 1000, and Pluronic F127 were evaluated during the dosage form development. The entire formulation batches the F2 batch with optimal drug content having 87.1 nm particle size and 1 mV zeta potential. By using FTIR, SEM, TEM, DSC, and microscopic analysis, the formulation was assessed.

Keywords: Sorafenib Tosylate, Soluplus, TPGS, micelles, Thin film hydration, SEM, TEM, FTIR, DSC

Introduction

According to studies conducted around the world, the creation of innovative nano-sized particle drug delivery systems (DDS) has proven to have an important influence on the prevention, diagnosis, and treatment of disease. [3] Depending on the concentration and content of the polymers, it is simple to build micellar delivery systems smaller than 2 nm to 200 nm. Although micelles allow for deep tissue penetration for targeted medication administration, they typically break down quickly in the body. So it is difficult to deliver drugs from micellar nanocarriers over an extended period of time, particularly sustained drug delivery. Among polymeric nanoparticles, polymeric micelles prepared by the self-assembled structure of amphiphilic block co-polymers are undoubtedly one among the most well-known DDS. Due to a nanoshell shape (Core-shell) that is extremely water-soluble while still having

a hydrophobic core suited for hydrophobic medicines, micelles in particular are the subject of interest as prospective drug carriers. Due to the fact that many medications are frequently rendered insoluble in water, putting them onto drug carriers can significantly boost their solubility.

D-alpha-tocopheryl polyethylene glycol 1000 succinate or TPGS Tocofersolan is a medicinal ingredient that has been granted FDA authorization. It is a derivative of water-soluble vitamin E that is created when polyethylene glycol succinate 1000 and vitamin E succinate are esterified. In order to lessen side effects and boost treatment effectiveness, TPGS can induce apoptosis in cancer cells and demonstrates specific cytotoxic effects against them. With a CMC of 0.02 weight percent, Micelles can be produced by vitamin E TPGS 1000 for transportation of drugs or imaging molecules. Due to the sorafenib tosylate's assimilation into the mixed micelle's inner cores, the drug's release from the mixture exhibited a sustained release pattern. Given these benefits, our investigation showed that Advanced HCC can benefit by employing SF micelles as a therapeutic agent. [4]

A naturally amphiphilic structure can be found in Soluplus also known as polyvinyl caprolactam-polyvinyl acetate-polyethylene glycol graft co-polymer (PCL-PVAc-PEG). It is been demonstrated that when its concentration exceeds the critical micelle concentration (CMC) required to form micelles, it exhibits dazzling features that could increase the ability of drugs that aren't easily soluble in water to be administered orally. [5]

This research outlines, enhancement of the solubility which helps in improvement of bioavailability of sorafenib tosylate by a novel micelle formulation, based on the flaw in sorafenib tosylate and the benefit of Polymeric micells. By employing the method of thin-film hydration, sorafenib tosylate-loaded Soluplus®/TPGS 1000 mixed micelles were created.

Material and Method

Materials:

Sorafenib Tosylate was obtained as a gift from Cipla R&D (Mumbai, Maharashtra, India). TPGS Tocofersolan or D-alpha-tocopherol polyethylene glycol 1000 succinate, or vitamin E TPGS 1000 was procured from PMC Isochem, Lavoisier, France. Soluplus was obtained from BASF, India. Dimethylformamide (DMF) and Deionized water was obtained from S.D. Fine chemicals, Mumbai & Ajinkya Enterprises, Pune respectively.

Preparation of Sorafenib micelles:

I. 5 mg of drug and both the polymer taken in a ratio of 4:1{TPGS (72 mg) + Soluplus (18 mg)} & dissolved in 3 ml Dimethylformamide.
II. Take all the ingredients in round-bottomed flask (RBF).
III. To create a solid matrix, At 40°C, the solvent was rotary evaporated for one hour.
IV. Any leftover DMF in the film was vacuumed out over the course of an entire night at room temperature.
V. To create a transparent micelle solution, the film formed at bottom of flask was then hydrated by deionized water (10ml) and again placed at rotary evaporator for 30 minutes.

VI. The fluid was then centrifuged for five minutes at 7000 RPM to remove any unencapsulated sorafenib medication. Sorafenib-loaded Soluplus®/TPGS 1000 mixed micelles as an opalescence suspension was obtained when the supernatant was removed.

Critical Micelles Concentration (CMC) determination

The CMC of Sorafenib-loaded polymeric mixed micelles in double-distilled was evaluated using an iodine ultraviolet-visible (UV-visible) spectroscopy technique, The utilisation of an iodine hydrophobic probe. 50 mL of deionized water were used to dissolve 0.5 gram of iodine (I2) and 1 gram of potassium iodide (KI) to create the solution of standard Potassium iodide/Iodine (KI/I2). Polymer solution test specimens were created with different concentrations varied between 0.00001% to 0.1%. 25 L of the standard solution was poured to each solution of the Soluplus®/TPGS 1000 mixture. Prior to measurement, the mixes were allowed to equilibrate for 12 hours while being kept at room temperature in a dark area. A UV-Vis spectrometer was used to evaluate the absorbance value of different concentrations of polymer at 366 nm. Plotting the graph of absorbance versus the copolymer content, the values for the CMC with different weight proportions of TPGS 1000 and Soluplus® (5:0, 4:1, 3:2, 2:3, 1:4, and 0:5) were determined. [6-7]

Characterization

Measurement of particle size:

Formulations for nanomicelles were analysed to establish the mean particle size and polydispersity index (PDI) of the size distribution using Horiba SZ100Z. The size distribution and average particle size were noted. The standard deviation (SD) for three different experiments is used to describe the mean size of each sample in the provided experimental data.

