IJNRD.ORG ISSN : 2456-4184
INTERNATIONAL JOURNAL OF NOVEL RESEARCH AND DEVELOPMENT (IJNRD) | IJNRD.ORG
An International Open Access, Peer-reviewed, Refereed Journal

Formulation Development, Optimization and Characterization of Folate Conjugated Solid Lipid Nanoparticle (FC-SLNs) for Effective Lung Cancer Targeting

(FC-SLNs for Effective Lung Cancer Targeting)

Vibha Rajoriya[1], Vaibhav Rajoriya[2], Ajay Singh Thakur[2], Yogesh Sharma[2], Ramdarshan Parashar[2], Surbhi Chourasiya[3], Kamlesh Patel[3], Prince Yadav[3], Avani Mishra[2],

[1]M. Pharm., Vedic Institute of Pharmaceutical Education and Research, Sagar, Madhya Pradesh
[2]Associate Professor, Vedic Institute of Pharmaceutical Education and Research, Sagar, Madhya Pradesh
[3]Assistant Professor, Vedic Institute of Pharmaceutical Education and Research, Sagar, Madhya Pradesh

Abstract: The object of the paper is preparation, optimization and characterization of folate conjugated solid lipid nanoparticle (FC-SLNs) for lung cancer targeting potential. The Prepared formulation was optimized for various parameters i.e., particle size, PDI, zeta potential and characterized by Scanning Electron Microscopic (SEM) and Transmission Electron Microscopic (TEM) technique. The folate conjugated (FC-SLNs) and non-conjugated (P-SLNs) formulations have been prepared by solvent evaporation method. The P-SLNs and FC-SLNs have shown the particle size 190.1±1.9 and 231.3±2.3 nm with a zeta potential of 13.41±0.3 and -4.2±0.2 mV respectively. The *In-vitro* drug release studies at pH 4 and 6.4 were carried out in the view that the tumor vicinity is relatively acidic in nature. The rapid release behavior of the drug from SLNs formulations at pH 4 supports the hypothesis that the SLNs formulation will release the drug in higher acidic condition, whenever it will reach the target site i.e. tumor; as the pH in such environment is generally below 7. The characterized parameter and drug release data suggested that FC-SLNs are a safe, stable, and potentially promising drug delivery system for lung targeting.

Index Terms - **Solid lipid nanoparticle, folate, lung cancer, paclitaxel, zeta potential, drug release**

INTRODUCTION

In lung cancer, over 7,36,000 people are expected to succumb to the disease in 2016 while the figure is estimated to shoot up to 8,80,000 by 2020. ICMR stated that India is likely to have over 17,30,000 new cases of cancer and over 8,80,000 deaths due to the disease by 2020 with cancers of breast, lung and cervix [Siegel and Miller, 2017]. In 12th 5-year plan, Ministry of Health, Government of India is focusing on four non-communicable diseases (cancer, diabetes mellitus, hypertension and stroke) [Chang, 2011]. This is because, currently more than 10 lakh new patients are confirmed to have cancer on biopsy every year and our citizens are facing a tremendous increase in the projected incidence of various cancers. Lung cancer is one of the commonest cancers and cause of cancer related deaths all over the world. It accounts for 13 percent of all new cancer cases and 19% of cancer related deaths worldwide [Paumier and LePechoux, 2010].

In India, lung cancer constitutes 6.9% of all new cancer cases and 9.3 percent of all cancer related deaths in both sexes, it is the commonest cancer and cause of cancer related mortality in men, with the highest reported incidences from Mizoram in both males and females (age adjusted rate 28.3 and 28.7 per 100,000 population in males and females, respectively). The time trends of lung cancer show a significant rise in Delhi, Chennai and Bengaluru in both sexes. The incidence and pattern of lung cancer differ as per geographic region and ethnicity and largely reflect the prevalence and pattern of smoking. The overall 5-year survival rate of lung cancer is dismal with approximately 15 percent in developed countries and 5 percent in developing countries [Sunderland et al., 2006]. Lung tumors are one of the main types of cancer and the first cause of death due to cancer in the United States, thus, the achievement of an effective and specific treatment is challenging.

NEED OF THE STUDY

The present chemotherapy for the treatment of lung cancer is not efficient by the procedure that involved, drug administered to the patient by the intravenous route by saline, result happen that it will kill the huge number of normal cells with smaller number of cancerous cells [Zhang et al., 2013]. The occurrence of side effect and the baldness is happened in the prime stage of chemotherapy of cancer. Taken these points, into consideration, the site specific and targeted delivery of anticancer drugs is

needed for further the improvement of bioavailability of the drug as well as diminished the side effect related to the anticancer drug. Exploring the anticancer drug by the nanoparticulate system for a site-specific target is a potential approach in the field of nanotechnology. Current research efforts made Solid-lipid Nanoparticle (SLNs) as a carrier to target lung cancer and other cancer sites [Kim et al., 2015]. SLNs have been a potential drug carrier for the lipophilic drug, it provides better drug entrapment, better stability, enhance cellular uptake and availability of drugs towards the targeting tissue and cells [Vaidya et al., 2009]. Paclitaxel (P) is a mitotic inhibitor is now widely used in the treatment of different types of cancers, including lungs, ovarian, breast, head, neck and advanced forms of Kaposi's sarcoma [Mansoori et al., 2010]. Paclitaxel promotes tubulin polymerization and stabilizes microtubules towards depolymerization.

