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RESEARCH ARTICLE

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A NOVEL APPROCH FOR DEVELOPMENT AND CHARECTRIZATION OF ETOPOSIDE LOADED SOLID LIPID NANOPARTICLES

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ABSTRACT

In recent years Solid Lipid Nanoparticles are regarded as alternative carrier systems to traditional colloidal systems. Drug loaded of twelve batches of different ratios of SLNs were prepared by solvent displacement method. Differing theoretical loading of ET 10, 20, 30 and 40% w/w of polymers respectively**.** The obtained ETSLN nanoparticles formulations ET1, ET2, ET3, ET4, ET5,ET6, ET7, ET8, ET9, ET10, ET11 and ET12 were evaluated for percentage yield, drug loading, percentage entrapment efficiency, particle size, surface morphology, *in vitro* release rate studies. ET4 shows maximum percentage yield of 84.73%, entrapment efficiency of 19.80±0.35 and particle size of 0.198±0.031. ETSLN were superficially spherical in shape with smooth surface and shows sustained release pattern for up to 90 hrs.

KEYWORDS

SLN's, Etoposide, Drug Loading, colloidal systems, Microemulsification.

INTRODUCTION

Etoposide is an epipodophyllotoxin antineoplastic agent, which acts by forming a ternary complex with topoisomerase II and DNA, causing DNA breaks and cell death.[1] It is used in the treatment of several tumors including small cell lung cancer, testicular cancer, lymphomas, and leukemias.[2] Etoposide is a highly hydrophobic molecule with lipophilic properties, and the formulation of etoposide is quite challenging. The major limiting step in the formulation of etoposide is its lipophilicity, and vehicles used as solubilizers are associated with adverse side effects such as hypotension, anaphylaxis, bronchospasm, etc.[3,4] Etoposide commercial formulations upon intravenous administration eliminated rapidly from the blood circulation and produced myelosuppression.[5] However, the effects of etoposide depend not only on its concentration but also on the duration of action.[6] Etoposide encapsulated in cationic liposomes produced improved anticancer activity and reduced myelosuppression toxicity.[5] In cancer chemotherapy, many types of carrier systems such as liposomes,[7] microcapsules,[8] lipid emulsions,[9] macromolecules,[10] and solid lipid nanoparticles,[11] have been investigated to find a means of delivering antitumor agents to target sites.

Parenteral drug delivery took a major leap after successful development of the submicronic parenteral fat emulsion (Intralipid) in 1960s. Quick commercialization of submicron emulsion based products, such as Diazemuls (Diazepam) and Diprivan (Propofol), was the indicator of the interest of pharmaceutical industry in colloidal carriers. Since then, there have been continuous efforts to develop novel colloidal nanocarriers for improved parenteral delivery. The concept of lipid nanoparticles for injectable delivery was developed from submicron sized parenteral fat o/w emulsion used for parenteral nutrition viz Intralipid in 1960s[12].

Liposomes represent the first generation of the novel colloidal carriers, which revolutionized the scenario in parenteral drug delivery. Liposomes offered several advantages such as encapsulation of hydrophobic and hydrophilic drugs, controlled drug release and reduction in toxicity/increased therapeutic efficacy of drugs most of which were not offered by submicronic emulsions. The successful commercialization various injectable liposomal products such as AmBisome® (Amphotericin B), Doxi®/Caelyx® (Doxorubicin)[13] and DaunoXome® (Daunorubicin)[14] and a large array of investigational products clearly indicates the potential advantages of liposomes as novel lipid colloidal carriers. However, complexity associated with the manufacturing of liposomes, difficulties in scale-up, limited physical stability and enormous cost of the liposomal formulation are the major barriers in the successful commercialization of liposomes[12].Solid lipid nanoparticles (SLN) are colloidal particles of a lipid matrix that is solid at body temperature. They were first introduced by Muller et al. in 1993[15,16] produced by high pressure homogenization and in parallel by Gasco by diluting warm microemulsion[17]. SLN have been exploited for delivery of actives via the dermal[18,] peroral[10], parenteral, ocular, pulmonary and rectal route. Upon administration of SLN via the parenteral route of administration, improved bioavailability, targeting, enhanced cytotoxicity against multidrug resistant cancer cells have been observed. Hence, there is need for the development of novel colloidal approached for engineered specificity of etoposide loaded solid lipid nanoparticles by using Dipalmitoylposphatidylcholine (DPPC), Distearoylphosphatidylcholine (DSPC) and Phosphatidylcholine based lipid polymers and their *in vitro* and *in vivo* characterization.