Zeta potential measurement:

Beckman Coulter (Beckman Coulter DelsaTM Nano Common) was used to assess the formulation's zeta potential.

Entrapment efficiency:

Using a Microcentrifuge (Remi), a small amount of the nano dispersion was centrifuged at 10,000 rpm for one hour. By evaluating absorbance of adequately diluted supernatant solution at 264 nm with a UV spectrophotometer (Jasco V530) and comparing it to a blank/control nano dispersion, the amount of unincorporated medication was determined after the supernatant was removed. Wang Y. et al. et al., 2013; Gan et al., 2009) The following equation was used to determine entrapment efficiency. [8]

$$\text{Entrapment Efficiency (\%)} = \frac{\text{W (initial amt of drug)} - \text{W (free amt of drug)}}{\text{W (Initial amt of drug)}} \times 100$$

Critical Micelle Concentration (CMC):

The Critical Micelle Concentration of Sorafenib Tosylate containing polymeric micelles in double distilled water was determined using an iodine UV spectroscopy technique, as was previously described. 50 mL of deionized water were used to dissolve 0.5 gram of iodine (I2) and 1 gram of potassium iodide (KI) to create the solution of standard Potassium iodide/Iodine (KI/I2). Polymer solution test specimens were created with different concentrations varied between 0.00001% to 0.1%. 25 L of the standard solution was poured to each solution of the Soluplus®/TPGS 1000 mixture. Prior to measurement, the mixes were allowed to equilibrate for 12 hours while being kept at room temperature in a dark area. A UV-Vis spectrometer was used to evaluate the absorbance value of different concentrations of polymer at 366 nm. Plotting the graph of absorbance versus the copolymer content, the values for the CMC with different weight proportions of TPGS 1000 and Soluplus® (5:0, 4:1, 3:2, 2:3, 1:4, and 0:5) were determined. [7]

FT-IR Study:

To evaluate for drug-polymer compatibility, FT-IR spectra of pure drug (Sorafenib Tosylate) and drug-polymer nanomicelles were taken. In order to determine whether the primary peaks of FT-IR spectrum of pure drugs had changed, the FT-IR spectruum of nanomiclles were compared to those of pure drugs.

Differential scanning calorimetric analysis (DSC):

On a differential scanning calorimeter (DSC Mettle STARS SW 9.20, Switzerland) with an intra-cooler, DSC measurements of a formulation with a 4:1 ratio were successfully carried out. 50 ml/min of nitrogen gas was utilised to purge and maintain inert atmosphere. The sample was heated from 40 to 240 degrees Celsius under nitrogen gas stream (20 ml/min), heating it at a rate of 10 degrees per minute using all precisely weighed samples (approximately 3-5mg of samples) that they resided in a pan made with sealed aluminium. As a guide, an empty pan was used. (Chacko et al. 2012).

Transmission Electron Microscopy (TEM):

TEM (JEM-100CX electron microscope) was used to analyse the surface morphology of Sorafenib tosylate-loaded Soluplus®/TPGS. The mixture was poured onto a grid made of copper that was previously coated with carbon prior being treated for 10 seconds with a phosphotungstic acid solution (2%, w/v). After removing the remaining solution, the formulation was kept to dry at room temperature before being shot with a TEM while accelerating.

Scanning electron microscopy (SEM):

Using a scanning electron microscope (SEM) (JEOL, JSM 6370), the morphological characteristics of the Sorafenib tosylate-loaded Soluplus®/TPGS formulation were examined. One sample drop was applied to a slide, and any extra liquid was allowed to dry at ambient temperature [8]. Double-coated adhesive tape was used to secure the slide to the specimen holder. Gold was then applied to the slide under vacuum using a sputter coater for 10 minutes to create a consistent coating that would allow for high-quality SEM photos. With a load

current of roughly 80MA and a modest accelerating voltage of about 15KV, the SEM was operated. (Hwang et al., 2002).

