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## Colloidal drug delivery system for tumor specificity of paclitaxel in mice

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### ABSTRACT

Chemotherapy is one line for treatment of various cancers. Various chemotherapeutic agents which are used for this are associated with cytotoxic effects on cancer cells as well as on normal cells. Paclitaxel is a potent chemotherapeutic agent used in the treatment of various malignancies. Along with its limited bioavailability due to its poor aqueous solubility, it also shows deleterious effects on various organs. Solid Lipid Nanoparticles (SLNs) are colloidal carriers which shown a promising utility in the delivery of various drugs in controlled manner with high degree of specificity. In this study paclitaxel SLNs were prepared using stearic acid and its combination with phospholipid, which were subjected to various evaluation studies. Drug entrapment was found to be 38.90 to 62.20 and 32.80 to 51.29 percent for stearic acid and its combination with phospholipid respectively. At the end of 48 hrs in vitro drug release found to be 69.50% to 79.35 % and 64.88% to 73.55% for stearic acid and its combination with phospholipid respectively. Biodistribution studies in EAC xenografted mouse model showed that SLNs significantly decreased the uptake in organs like liver, spleen and lung while increasing the uptake in tumor tissues after injection compared with pure drug. Moreover, the SLNs prepared with combination of lipids showed greater tumor growth inhibition effect in in-vivo studies. Study showed that the breast cancer tumor specificity of paclitaxel can be improved by delivering it in SLN.

**Key Words:** Anti- cancer drug, SLN, Paclitaxel, stearic acid, DPPG.Na.

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### INTRODUCTION

An ideal drug delivery system should improve therapeutic index of drug as well as it should improve patient compliance [1]. Colloidal drug carriers have attracted increasing attention during recent years to achieve the objective of modern drug therapy. Investigated system includes solid lipid nanoparticles, nanoemulsion, liposomes and nanosuspension. The increasing interest gained by SLNs as a colloidal drug carrier is due to their properties like possible targeting by suitable modification, good protection of encapsulated drug, high encapsulation load, no biotoxicity of carrier and ease of production and scale up at low cost [2]. Therefore solid lipid nanoparticles have been proposed as a drug delivery system for a number of drugs [3-5]. The SLN were found to an ideal carriers for lipophilic drug for better stability and release retardation [6]. Bioavailability of poorly soluble drug was increased when they were incorporated in SLN [7]

Paclitaxel is natural compound found in the bark of pacific yew trees. It binds to the tubulin and thus inhibits the regular separation of chromosomes in dividing cells. It belongs to the group of cytostatic agents. Antiproliferative taxanes such as Paclitaxel seem to be suitable due to their high lipophilicity and tight binding to various cell constituents, resulting in effective local retention at the site of delivery [8]. Paclitaxel is administered using vehicle, cremophor EL surfactant & ethanol mixture, marketed under the trade name Taxol. However the major problem associated with this formulation is that patients commonly experience an anaphylactic reaction, believed to cause non-linear pharmacokinetics, making dose escalation problematic.

Paclitaxel being an antineoplastic agent it has shown number of untoward effects on the normal tissues. These effects are due to its pharmacokinetic performance and ultimately can be reduced by providing drug at specific site and less to other tissues. The successful administration of paclitaxel requires a formulation which is devoid of toxic adjuvants, release the drug for extended period of time and having good site specificity and devoid of stability problems.[9]. To meet above goals researchers attempted to formulate paclitaxel in the form of liposome's,[10],water soluble prodrug [11],enzyme activation prodrug [12],albumin conjugates[13],complexes with cyclodextrins[14],and parenteral emulsion[15].

All these formulation were associated with limited applicability in terms of drug loading, stability of formulation, site specificity and large scale production.

Hence, in the present work an attempt is being made to provide an alternative colloidal drug delivery system for Paclitaxel in the form of solid lipid nanoparticles.

## MATERIALS AND METHODS

Paclitaxel (IP grade) was supplied by Unan Hande Biotech Co. Ltd. China; Lipid- stearic acid, was purchased from S.D.Fine Chemicals, Mumbai. 1,2,Dipalmitoyel-SN-Glycero-3-Phospho Glycerol, Sodium. was procured from Genzyme, Germany. Sodium glycholate and Soya lecithin were purchased from Across Organics, New Jersey, USA. Potassium dihydrogen phosphate was procured from Ranbaxy Fine Chemicals New Delhi. Ethanol and other solvents were purchased from local suppliers. All the chemicals were used as supplied, without further purification.