Active targeting employs the use of ligands on the carrier surface for specific recognition by the cell surface receptors [Muller et al., 2011] accomplished limiting drug distribution towards the normal cell, result in reducing the side effects and directed towards target organ by the cell recognition [Parkar et al., 2005]. Folate (FA) can enter cells through a receptor-mediated endocytosis pathway. Folate receptors (FR) are over-expressed in many human cancer cell surfaces on an order of 100 times more than normal, healthy cells and tissue. It was also reported that cancers of the pancreas, testicles, bladder, prostate, and liver often do not show elevated levels of folate receptors [Zwicke et al., 2012]. The folate receptor is a glycosyl phosphatidyl inositol, anchored cell surface receptor suitable for targeting because of its relatively low expression level in healthy tissues and high expression in the cancerous cell through a carrier protein termed as reduced folate carrier via receptor-mediated endocytosis facilities by folate receptor [Chan et al., 2010]. The endocytosis mediated by FR can be used to absorb the FA, FA conjugate, and FA antagonists effectively. Therefore, the FR is a good target for the target delivery system for many solid tumors [Chan et al., 2007].

In this regard, folate conjugated solid-lipid nanoparticle (FC-SLNs) has shown the signs of targeting approach; particularly to carcinoma cells and good biocompatibility are of particular interest to select solid lipid nanoparticle as a carrier and targeting vehicle.

MATERIAL AND METHOD

Targeted drug delivery of nanocarrier is particularly a challenge in Nanomedicine. The newly fabricated F-SLNs may improve the bioavailability, reduce the side effect as well as promote the targeting propensity. The polymer used for the fabrication of SLNs, is biodegradable in nature and cyto-compatible. The prepared FC-SLNs formulation will shown the particle size in the range of 100-300nm that is suitable for the carcinoma targeting. Active targeting employs the use of ligands on the carrier surface for specific recognition by the cell surface receptors accomplished limiting drug distribution towards the normal cell, result in reducing the side effects and directed towards target organ by the cell recognition [Li et al., 2011]. Folate receptors (FR) are over-expressed in many human cancer cell surfaces on an order of 100 times more than normal, healthy cells and tissue, but rarely found on normal cell surfaces [Sahu et al., 2015].

Materials

The drug Paclitaxel was a generous gift from Neon Laboratory, Mumbai, Maharashtra (India). FA-PEG-DSPE was obtained as a gift sample from Lipoid Ludwigshafen (Germany). Tristearin, soya PC, FA-PEG-DSPE, Sephadex G-50, HPLC vials, anti-clot blood collecting vials, HPLC grade acetonitrile, ethanol and dimethyl sulfoxide (DMSO) was purchased from Hi-media Laboratories Pvt. Ltd, Mumbai (India). Dialyses tubing (mol. Wt. 12,000 Da) were procured from Sigma-Aldrich, India. All other chemicals used were of reagent grade. Triple distilled water was used throughout the study.

Preparation of P-SLNs and FC-SLNs

The FC-SLNs was prepared by the hot homogenization-based ethanol injection method as reported by Rajoriya et al., 2021 [Rajoriya et al., 2021]. Briefly, Tristearin, Soya PC, and FA-PEG-DSPE was dissolved in ethanol at a concentration of 10mg/ml, Paclitaxel (1%) was added into the mix. The melt was rapidly injected through a syringe at a current flow rate of 5ml/min into a stirred aqueous phase containing poloxamer 188 (1% w/v). Both phases were pre-warmed and maintained at 70˚C during the mixing. The temperature must be maintained at 70°C throughout the experiment [Houghton et al., 2007]. The suspension was mechanically stirred (Remi, Mumbai, India) at 3000 rpm for 15 min and then sonicated for 5 min using a probe-sonicator to form FC-SLNs. The same procedure is adopted for the preparation of P-SLNs but DSPE used in-spite to FA-PEG-DSPE. The product is lyophilized to obtained powder material.

Optimization of P-SLNs Formulation

P-SLNs formulation was optimized for optimization of formulation variables i.e. polymer ratio, drug and lipid ratio, lipid and Lipid and stearyl-amine (SA) ratio, surfactant concentration and Process variables i.e., stirring speed, stirring time and sonication greatly affect the characteristic of SLNs formulation.

Characterization of P-SLNs and FC-SLNs

Particle morphology

Transmission electron microscopy (TEM) of both SLNs formulation was studied to characterize them in terms of size, shape and surface morphology. In that one drop (10µL) of SLNs formulation as the suspension was placed on a copper grid (300 mesh), after negative staining with uranyl acetate solution (1%) for two min, remaining excess fluid was removed by touching the edge with filter paper [Rahiminejad et al., 2019]**.** The copper grid was allowed to dry at room temperature and was examined under an acceleration voltage of 30kV with the transmission electron microscope and

photomicrographs were taken at a suitable magnification. The morphology of P-SLNs and F-SLNs was observed using transmission electron microscope (TEM) (Philips Morgagni 268, Eindhoven, Netherlands) from All India Institute of Medical Sciences (AIIMS), New Delhi.

Particle size, polydispersity index and zeta potential

Characterization of both SLNs formulation in term of particle size and PDI was done by photon correlation spectroscopy [Gawde et al., 2017] using a zeta-sizer (DTS Ver. 4.10, Malvern Instruments, England) from CSIR-CDRI, Lucknow (U.P.). The samples were kept in polystyrene cuvettes diluted with 1:9 (v/v); filtered deionised water at 25°C and analysed as a 90° fixed angle. The zeta potential was determined by laser doppler anemometry using a Malvern Zeta-sizer (DTS Ver.4.10, Malvern Instruments, England, UK) also called doppler electrophoretic light scatter analyser. Both SLNs formulation was dispersed in deionised water, placed in the electrophoretic cell with an electric field of 15.24 V/cm and the zeta potential was determined. The zeta potential is the potential difference between the dispersion medium and the stationary layer of fluid attached to the dispersed particle. The stability of particle dispersion will depend upon the balance of the repulsive and attractive forces that exist between particles as they approach one another.