In vitro release of Sorafenib tosylate:

In Phosphate buffer solution (pH 7.4) with 0.5% Tween 80, which approximated the pH value at 37°C for physiological environment as well as tumour microenvironment, respectively, the in vitro behaviour of the drug was examined. Over the course of 72 hours, sorafenib tosylate released from Soluplus®/TPGS 1000 mixed micelles and free sorafenib tosylate mixture (used as a control and dissolved in water) were both subjected to dialysis. In a nutshell, a dialysis membrane bag that has swelled was filled with 2 mililitre of sorafenib tosylate-loaded Soluplus®/TPGS 1000 mixed micelles and free sorafenib tosylate mixture with an equal sorafenib tosylate concentration of 0.5 mg/ml. Additionally, release media (200ml) was introduced to an orbital shaker, where the dialysis bag was incubated at 37°C while being stirred at 100 rpm. At the predetermined time intervals (0.5, 1, 2, 4, 6, 8, 12, 24, 48, and 72 h), 2 ml samples was pipette out and filtered through a 0.22 m membrane. The whole release medium was then displaced with a fresh medium volume in an equal amount that had been preheated to 37 °C. [7]

Stability of sorafenib tosylate micelles:

Over the course of a week, the stability of SF polymeric mixed micelles was examined at 4°C and at room temperature (37°C). Particle size and polydispersity index (PDI) measurements of polymeric mixed micelles were taken at the designated intervals (0.5, 1, 2, 4, 6, 8, 12, 24, 48 h). Additionally, after being pre-frozen at 80°C in the refrigerator for 0.5 hours, the optimised sorafenib tosylate loaded polymeric mixed micelle solution lyophilized at temperature 50°C and pressure of 0.01 mbar. The lyophilized powder was removed from the freeze drier one day later and kept at 4°C. To evaluate the particle size and PDI, Three months later, the polymeric mixed micelles powder was regenerated in water. [10]

Stability under storage condition:

Sorafenib tosylate loaded mixed micellar solution is freshly made and then put into glass vials where it is kept chilled at 4°C for 45 days. After 45 days, the size and zeta potential of the micelles were analysed to figure out the effect of storage conditions on the stability of the formulation.

Results and discussion

Micelle characterization

In the current work, six distinct Soluplus®/TPGS 1000 proportions (5:0, 4:1, 3:2, 2:1, 1:4, and 0:5) were used to create blank and sorafenib tosylate drug-loaded polymeric mixed micelles in order to assess their effects on micelle characterisation. Table 1 displays mean particle sizes, PDI, and zeta potential. Particle sizes in the nano range were present in all batches. The range of the particle size was determined to be between 70.4 ± 13.768 and 95.9 ± 3.549. The size of the micelles is another aspect that can affect sorafenib tosylate's bioactivity. The 100 nm-sized polymeric micelles developed during this study were satisfactory to avoid the reticuloendothelial system's (RES) detection and phagocytosis, enabling them to remain in the blood or systemic circulation for a longer time frame and

passively target solid tumours through the improved penetration and retention effect. The Soluplus®/TPGS 1000 (4:1) preparation has a larger drug loading and a preferred particle size for greater bioavailability depending upon characterisation parameters of micelles.

Table 1: Characterization of Sorafenib tosylate-loaded Soluplus®/TPGS 1000 mixed micelles

Sr.No	Formulation Code	Soluplus: TPGS 1000 concentration (% w/v: % w/v)	Particle size(nm) ± SD	PI ± SD (n=3)	Zeta potential ± SD
1	F1	5:0	95.9 ± 3.549	0.136 ± 1.23	4.3 ± 0.5312
2	**F2**	**4:1**	**87.1 ± 1.9442**	**0.152 ± 0.025**	**1 ± 0.1633**
3	F3	3:2	69.4 ± 8.1197	0.317 ± 0.048	0.64 ± 0.0741
4	F4	2:3	95.3 ± 8.1939	−6.16 ± 0.84	3.2 ± 0.6018
5	F5	1:4	70.4 ± 13.768	0.324 ± 0.029	0.4 ± 0.4497
6	F6	0:5	59.6 ± 10.391	-6.35 ± 0.78	-6.35 ± 1.0925

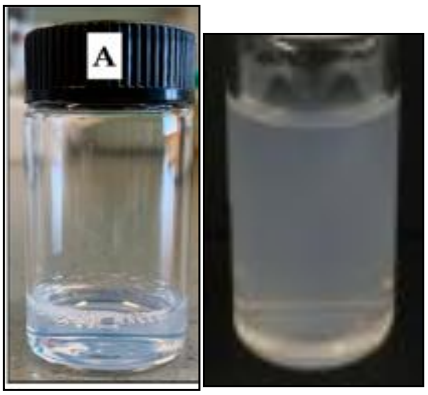

Figure 1: (A) Empty Mixed polymeric micelles, (B) Sorafenib Loaded Mixed polymeric micelles