MATERIALS AND METHODS

Etoposide was obtained as gift sample by Naprod Life science, Mumbai. Phosphatidylcholine 99%, Soya-Lecithin, Taurocholic acid, Glucose, Chloroform, Methanol, Ethanol Sodium chloride were obtained as gift sample from S.D. Fine Chem Ltd., Mumbai, Dipalmitoylposphatidylcholine,Distearoylphos phatidylcholine was obtained as gift sample from Sigma Aldrich, Bangalore India and Potassium dihydrogen phosphate, Di-sodium hydrogen phosphate was obtained as gift sample from Thermo Fisher Scientific India Pvt. Ltd., Mumbai

Methods:

Drug free and ET loaded solid lipid nanoparticles were prepared by solvent displacement method. Twelve different types of systems ET1, ET2, ET3, ET4, ET5,ET6, ET7, ET8, ET9, ET10, ET11 and ET12 were prepared, differing in the theoretical loading of ET 10, 20, 30 and 40% w/w of polymers respectively and then were evaluated for percentage yield, drug loading, percentage entrapment efficiency, particle size, surface morphology, *in vitro* release rate studies[2].

Percentage yield:

The lyophilized nanoparticles from each formulation were weighed and the respective percentage yield was calculated using the following formula[3].

Percentage yield **=** wt of nanoparticles obtained**/**wt of drug, polymer, glucose used X100

Drug loading:

Accurately weighed equivalent 10.0 mg of each ETSLN loaded nanoparticles formulations were dissolved in 15.0 ml of Dichloromethane and to this mobile phase 60:40 Acetonitrile: Millipore water was added. DCM was evaporated completely, the solution was filtered by using 0.22µm nylon membrane filter. Clear solution was suitably diluted and injected 50µl in to HPLC system drug loading was detected at 230 nm by developed HPLC method. The obtained drug loading data are given in Table 4. The percentage drug loading was calculated by using following equation[4,]

Percentage drug loading = weight of drug in nanoparticles**/** Weight of nanoparticles taken X 100

Percentage entrapment efficiency:

To determine ET entrapment in nanoparticles were analyzed for ET content using HPLC and % entrapment efficiency (EE) was calculated using following equation[,4]. The obtained percentage entrapment efficiency data are given in Table 4.

% Yield = 1- Free drug/theoretical drug loaded X 100

Particle size analysis:

In order to analyze particle size drug loaded lyophilized nanoparticles were dispersed in deionized water, vortexed for 10 min and sonicated for 5 min before sampling. Particle size was determined by laser scattering light using Malvern Laser Analyzer Instruments[1]. The obtained results are shown in Figure 1.

Scanning electron microscopic analysis (SEM analysis):

The shape and surface characteristics of nanoparticles were visualized using scanning electron microscopy, JSM-848, Joel, Japan The coated nanoparticles were finally characterized for surface morphology under suitable magnification[5,6,9]. The SEM results are shown in Figure 4.

Differential scanning calorimetric analysis (DSC):

Differential scanning calorimetry (DSC) analysis of pure ET, pure polymers, physical mixture of polymers and drug and ET loaded SLN nanoparticulates formulations was conducted to ascertain the compatibility of drug with the polymer using Mettler Toledo DSC 822[e11,12,13]. The obtained DSC thermograms are shown in Figure 3.