***In-vitro* drug release study**

The *in-vitro* drug release of entrapped paclitaxel from SLNs formulation was determined using dialysis tube. The SLNs formulation was first separated from free drug by passing through Sephadex column and then centrifugation. Separated SLNs formulation (5ml) was taken in to the dialysis tube (molecular weight cut off 12,000 Da, Hi Media, India) and placed in a beaker containing 50 ml of PBS (pH 7.4). The beaker was placed over a magnetic stirrer and the temperature was maintained at 37±2˚C throughout the procedure [Siddiqui et al. 2012]. Samples were withdrawn at definite time intervals and replaced with same volume of phosphate buffer. It was then spectrophotometric analysed for drug content by measuring absorbance at 237.0 nm against blank. Same procedure utilized for three other PBS medium (pH 4.0, 6.4 and 8.0).

Statistical analysis

Statistical analysis was performed with graph pad In-stat software (version 3.00 graph pad software, San Diego California USA) using one-way ANOVA followed by Tukey-Kramer multiple comparison test. The difference with $p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

P-SLNs formulation was optimized for formulation variables i.e., polymer ratio, drug and lipid ratio, lipid and Lipid and stearyl-amine ratio, surfactant concentration and process variables i.e., stirring speed, stirring time and sonication time.

Optimization of Lipid Ratio

For optimization of lipids ratio, SLNs were prepared with varying ratio of three lipids i.e. Tristearin, mPEG-DSPE and Soya PC (viz. 1:0.5, 1:1, 1:1.5, 1:2 % w/w), while keep all other parameters constant. Optimization was done on the basis of average particle size and poly dispersity index (PDI) of SLNs, which were determined using Malvern Zetasizer (Malvern, UK). The result represent in Figure 1.

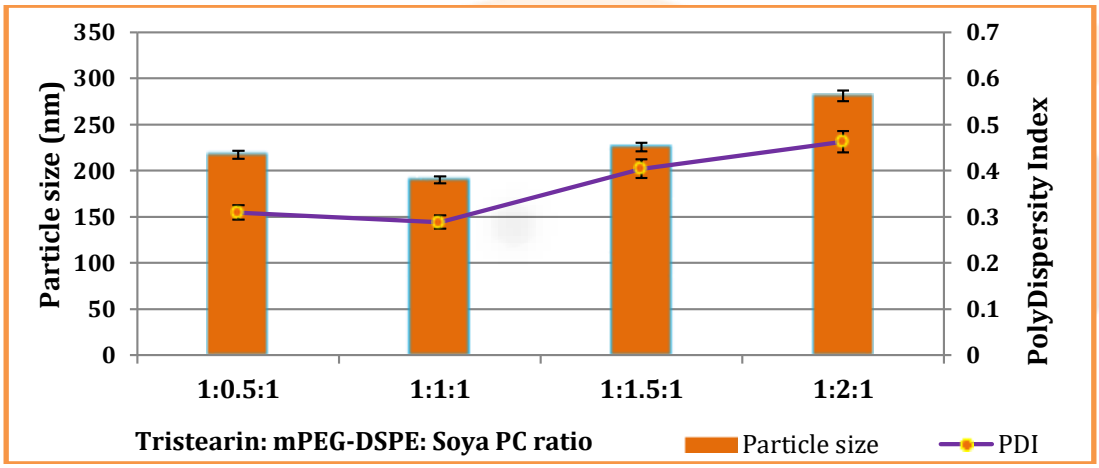


Figure 1: Optimization of Lipid ratio

(II) Optimization of drug and lipid ratio

For optimization of drug and lipid ratio, the SLNs formulation V_2 was selected and different SLNs formulations were prepared with ratio of 5:100, 10:100, 15:100, 20:100 % w/w of drug (Paclitaxel) and lipids (Tristearin/mPEG-DSPE/Soya PC ratio), keeping the other parameters constant . Optimization was done on the basis of average particle size of SLNs and percent drug entrapment. The drug entrapment efficiency was determined by dialysis tube technique. The observations are shown in Figure 2.

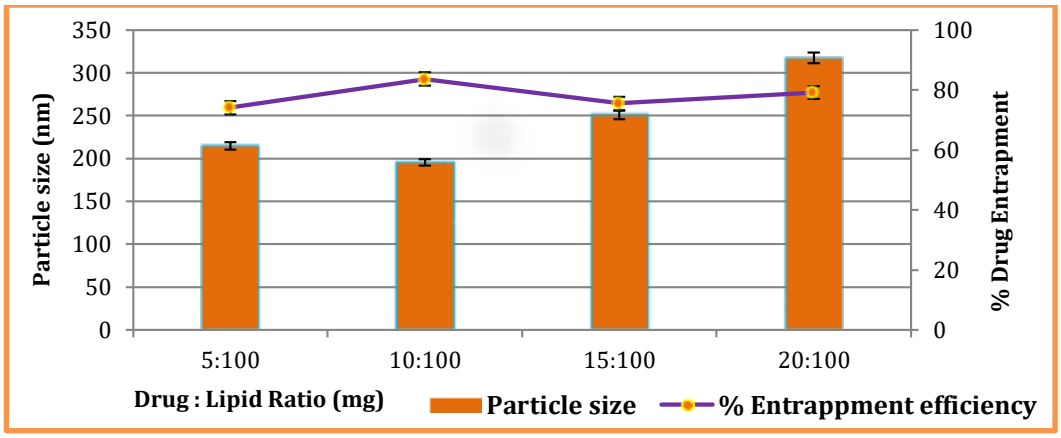


Figure 2: Optimization of drug and lipids ratio

(III) Optimization of Lipid and Stearyl-amine (SA)