### PREPARATION OF PACLITAXEL LOADED SOLID LIPID NANOPARTICLES

The formulation chart for formulating SLN's is shown in table no.1. Solid lipid nanoparticles of Paclitaxel were prepared by micro emulsification technique using soy lecithin as surfactant, sodium glycholate as co surfactant and 0.1 N HCl as liquid manufacturing vehicle. Lipid was melted at a temperature of 80°C to the melted lipid. Paclitaxel drug was added with 5 min. stirring followed by sonication. To this mixture soy lecithin was added and stirred for 2 minutes. Aqueous phase containing co surfactant sodium glycholate was heated at 80°C and added to melted lipid phase mechanical stirring at 80°C for 10-15 minutes, formed o/w microemulsion. Methanol, acetonitrile and tert.butyl methyl ether all were of HPLC grade. The micro emulsion was carefully added drop wise into ice cold water in a beaker with continuous stirring a 5 cc glass syringe fitted with 21 gauge needle was used to control the particle size. The mixture was stirred at 3000rpm and sln dispersion was stirred for 3 hrs after complete addition of microemulsion. The sln dispersion was subjected to ultrasonication for 10 minutes [16].

Table No.1 Formulation Chart For Drug Loaded SLN preparation.

Code	DRUG : STEARIC ACID	DRUG : STEARIC ACID+ DPPG.Na	STEARIC ACID: DPPG.NA
A1	1:10	-----	-----
A2	1:15	-----	-----
A3	1:20	-----	-----
B1	-----	1:10	1:0.25
B2	-----	1:15	1:0.5
B3	-----	1:20	1:1

Collected formulations were lyophilized to get free flowing product. Lyophilized particles were sterilized by autoclaving and stored in glass containers which were sterilized previously by autoclaving [17].

### CHARACTERIZATION OF PACLITAXEL LOADED SLN

#### Particle Size Analysis:

The size distributions along the volume mean diameter of the nanoparticles were measured by Dynamic Light Scattering Particle Size Analyzer (Nanotrac Particle Size Analyzer). The range of the analyzer is 0.8 nm to 6.54 µm [18].

#### Percentage Yield

The lyophilized nanoparticles from each formulation were weighed and the respective percentage yield was calculated using the formula no. [1]

$$\text{Percentage Yield} = \frac{\text{Wt of SLN obtained}}{\text{Wt of drug and lipid used}} \times$$

#### Determination of Entrapment Efficiency Percentage

Entrapment efficiency of Paclitaxel loaded SLN was estimated by centrifugation method. The prepared SLNs were placed in centrifugation tube and centrifuged at 15000 rpm for 30 min. The supernatant (1ml) was withdrawn and diluted with methanol. The untrapped Paclitaxel was determined by UV spectrophotometer at 227 nm. The samples from the supernatant were diluted suitably before going for absorbance measurement. The free Paclitaxel in the supernatant gives the total amount of untrapped drug. Encapsulation efficiency is expressed as the percent of drug trapped and was calculated using equation no. [2]. Concentration of drug was calculated from equation of straight line obtained for standard curve for paclitaxel.

$$\% \text{ EE} = \frac{\text{Total amount of drug} - \text{Free dissolved drug}}{\text{Total amount of drug}} \times 100 \dots [2]$$

#### SEM Study

Scanning electron microscopy was done to study the particle surface morphology and shape. SEM was done by using JSM-T330A, JEOL, Hindal, India.

#### Zeta Potential Determination

Zeta potential was measured by using Zetatrak after appropriate dilution with distilled deionised water. [19].

#### In Vitro Drug Release Studies

The release of paclitaxel from the nanoparticles was measured in triplicate in PBS (PH 7.4) and was compared with its marketed formulation Taxol®. Sample equivalent to 10 mg of paclitaxel was taken into a tube with both ends open. One end of the tube is closed with dialysis membrane. The tube containing drug-loaded SLNs/ Taxol® was kept in a beaker containing 200 ml of PBS pH 7.4. The tube is arranged in such a way that, it just touches the surface of buffer solution. The whole set up is place on a magnetic stirrer and rotated at 50 rpm. The temperature of buffer is maintained at 37±1 °C. 2 ml aliquot of release medium were withdrawn at time intervals of 0.5, 1, 2, 4, 8, 16, 24, and 48 hrs and replaced by the same volume of PBS. These samples were filtered through 0.45 µm membrane filter. The filtrate was diluted appropriately with methanol and estimated by UV-Visible spectrophotometer at 227 nm [20]. Concentration of drug was calculated from equation of straight line obtained for standard curve for paclitaxel.

#### In Vivo tissue targeting study:

This study was carried out after obtaining the due permission for conducting experiments from institutional ethics committee which is registered for "Teaching and Research on Animals".