In vitro drug release studies:

The *in vitro* release studies of ETSLN nanoparticles were carried out at 37±2^{o}C in phosphate buffer saline (PBS) pH 7.4 buffer media for a period of 90 hrs. The 30 ml screw capped bottles, containing ETSLN

nanoparticles in 20 ml of PBS pH 7.4 as release medium, were fixed in holders in water bath shaker and temperature was maintained at 37±2°C. The platform was allowed to vibrate horizontally at an average speed of 100 rpm to induce mixing in the release medium. At periodic intervals of every 1 h, 2.0 ml of the release medium was sampled and replaced with fresh 2.0 ml of release medium to provide the necessary sink condition. The samples were further diluted with mobile phase. The cumulative percentage drug release was calculated to establish the drug release profile of the ET loaded nanoparticles[10, 14, 15]. The obtained release profile data are shown in Figure 5 and Table 4.

Stability studies:

According to ICH guidelines, ET containing nanoparticles were stored at elevated temperature and relative humidity (25±2°C/60%±5% RH, 40±2°C/75%±5% RH) in a stability analysis chamber over a period of 3 months.[10] Freshly prepared nanoparticles were stored at 5±3°C used as control. Samples were kept for 90 days for stability analysis and after 90 days, drug loading of nanoparticles were compared with those of the control formulations[16,17,18]. The results of stability study are tabulated in Table 6.

RESULTS AND DISCUSSION

Percentage yield:

The yield of nanoparticles formulations given in Table 4 was found to be increased with increasing in drug loading. The loss of yield might be due to recovery problem and adherence of formulation due to sticky nature of lipid polymer. Comparative loss in ET1 is more than ET3 due to presence of comparatively large quantity of polymer in F1.

Table No 1
The % yield of Nanoparticles

Formulations	Yield (% w/w)*
ET1	70.00
ET2	73.89
ET3	76.90
ET4	84.73
ET5	72.34
ET6	75.65
ET7	82.78
ET8	72.46
ET9	75.45
ET10	80.28
ET11	74.67
ET12	82.34

Percentage entrapment efficiency:

The entrapment efficiency and drug loading of ET in the lipid based nanoparticles was found in the range of 86-90%. This can be explained by the high lipophilicity of the ET, minimizing its loss into external water phase as ET solubility in water is very less.

Table No.2
% of drug entrapment

Formulations	Drug Loading (%±S.D.)*
ET1	9.31±0.10
ET2	13.46±0.47
ET3	17.34±0.31
ET4	19.80±0.35
ET5	9.56±0.23
ET6	12.56±0.45
ET7	15.46±0.65
ET8	11 .23±0.45
ET9	12.34±0.76
ET10	15.34±0.38
ET11	13.34±0.56
ET12	19.23±0.28

Particle Size:
Table 3 shows Nanosized particles of range in between 150-225 nm were obtained. Particle size of the nanoparticles formulation was observed to be increased slightly with the increase in ET. Distribution of particles size range was observed narrow for ET4, ET7, ET12 compared to other formulations in Figure 1.

Table No.3
Particle Size ET3 Formulation.

Formulations	Mean Particle size (µm±S.D.)*
ET1	0.542±0.016
ET2	0.412±0.023
ET3	0.156±0.019
ET4	0.198±0.031
ET5	0.603±0.018
ET6	0.487±0.034
ET7	0.278±0.028
ET8	0..523±0.018
ET9	0.398±0.035
ET10	0.167±0.016
ET11	0.489±0.023
ET12	0.204±0.016