Formulation D_2V_2 was selected for the optimization of lipid and Stearyl-amine ratio while keeping other parameters as constants. Lipid and Stearyl-amine were taken in different ratios 100:5, 100:10, 100:15 and 100:20. The observations is represented in Figure 3.

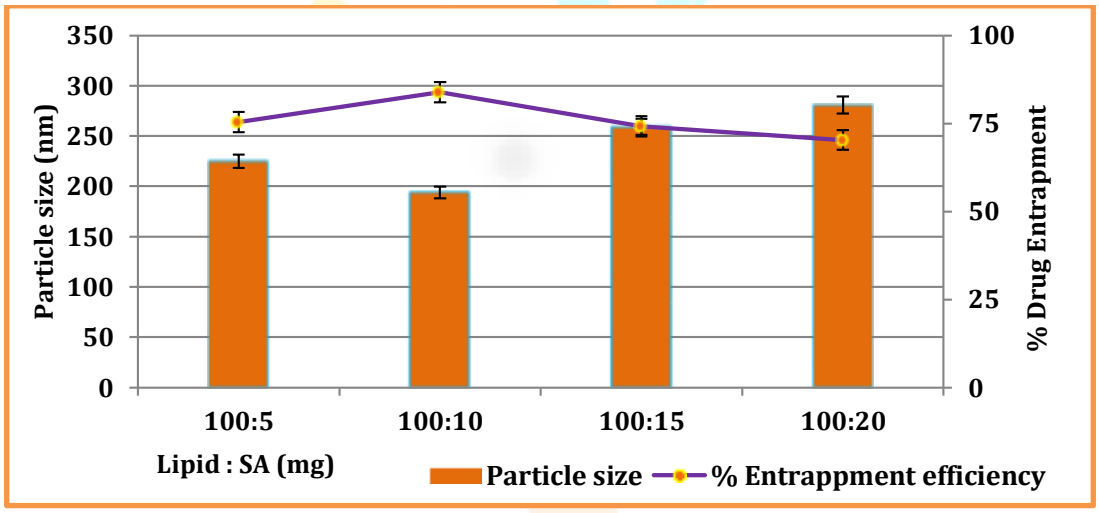


Figure 3: Optimization of Lipid/stearyl-amine ratio

(IV) Optimization of surfactant Concentration

For optimization of concentration of Polaxamer-188, formulation $D_2V_2S_2$ was selected on the basis of particle size and drug entrapment. Keeping the other parameters constant, SLNs formulations were prepared using different concentrations of Polaxamer-188 in aqueous medium. The effects of emulsifier concentration on the particle size & entrapment efficiency and result represent in Figure 4.

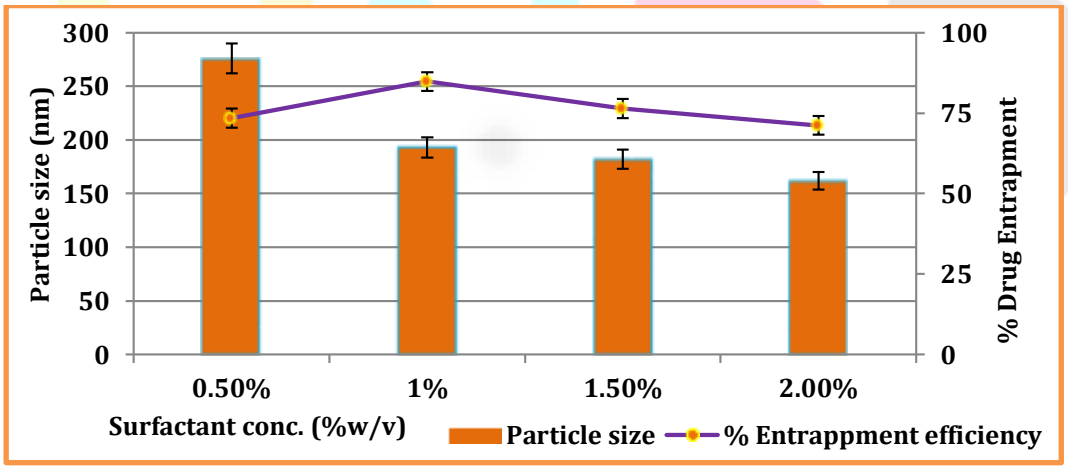


Figure 4: Optimization of surfactant concentration

Optimization of Process Variables

Process variables i.e., stirring speed, stirring time and Sonication greatly affect the characteristic of SLNs formulation. **Formulation $D_2V_2S_2P_2$** was selected as the optimized formulation on the basis of formulation variables in order to optimize the process variables like stirring speed and time, Sonication etc.

Optimization of stirring speed

Stirring speed was varied from 1000 to 4000 rpm for SLNs using the same formula of optimized formulation parameters. The particle size and percent drug entrapment of prepared SLNs were determined. The observations are shown in Figure 5.