To perform tissue targeting studies, female Swiss Albino mice (weighing 25-30 Gms) were divided in 3 groups six in each group. Each mouse was inoculated with Ehrlich Ascites Carcinoma cell line at a number of 1 to 2×10<sup>6</sup> subcutaneously at the right thigh of each mouse using a 1.0 mL syringe. Animals were kept in a SPF facility and had free access to food and water. After the inoculation, tumor allowed to attain volume of 100-200 mm<sup>3</sup> and then group I received SLN formulation prepared by using stearic acid (7.5 mg/ kg) (with optimum *in-vitro* release characteristic), after redispersing it into phosphate saline buffer pH 7.4.

Group II received SLN formulation prepared by using lipid combination of stearic acid + DPPG, Na (7.5mg/kg) (with optimum *in-vitro* release characteristic), after redispersing it into phosphate saline buffer pH 7.4. Group III received marketed formulation Taxol® (7.5mg/kg) as a control.

The mice were sacrificed after 24 hours of drug administration, by cervical dislocation in order to obtain the organs. The organs heart, lungs, spleen, liver and kidneys and tumor were harvested, washed with physiological solution (0.9% NaCl solution) weighed and stored at -20 °C until drug analysis by HPLC. Data is expressed as amount of paclitaxel per gram of tissue.

#### HPLC Analysis Condition:

Phenomenex Bondclone reversed-phase C18 column with particle size of 10 µm was used as analytical column. The column temperature was 30 °C. The control of the HPLC system and data collection was done by a computer equipped with spinchrome software. The flow rate was 1.3 ml/min and an injection volume of 20 µl was used. The

mobile phase involved a mixture of H<sub>2</sub>O: ACN (Water: Acetonitril) (60:40 v/v). The retention time was 6.1 min for Paclitaxel and 8.3 min for Carbamazepine, the internal standard.

*Preparation standard paclitaxel solution*

A standard stock solution containing paclitaxel (1 mg/ml) was prepared in H<sub>2</sub>O: ACN (60:40) and stored at 4 °C., working standard stock solutions were prepared from the stock solution by sequential dilution with H<sub>2</sub>O: ACN (60:40) to yield final concentration range 1 to 20 µg/ml.

*Preparation of standard tissue stock solution:*

To prepare standard tissue stock solution, tissue samples of untreated female Swiss Albino mice were collected and were homogenized in tissue homogenizer by adding 10 time volumes of distilled water containing 4% bovine serum albumin for 5 minute. The supernant was collected and was spiked with 50microlitre of internal standard methanolic solution of carbamazepine (5mcg/ml).The sample was then extracted twice with 2ml ethyl acetate. The ethyl acetate fractions were combined and were dried. Dried residue was dissolved in 100 microlitre of zinc sulphate solution, centrifuged and supernant was collected.

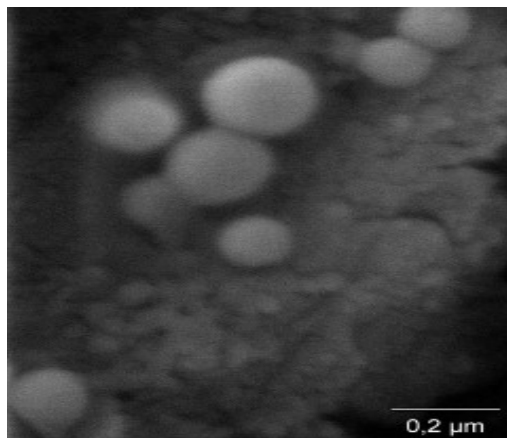
To this collected supernant liquid standard paclitaxel solution was added to get the concentration in the range of 0.1 to 20 mcg/ml. This solution was injected in chromatographic column and recorded the chromatogram at 227 nm.

## RESULTS AND DISCUSSION

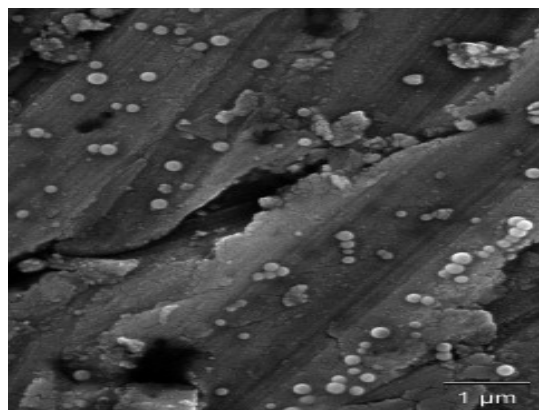
*Particle size analysis*

The size distributions along with the volume mean diameter of the nanoparticles were measured by Dynamic Light Scattering Particle Size Analyzer. Large particle size was found for formulations which were prepared by using combination of stearic acid and phospholipid. Further as the proportion of lipid functions raised there is an increase in average particle size. The combination of lipid with phospholipids and there increased concentration gives increased accumulation of lipid contents on core material resulting in increased particle size. The average particle sizes of all six formulations were listed in Table No. 2.