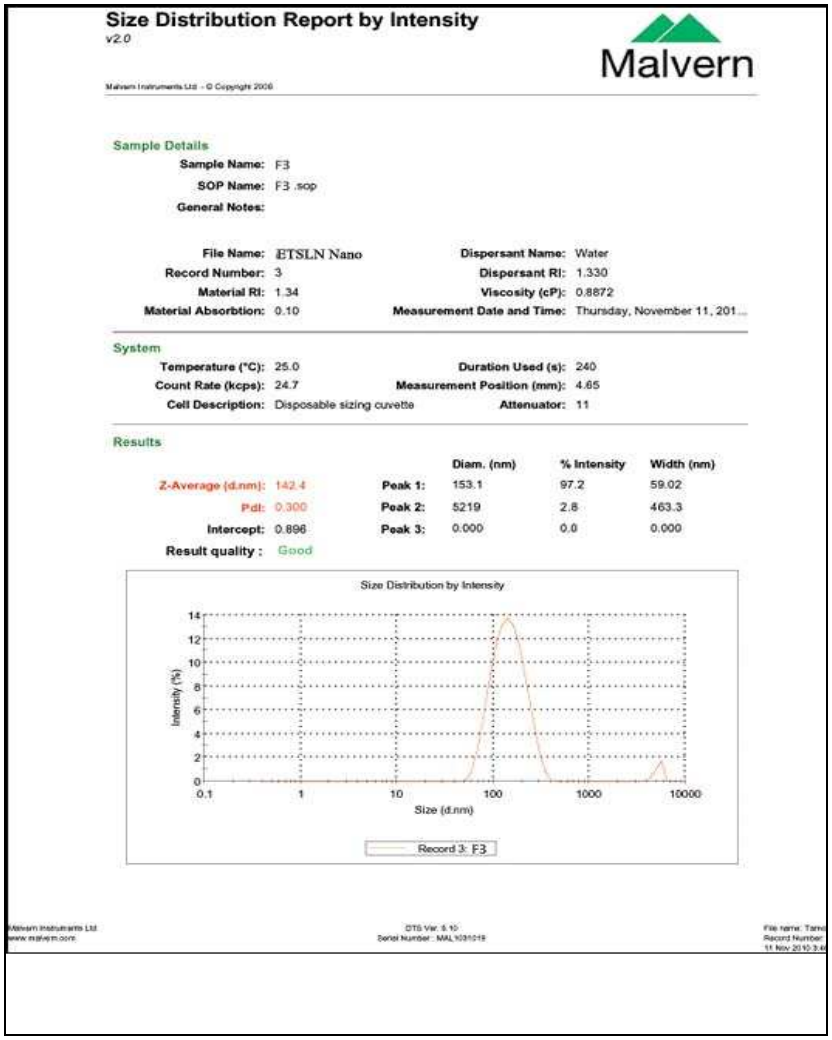

Fig .1
Typical particle size analysis of ET1 –ET12 Formulation

Surface morphology SEM:

Figure 2 showed the typical SEM photograph of the nanoparticles formulation demonstrating the particle size distribution pattern of formulation. Figure showed that ETSLN were superficially spherical in shape with smooth surface. The particles are observed aggregated might be due to the sticky nature of polymer.

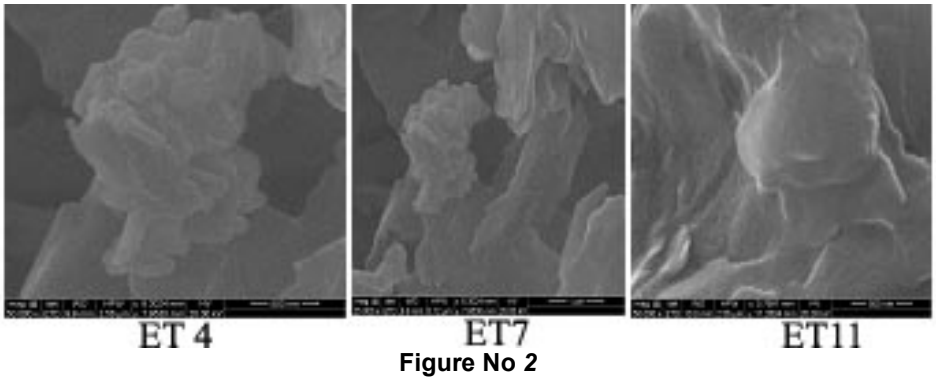

Figure No 2
***Typical scanning electron micrographs of drug loaded* Nanoparticles.**