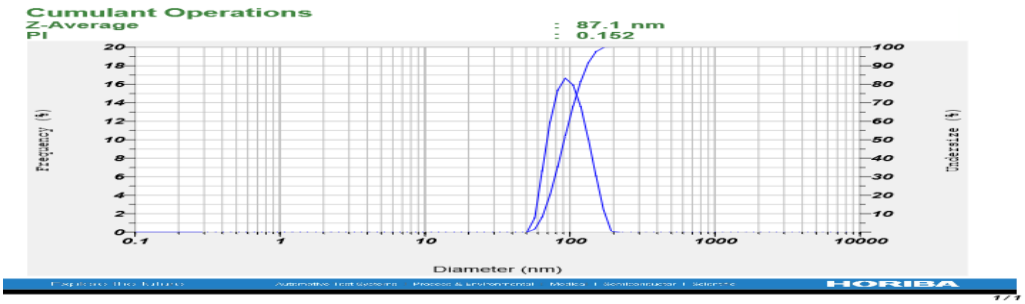

Figure 2: Particle size representation graph with Horiba particle size analyzer of formulation F2 (4:1)

Zeta potential of the optimised formulation was discovered to be 1 ± 0.1633 mV. Zeta potential should be between -30 and -60 or between +30 and +60 for nanomicelles to remain stable.

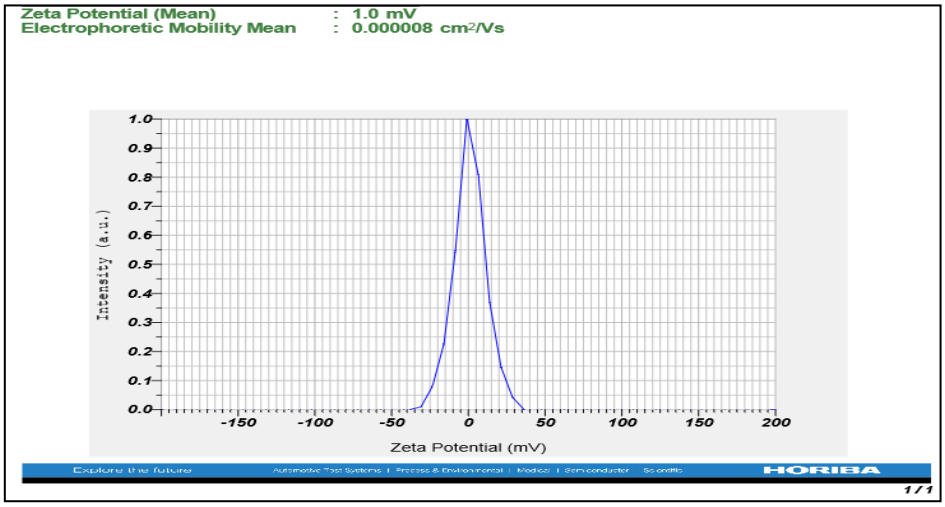

Figure 3: Zeta potential graph of optimized batch F2

Critical Micelle Concentration (CMC)

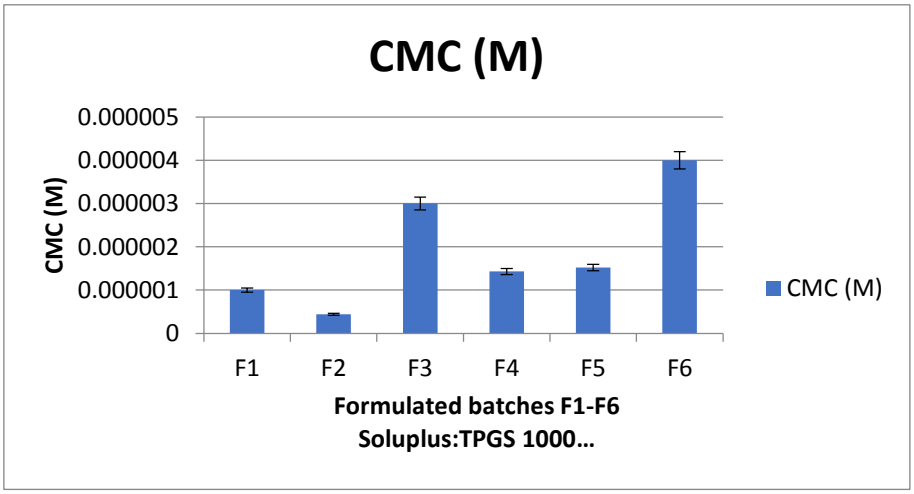

Figure 4: CMC values for Soluplus/TPGS 1000 mixed polymeric micelles with sorafenib