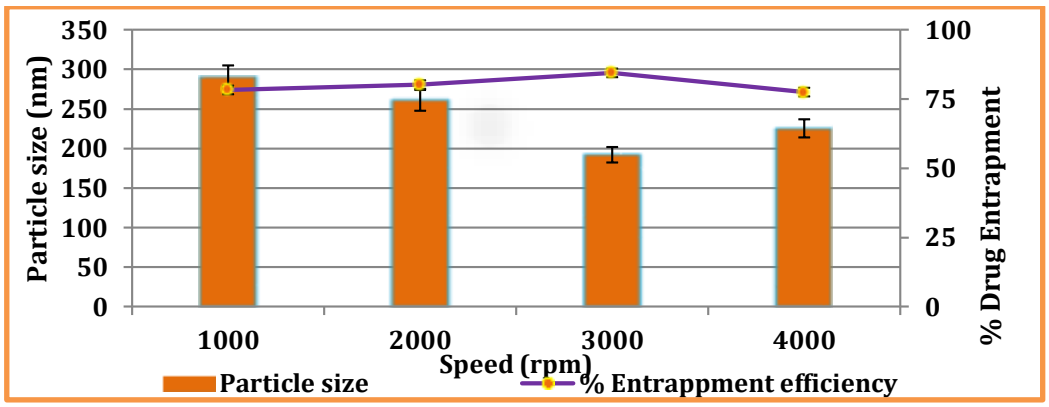


Figure 5: Optimization of stirring speed

Optimization of stirring time

For the optimization of stirring time, **$D_2V_2S_2P_2R_3$** was selected while other process variables were kept constant. The SLNs dispersion was prepared by stirring for different time periods viz. 15, 30, 45 and 60 min. Further the particle size and percent drug entrapment were determined which are shown in Figure 6.

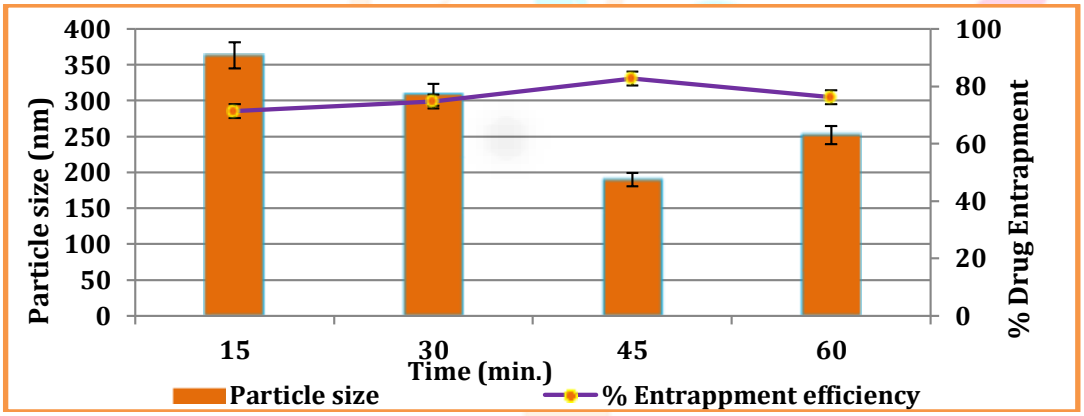


Figure 6: Optimization of stirring time

Optimization of Sonication time

Formulation **$D_2V_2S_2P_2R_3T_3$** was selected for the optimization of Sonication time. SLNs formulations were prepared with varying Sonication time period i.e. 1, 2, 3, 4 minutes by probe sonicator (Imeco, Ultrasonic India), while keeping all the other variables constant. Average particle size was determined using Zetasizer 3000HS (Malvern, UK) and percent drug entrapment was also determined and shown in Figure 7.

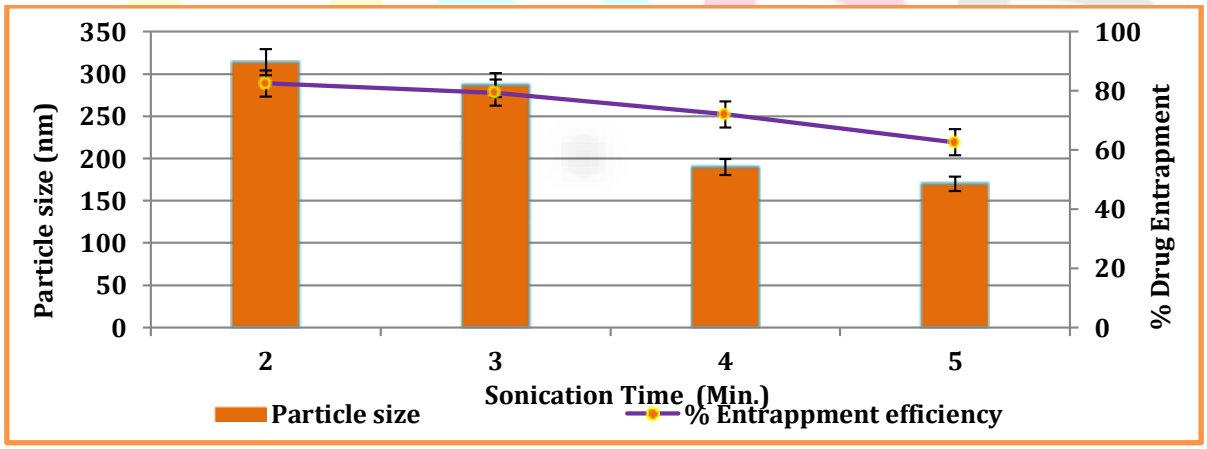


Figure 7: Optimization of Sonication time

C. Optimized Parameter

On the basis of above discussed formulation and process variables the SLNs formulation **$D_2V_2S_2P_2R_3T_3Y$**was selected as the optimized formulation and the parameters used for the preparation of this optimized formulation are shown in the Table 7.8.