**Fig.No.1 Scanning Electron Microscopy of formulation A3**



**Fig.No.2 Scanning Electron Microscopy of formulation B3**



### Percentage yield

Total yield of sln for all six batches was found to increase as the lipid concentration was increased. Nanoparticles prepared with combination of lipid with phospholipid shown high production yield which indicates that more utilization of processing components with reduced losses. Indicating lipid concentration is vital for high yield. The results of percent practical yield are shown in Table No. 2.

### Determination of Entrapment Efficiency Percentage

The % drug entrapment in Paclitaxel-SLN was found to 44.95, 50.40, and 58.84 for formulation A1, A2, and A3 respectively. It was found to be 46.8, 53.76 and 63.99 percent for B1, B2 and B3 batches. As the lipid concentration was increased from 100-300mg, the encapsulation efficiency was increased. With incorporation of lipid with phospholipid encapsulation efficiency was increased slightly for all three batches. High lipid concentration resulted in maximum drug entrapment. The increased EE is due to more availability of encapsulating lipid for drug which ultimately raised the encapsulation of drug. The data is given in Table No2.

### Surface Morphology

Shape and surface morphology of nanoparticles was done by Scanning Electron Microscopy (JSM-T330A, JEOL). SEM photograph of selected formulations, A3 and B3 is shown in Figure 1 and 2. The paclitaxel SLNs have shown smooth surface and spherical shape.

### Zeta Potential Determination

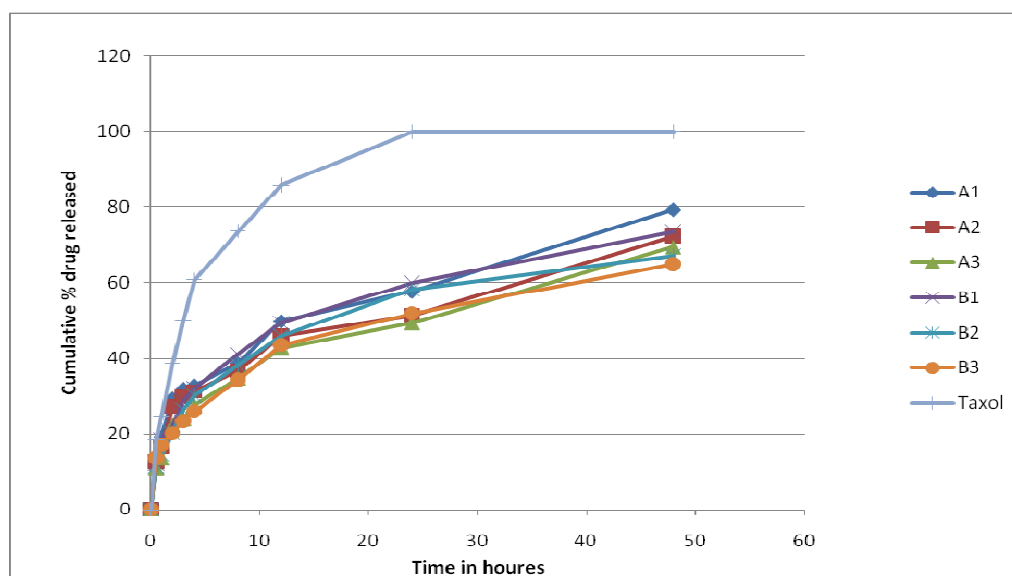
The stability study of the nanoparticle was evaluated by measuring the zeta potential of the SLNs by the zeta meter. The B3 batch shown high zeta potential value attributed to high percentage of phospholipid. The high zeta potential value indicate better stability of product as particles continue to repel each other and remain non aggregated [21]. The results are tabulated in Table 2

Table No.2. Mean particle size, Zeta-potential, % entrapment efficiency and % yield of SLN formulations.