Identification of drug

- Identification by HPLC Chromatographic method

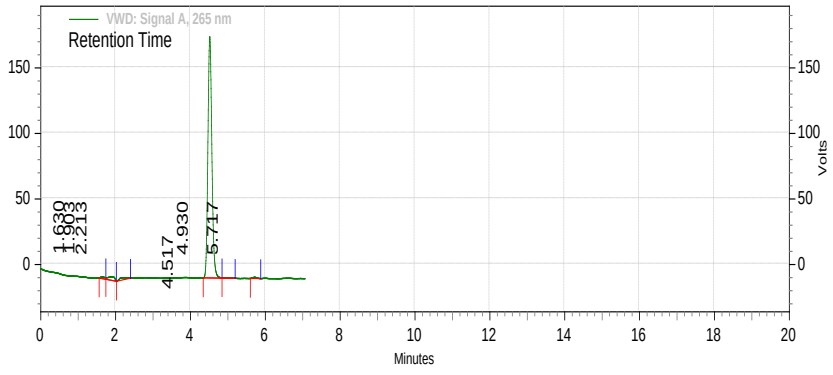

Figure 4: HPLC chromatogram of Sorafenib Tosylate

- FTIR spectrum of drug

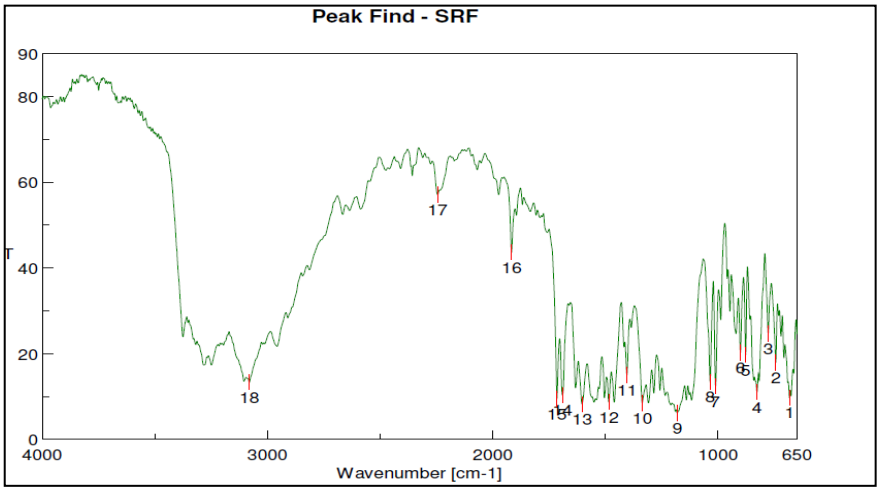

Figure 5: FTIR spectrum of Sorafenib tosylate

- Differential scanning calorimetric analysis

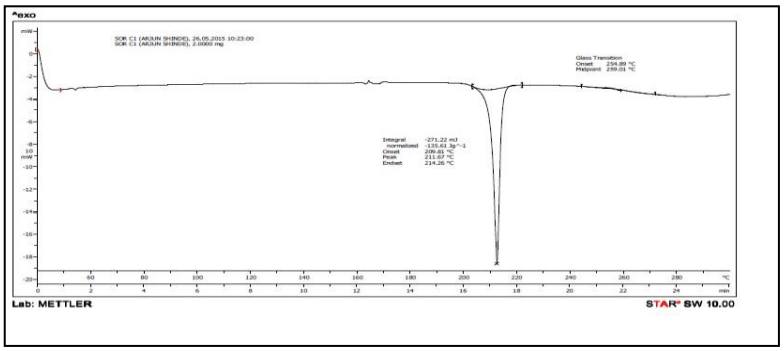

Figure 6: Differential scanning calorimetry (DSC) of sorafenib tosylate

10 °C/min heating rate and under nitrogen environment, the curve was measured from 20 to 500 °C. The mixture melted at a temperature of 223.68 °C.

- X-Ray Diffraction (XRD):

The aim of the XRD investigation was to ascertain the nature of medication. Graph of 2θ versus peak intensity was plotted. The nature of drug was found to be crystalline.