In vitro drug release studies:

Figure 3 showed the release behavior of ETSLN nanoparticles demonstrated sustained release pattern for up to 90 hrs. At the initial stage, lipid based nanoparticles burst effect related to the drug entrapped near the surface of the nanoparticles[7,8] was remarkably small, and it was followed by a very slow release stage.

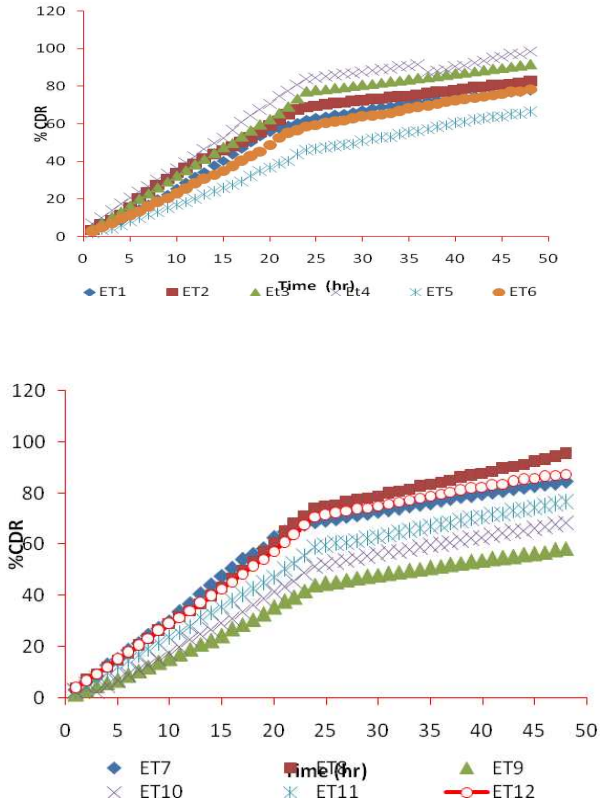

Figure No. 3

Release profile of ETSLN nanoparticles ET1, ET2, ET3, ET4, ET5, ET6, ET7, ET8, ET9, ET10, ET11 and ET12.

Stability studies:

The ETSLN nanoparticles were subjected to stability analysis over a period of 3 months. The obtained results of PTX content in nanoparticles after 15, 30, 60 and 90 days of stability studies for each formulation are tabulated in Table 4.Drug loading data of freshly prepared formulations stored at 5±3°C were considered for the control and compared against samples subjected to stability studies[6,7].There was no effective change in the ET content in the formulations stored at 25°±2°C/60%±5% RH at the end of 90 days. However, the samples kept at 40°±2°C/75%±5% RH, significant reduction in amount of ET was determined at the end of 15, 30, 60 and 90 days in each formulation. This might be due to the degradation of both drug and lipid polymer having a low glass transition temperature and low melting point was concluded.

Table 4
The results of stability study

Time (days)	Formulations	Drug Loading (% w/w)		
		Control 5±3°C	25±2°C / 60%±5% RH	40±2°C / 75%±5% RH
	ET1	4.51	4.69	3.15
	ET2	9.89	9.59	8.01
	ET3	17.84	18.04	17.34
	ET4	28.59	27.09	26.97
	ET5	8.80	8.58	8.42
	ET6	19.39	19.12	19.01
	ET7	29.70	29.43	29.33
	ET8	8.45	8.24	8.12
	ET9	19.03	18.76	18.62
	ET10	29.65	29.43	29.32
	ET11	18.48	18.24	18.10
	ET12	30.05	29.83	29.65

90 DAYS

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