Table 7.8: Optimized Parameters of SLNs formulation $D_2V_2S_2P_2R_3T_3Y$

PARAMETER	OPTIMIZED VALUE
Tristearin: mPEG-DSPE: Soya PC (Lipid Ratio)	1 :1 :1
Drug: Lipid ratio	10:100 mg
Lipid: Stearylamine ratio	100 : 10 mg
Surfactant (Poloxamer-188) concentration	1 %w/v
Stirring speed	3000 rpm
Stirring time	45 min
Sonication time	4 min

$D_2V_2S_2P_2R_3T_3Y$; D = Drug, V = Lipid /DSPE ratio; S= Lipid/Stearylamine ratio; P = Concentration of poloxamer-188; R = rpm (stirring speed); T = Stirring time, Y = Sonication time

Characterization

The P-SLNs were prepared by hot homogenization-based ethanol injection method. The particle size and PDI data was represented in Fig. 8 and data represented in Table 1. The average particle size of the P-SLNs was found to be 190.1 nm with a PDI of 0.410 (Fig. 8a). The average particle size of the FC-SLNs was found to be 231.3 nm with a PDI of 0.621 (Fig. 8b). Zeta-potential (mV) was recorded as 13.41±0.3 and -4.27± 0.2, for P-SLNs and FC-SLNs respectively. The negative surface charge on the FC-SLNs is superlative for drug targeting as they have least probability to cause immune activation or particle opsonization and clearance.

Table 1: Characterization of optimized P-SLNs and F-SLNs

Formulation Code	Particle size (nm)	Zeta-potential(mV)	PDI
P-SLNs	190.1±1.9	13.41 ± 0.3	0.410±0.13
F-SLNs	231.3±2.3	- 4.27± 0.2	0.621±0.12

*tristearin:soyapc:mpeg-dspe,1:1:1; drug: lipid ratio,10:100 mg; lipid: stearylamine ratio,100:10 mg; surfactant concentration,1 % w/v; stirring speed, 3000 rpm(1006 g); stirring time, 45 min; sonication time, 4 min; values are expressed as mean ±s.d. n=3.

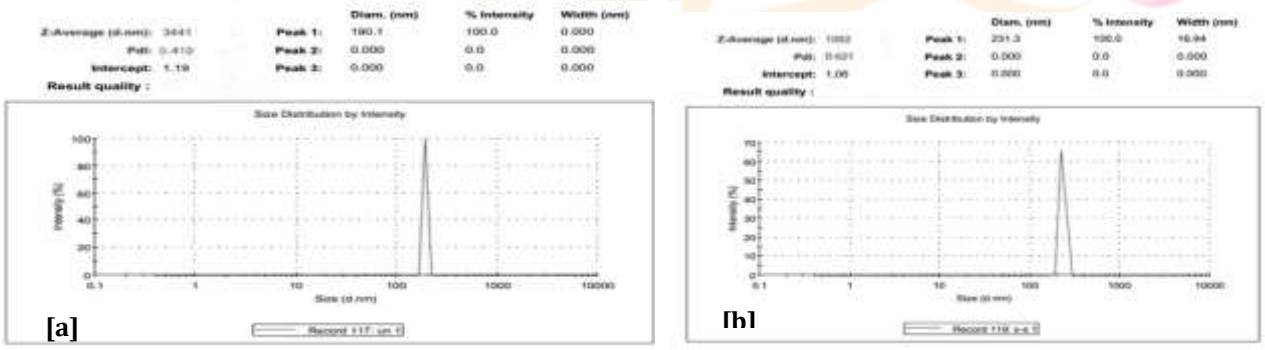


Figure 8: Particle size and poly-dispersity Index of (a) P-SLNs and (b) FC-SLNs

Morphology

The nanometric size of P-SLNs and FC-SLNs was observed using transmission electron microscope (TEM) and was shown in **Fig. 9a and 9b** respectively. The particle size of FC-SLNs was found to be greater than, in comparison to P-SLNs which may be due to the folate conjugation on SLNs surface. The data are in agreement with TEM studies that has shown increase the size of the particle, upon folate conjugation..

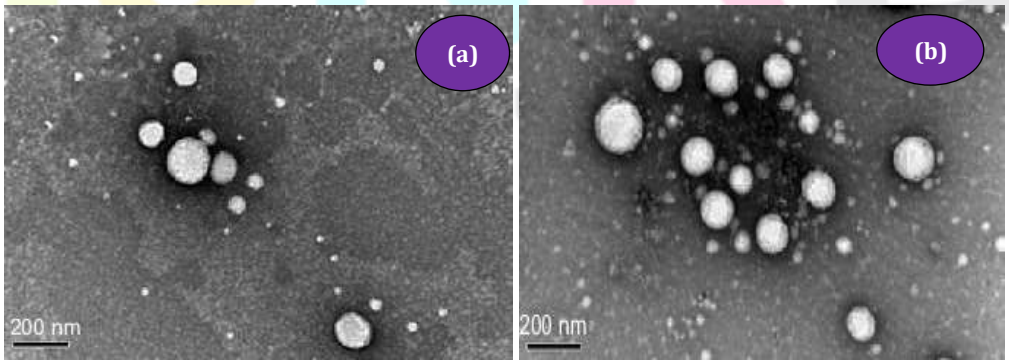


Figure 9. TEM image of (a) P-SLNs and (b) FC-SLNs

The surface morphology of P-SLNs and FC-SLNs was observed using Scanning electron microscope (SEM). In P-SLNs **(Fig. 10(a)** exhibited relatively smoother surface as compared to FC-SLNs (**Fig. 10b)** could be due to the substitution of the amino group of P-SLNs.