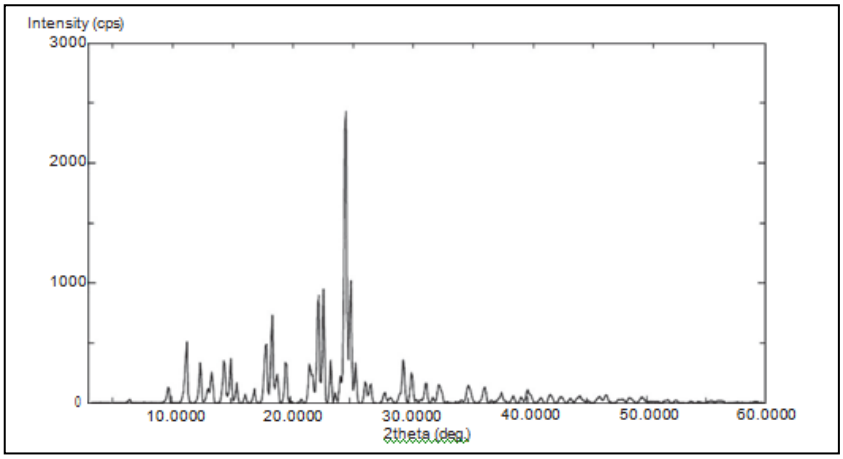

Figure 7: XRD diffractogram of Sorafenib tosylate

- Scanning electron Microscopy

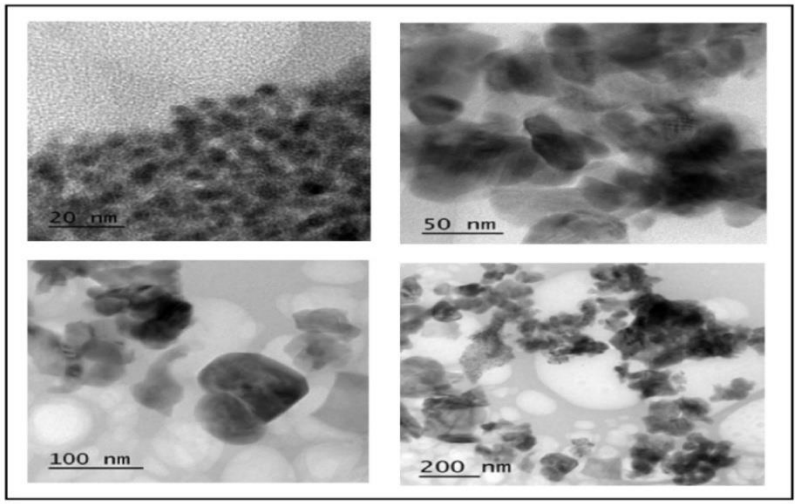

Figure 8: Scanning electron microscope of SRF

Drug loading and encapsulation efficiency(EE%)

UV-Vis spectrophotometry was used to measure the drug loading denoted by DL and encapsulation efficiency denoted by EE% or %EE, at 265 nm, found to be as maximum absorbance. Prior to analysis, the polymeric micelle mixture was appropriately diluted using DMF. Equations below were used to determine DL% and EE%:

DL% = Weight of the drug in micelle
/Weight of the polymer and drug × 100

EE% = Weight of the drug in micelle
/Weight of the feeding drug × 100

Table 12: Drug loading and Entrapment efficiency of sorafenib loaded Soluplus®/TPGS 1000 mixed micelles

Sr.No	**Formulation Code**	**Soluplus: TPGS 1000 proportion (% w/v:% w/v)**	**Drug Loading (DL) ± SD**	**(EE %) ± SD**
1	F1	5:0	0.650 ± 0.094	12.97 ± 1.89
2	**F2**	**4:1**	**4.630 ± 0.085**	**92.59 ± 1.71**
3	F3	3:2	4.539 ± 0.015	85.1 ± 1.50
4	F4	2:3	3.224 ± 0.045	87.95 ± 1.95
5	F5	1:4	3.331 ± 0.253	39.4 ± 0.20
6	F6	0:5	2.07 ± 0.02	49.97 ± 1.89

Microscopic Evaluation of optimized batch

Microscopic evaluation shows us micelles are in spherical shape and drug is incorporated into it.

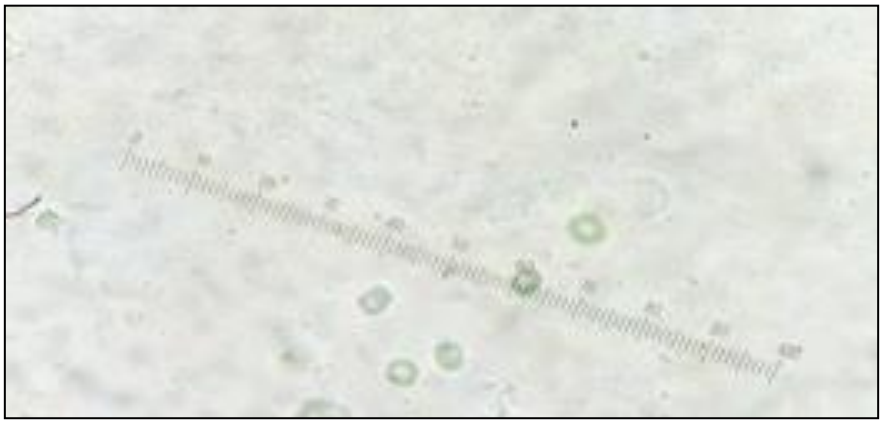

Figure 9: Microscopic evaluation of micelles into 100x magnification

Drug release study

Physiological saline comprising of 0.5% Tween 80 (w/v), the table illustrates the in vitro study of sorafenib Tosylate from Soluplus®/TPGS 1000 (4:1) mixed micelles systems. Within 72 hours, around 95% of the medication in Soluplus®/TPGS 1000 (4:1) micelles systems disappeared from the dialysis bag. Due to the steady inclusion of hydrophobic sorafenib Tosylate in the core of the polymeric mixed micelles, sorafenib Tosylate-loaded Soluplus®/TPGS 1000 mixed micelles had a strong maintained-release characteristic. Sorafenib Tosylate's sustained release may offer a constant drug concentration and prolong the duration of its therapeutic effects.