Formulation code	Mean particle size (nm)	Zeta-potential (mV)	% Entrapment efficiency	% Yield
A1	198±2.6	-4.58±0.017	44.95±1.5	30.63
A2	209±10.2	-4.95±0.017	50.40±2.1	40.43
A3	219±5.5	-5.13±0.017	58.84±1.8	48.47
B1	203± 7	-29.45±0.017	46.8±1.7	32.80
B2	276± 4.04	-32.35±0.017	53.76±2.3	45.67
B3	320± 6.6	-33.23±0.017	63.99±2.2	51.29

Note: Each value represents mean ± S.E. (n=3)

Figure No.3 In vitro dissolution profile of formulation A1,A2,A3,B1,B2,B3 and marketed formulation Taxol®



### In Vitro Drug Release Studies

In vitro drug release from the SLNs in phosphate buffer pH 7.4 was performed using dialysis bag diffusion technique. The in vitro drug release profile of SLNs formulations obtained from dialysis experiment is shown in Figure No. 2. It was observed that the drug release from the formulations slightly increases as the particle size of the

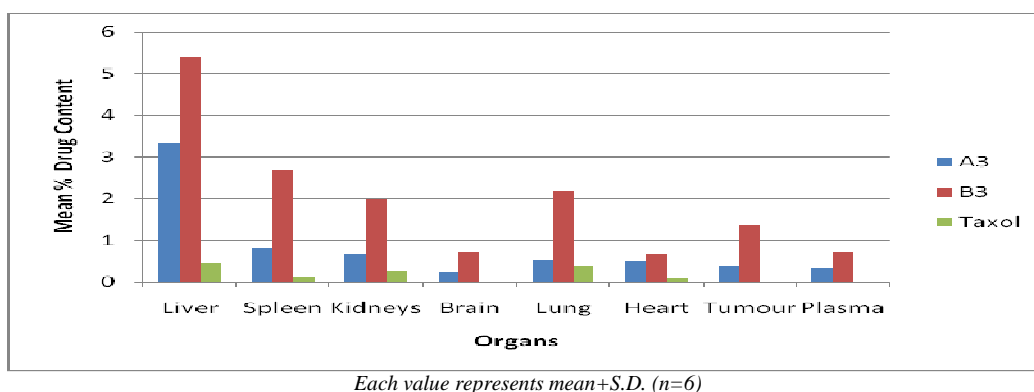
formulation decreases and all the six formulations showed a biphasic release with initial burst effect. The mechanism for the burst release may be attributed to the drug adsorbed on SLNs or due to leakage of drug from SLNs. [22] .All the formulation released the drug up to 48 hours which was very significant as compared with the marketed formulation Taxol which released nearly 100% drug within 24 hours.

The results obtained for *in vitro* release studies were studied in five models of data treatment as zero order rate kinetics, first order rate kinetics, Higuchi's classical diffusion equation, Peppas exponential equation, Hixson-Crowell erosion equation [23, 24].The criterion for selecting the appropriate model was chosen on the basis of goodness of fit test. Based on the highest regression values ( $r$ ), the best-fit model for all formulations was Higuchi Model. Peppas's model regression values are almost equal to Higuchi's model. With ' $n \sim 0.5$ ' value indicating non-fickian diffusion release.

#### ***In vivo* tissue targeting studies:**

To perform in vivo tissue targeting study formulation A3 and B3 were selected from respective class and were compared with Taxol®.The respective formulation were administered to EAC xenografted mouse and relative concentration of drug in different tissues and tumor was analysed.The results are represented as concentration of drug per gram of tissue weight. The data is shown in figure 4.

**Figure No.4: In vivo tissue targeting study of formulation A3,B3 and marketed formulation Taxol® After 24 hours of product administration in mice.**



The results were very promising for the SLN prepared with combination of lipid and phospholipid as compared with single lipid and marketed formulation Taxol®.Concentration of formulation B3 was found to be high in tumor when compared with A3 and Taxol after 24 hours.The amount of drug in RES organs was in order of B3<A3<Taxol.

Plasma drug concentration after 24 hours was found to be more for formulation B3 than A3 and Taxol® indicating increased availability of drug as compared with the marketed formulation.

Study showed that increased tumor localization of paxlitaxel can be obtained by using combination of lipid and phospholipid.Increased tumor localization can be attributed to EPR effect and by maintaining therapeutic concentration for long period.

#### **CONCLUSION**

The study showed that the colloidal drug carriers are having promising performance for loading of chemotherapeutic agent Paclitaxel. As the study showed that prepared formulation are having good surface morphology and loading efficiency. The entrapment efficiency, particle size and zeta potential value increased as lipid concentration was raised and gave retarded release with this increase in lipid concentration. The release profile was very promising as compared with the marketed formulation. The in vivo tissue targeting study showed that there is increased specificity of drug in tumor tissue when compared with marketed formulation. The future studies should be performed to assess the pharmacokinetic parameters of the formulations and to study scale up on large scale so as to give best alternative to existing formulation.



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