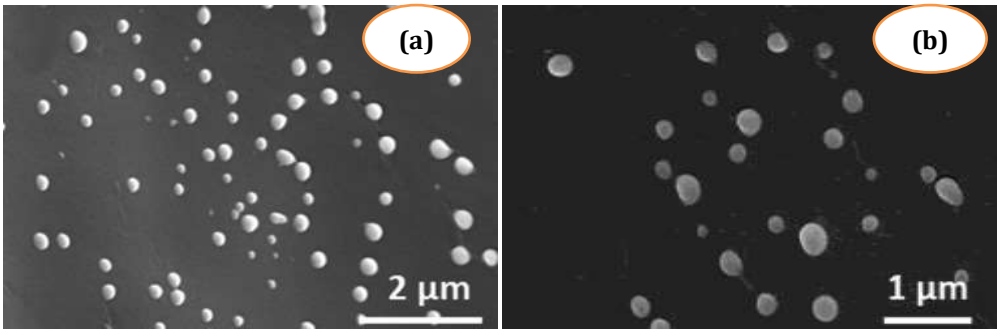
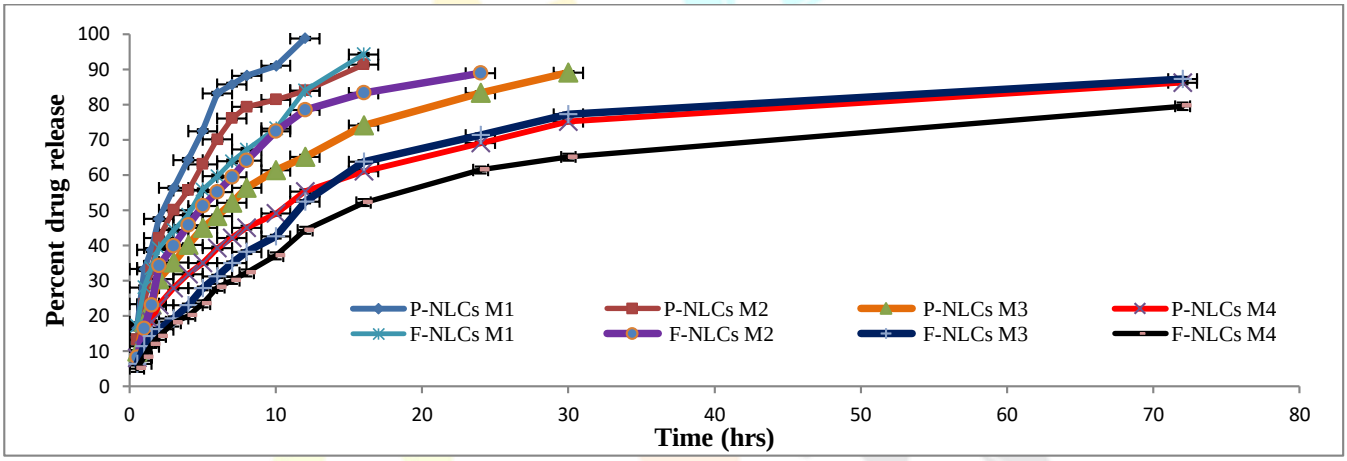


Figure 10: SEM image of (a) P-SLNs and (b) FC-SLNs

***In-vitro* drug release**

In-vitro drug release studies at pH 4 and 6.4 were carried out in the view that the tumor vicinity is relatively acidic in nature. The rapid release behavior of the drug from SLNs formulations at pH 4 supports the hypothesis that the SLNs formulation will release the drug in higher concentration, whenever it will reach the target site i.e. tumor; as the pH in such environment is generally below 7.



M1: Phosphate Buffer Saline pH 4.0; **M2:** Phosphate Buffer Saline pH 6.4; **M3:** Phosphate Buffer Saline pH 7.4 and **M4:** Phosphate Buffer Saline pH 8.0. Values represent mean ± SD (n=3): **P-SLNs:** Paclitaxel loaded Nano-lipid Construct; **FC -SLNs** Paclitaxel loaded folate conjugated Nano-lipid Construct

Figure 11: Drug release of SLNs formulation on different pH Environment (pH 4, 6.4, 7.4 and 8.0).

The FC-SLNs formulation has shown the different release pattern on different pH medium i.e. at pH 4.0 show 94.21% drug release after 16 hrs; at pH 6.4 show 88.92% of drug release after 16 hrs, at pH 7.4 shown 87.28% drug release after 30 hrs, at pH 8.0 shown 79.54% drug release after 72 hrs. These types of release behavior indicate that the FC-SLNs has shown the better release of drug at lower pH range 4 and 6.4 (acidic environment). This result is favoring our hypothesis that FC-SLNs formulation release the drug in higher concentration in lesser times when it reaches to tumor sites, where the pH is always be lesser than 7. The release data indicates that moreover at pH 7.4 and 8.0, the release patterns were sustained by FC-SLNs resulting in about 65.78%, 52.18% drug release in 16 hrs, respectively. The reason attributed to surface engineering of folic acid as it led to more sealing at the nanoparticle periphery and hydrophobic interactions which delayed the drug release. The release pattern by different pH medium or environment by the SLNs formulation was represented in Figure 11.