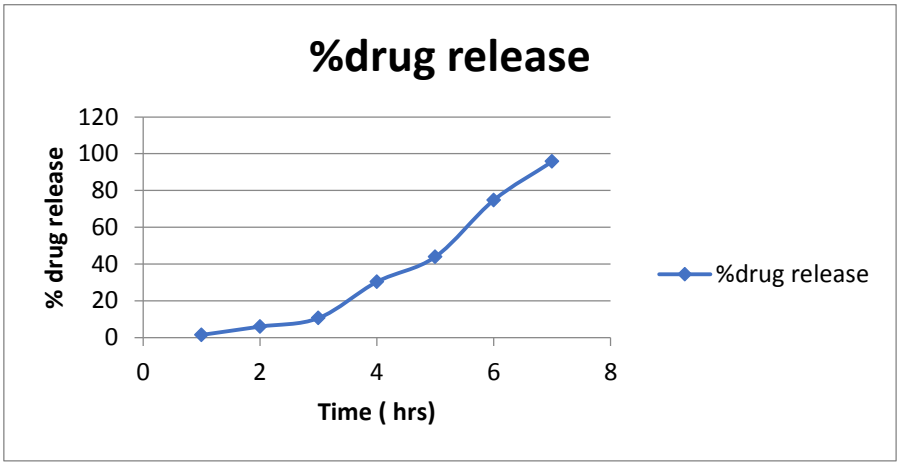

Figure 10: Release profiles of sorafenib tosylate from sorafenib-loaded Soluplus®/TPGS 1000 mixed micelles at 37 °C in phosphate buffer medium of pH 7.4 comprising of 0.5% Tween 80

Stability Study

- Particle Size

Soluplus®/TPGS 1000 (4:1) micelle were stored at refrigerated condition at 4°C and at room temperature at 37 °C to examine the stability on storage of the ideal formulation. Our findings revealed that the micelles remained stable after 7 days, since their size and PDI were constant at 4 °C and 37 °C throughout this time, and no drug precipitation was discovered.

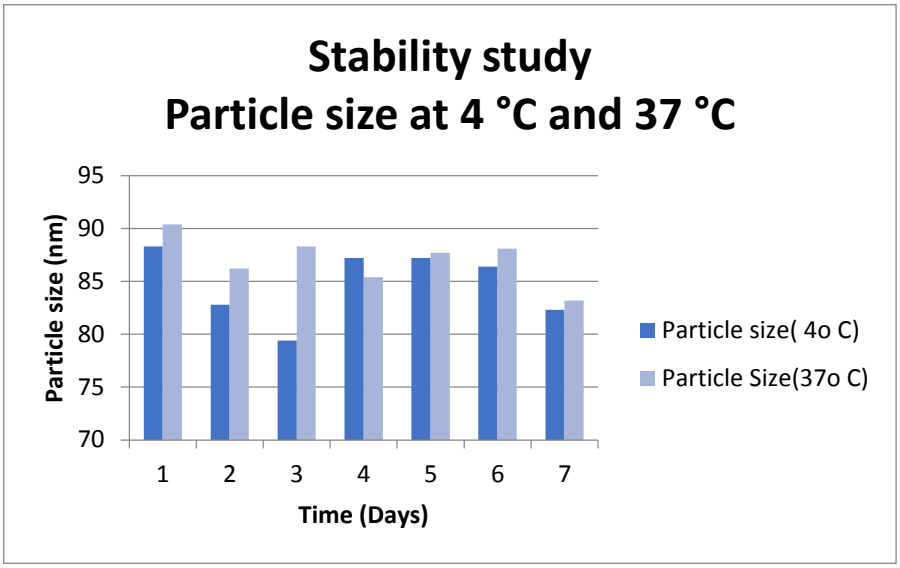

Figure 11: The particle size of Sorafenib tosylate-loaded polymeric mixed micelles at 4 °C and 37 °C after 7 days

- Zeta Potential

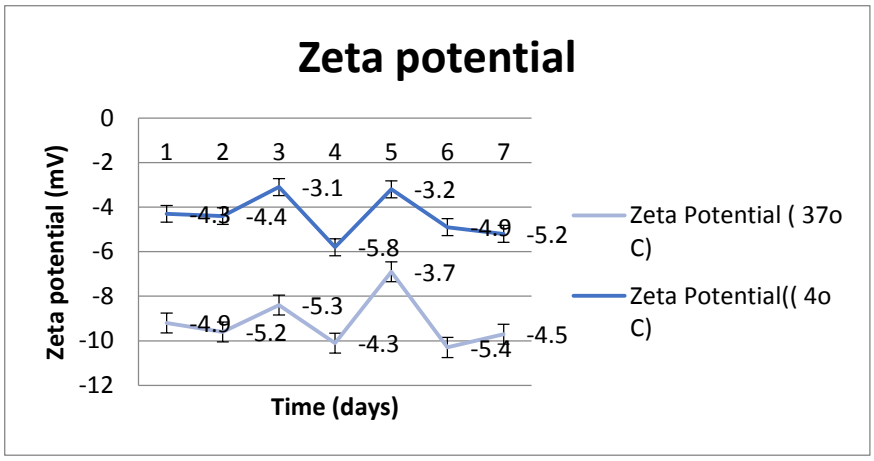

Figure 12: The Zeta Potential of Sorafenib tosylate-loaded polymeric mixed micelles at 4 °C and 37 °C after 7 days

- PDI

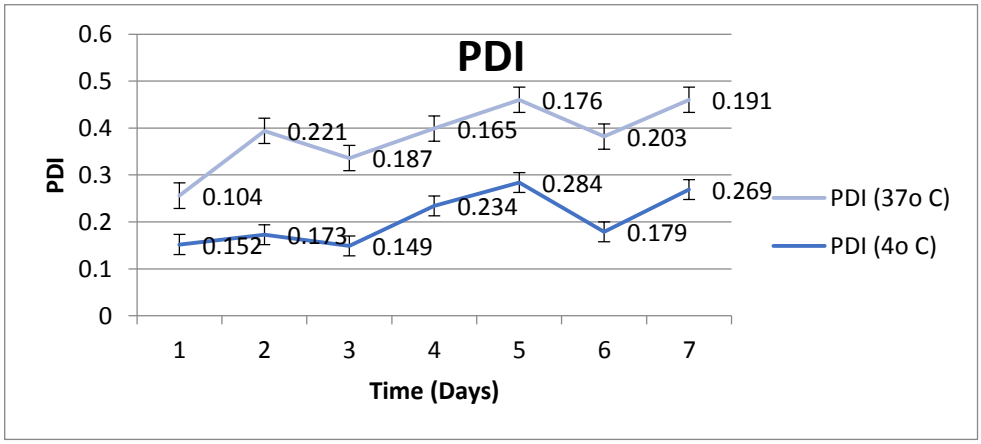

Figure 27: The PDI of Sorafenib tosylate-loaded polymeric mixed micelles at 4 °C and 37 °C after 7 days

Conclusion

In the present investigation, mixed polymeric micelles were produced that included the anticancer medication sorafenib tosylate, which is not easily soluble. The mixed micelles were prepared by method of thin-film hydration , which helped to increase the stability and effectiveness of drug solubilization. The F2 batch of the total formulation, with particle sizes of 87.1 nm and 1 mV zeta potential, contains the most medication.Using docking software; a drug's interaction with the VEGFR 2 receptor was studied. Due to the sorafenib tosylate's incorporation in the inner core of the polymeric mixed micelles, the drug's release from the mixture exhibited a sustained release pattern. Our research demonstrated that, when these advantages are taken into account, SF micelles have the capacity to be employed as a therapeutic agent for the treatment of advanced HCC.

References

1. Tsukamoto T, Kawasaki T, Yamauchi T: Saponins of Japanese Dioscoreaceae. V. On the structure of dioscin. Pharm Bull 1956, 4:35-42.
2. Cho J, Choi H, Lee J, Kim MS, Sohn HY, Lee DG: The antifungal activity and membrane-disruptive action of dioscin extracted from Dioscoreanipponica. BiochimBiophys Acta 2013, 1828**:**1153-1158.
3. Bhalekar, M.R., et al., Formulation of piperine solid lipid nanoparticles (SLN) for treatment of rheumatoid arthritis. Drug Dev Ind Pharm, 2017. 43(6): p. 1003-1010.
4. Shah, A.R.; Banerjee, R. Effect of d-tocopheryl polyethylene glycol 1000 succinate (TPGS) on surfactant monolayers. Colloids Surf. B Biointerfaces 2011, 85, 116–124.
5. Bernabeu, E.; Gonzalez, L.; Cagel, M.; Gergic, E.P.; Moretton, M.A.; Chiappetta, D.A. Novel Soluplus®—TPGS mixed micelles for encapsulation of paclitaxel with enhanced in vitro cytotoxicity on breast and ovarian cancer cell lines. Colloids Surf. B Biointerfaces 2016, 140, 403–411.
6. Ranieri GG-C G, Goffredo V, Patruno R et al. Sorafenib (BAY 43–9006) in hepatocellular carcinoma patients: from discovery to clinical development. Curr. Med. Chem. 19, 938–944 (2012)
7. C. Bothiraja, P. P. Joshi, G. Y. Dama and A. P. Pawar, Eur. J.Intern. Med.,D-a-Tocopheryl polyethylene glycol 1000succinate conjugated folic acid nanomicelles: towards enhanced bioavailability, stability, safety, prolonged drug release and synergized anticancer effect of plumbagin, 2011, 3, 39
8. Dou, J.; Zhang, H.; Liu, X.; Zhang, M.; Zhai, G. Preparation and evaluation in vitro and in vivo of docetaxel loaded mixed micelles for oral administration. Colloids Surf. B Biointerfaces 2014, 114, 20–27.
9. Zhu MH, Chen SC, Hua LB et al. Self-targeted salinomycin-loaded DSPE-PEG-methotrexate nanomicelles for targeting both head and neck squamous cell carcinoma cancer cells and cancer stem cells. Nanomedicine 12(4), 295–315 (2017).
10. Zhang, Z.; Lee, S.H.; Feng, S.S. Folate-decorated poly(lactide-co-glycolide)-vitamin E TPGS nanoparticles for targeted drug delivery. Biomaterials 2007, 28, 1889–1899.