Conclusion

In FC-SLNs formulation was productively developed and optimized. The FC-SLNs and P-SLNs were characterized for SEM and TEM. The Drug release of FC-SLNs and P-SLNs was performed in different pH medium. The rapid release behavior of the drug from SLNs formulations at pH 4 supports the hypothesis that the SLNs formulation will release the drug in higher concentration, whenever it will reach the target site i.e. tumor; as the pH in such environment is generally below 7. The Characterized parameter and drug release data suggested that FC-SLNs are a safe, stable, and potentially promising drug delivery system for lung targeting.

Abbreviations

FC-SLNs- Folate conjugated Solid Lipid Nanoparticle; PS-Paclitaxel drug solution; P-SLNs- Paclitaxel loaded Solid Lipid Nanoparticle; TEM: Transmission electron microscope

Acknowledgements

The author thanks, Neon Laboratory, Mumbai, India for providing gift samples of paclitaxel to carry out the work.

REFERENCES

1. Siegel RL, Miller KD, Jemal A (2017) Cancer statistics. A Cancer J. Clin. 67: 7-30.
2. Chang A (2011) Chemotherapy, chemo-resistance and the changing treatment landscape for NSCLC. Lung Cancer 71: 3-10
3. Paumier A, LePéchoux C (2010) Radiotherapy in small-cell lung cancer: where should it go? Lung Cancer 69: 133–140.

4. Sunderland CJ, Steiert M, Talmadge JE (2006) Targeted nanoparticle for detecting and treating cancer. Drug Dev. Res. 67: 70-93.
5. Zhang YL, Zhang, ZH, Jiang TY (2013) Cell uptake of paclitaxel solid lipid nanoparticle modified by cell-penetrating peptides in A549 cells. Die Pharmazie. 68: 47–53.
6. Kim SS, Harford JB, Pirollo KF (2015) Effective treatment of glioblastoma requires crossing the blood-brain barrier and targeting tumors including cancer stem cells: The promise of Nanomedicine. Biochem. Biophys. Res. Commun. 468: 485-489.
7. Vaidya B, Paliwal R, Rai S (2009). Cell-selective mitochondrial targeting: A new approach for cancer therapy. Cancer Ther. 7:141-148.
8. Mansoori GA, Brandenburg KS, Shakeri-Zadeh A (2010) A comparative study of two folate-conjugated gold nanoparticles for cancer nanotechnology applications. Cancers 2: 1911-1928.
9. Muller C, Schibli R (2011) Folic acid conjugates for nuclear imaging of folate receptor positive cancer. J. Nucl. Med. 52:1-4.
10. Parker N, Turk MJ, Westrick E (2005) Folate receptor expression in carcinomas and normal tissues determined by a quantitative radio-ligand binding assay. Anal. Biochem. 338: 284-293.
11. Zwicke GL, Mansoori GA, Jeffery CJ (2012). Utilizing the folate receptor for active targeting of cancer nanotherapeutics. Nano Rev. 3: 184-196.
12. J.M. Chan, L. Zhang, R. Tong, Spatiotemporal controlled delivery of nanoparticle to injured vasculature. Proc. Natl. Acad. Sci.107(5), 2213–2218 (2010).
13. Chan P, Kurisawa M, Chung JE (2007) Synthesis and characterization of Chitosan-g-poly (ethylene glycol)-folate as a non-viral carrier for tumor-targeted gene delivery, Biomaterials. 28: 540-549.
14. Li R, Eun JS, Lee MK (2011) Pharmacokinetics and biodistribution of paclitaxel loaded in pegylated solid lipid nanoparticles after intravenous administration. Arch. Pharm. Res. 34: 331–337.
15. Sahu PK, Mishra DK, Jain N, Rajoriya V, Jain AK (2015). Mannosylated solid lipid nanoparticles for lung targeted delivery of Paclitaxel. Drug. Dev. Indus. Pharm. 41(4): 1-10.
16. Rajoriya V, Kashaw V, Sushil Kumar Kashaw. Folate conjugated Solid Lipid Nanoparticle: formulation development, optimization, and characterization. Current Nanomedicine, 2021; 11(3): 186-199.
17. Houghton P, Fang R, Techatanawat I (2007) The sulphorhodamine (SRB) assay and other approaches to testing plant extracts and derived compounds for activities related to reputed anticancer activity. Methods. 42: 377–87.
18. Rahiminejad A, Dinarvand R, Johari B (2019) Preparation and investigation of indirubin-loaded SLN nanoparticles and their anti-cancer effects on human glioblastoma U87MG cells. Cell. Biol. Int. 43: 2-11.
19. Gawde KA, Kesharwani P, Sau S, Sarkar FH, Padhye S, Kashaw SK, Iyer AK (2017) Synthesis and characterization of folate decorated albumin bio-conjugate nanoparticles loaded with a synthetic curcumin difluorinated analogue. J. Coll. Int. Sci. 3(4): 716-726.
20. Siddiqui S, Paliwal S, Kohli K (2012) RP-HPLC Method for Estimation and Stress Degradation Study of Paclitaxel as per ICH Guidelines.Chromat. Sep. Techniq. 3.