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Research Article

Development and Characterization of a Particulate Drug Delivery System for Etoposide

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ABSTRACT

In the present study polymeric biodegradable nanoparticles (NPs) of Etoposide (ETP) were prepared by modified spontaneous emulsification solvent diffusion method using polylactic-co-glycolic acid (PLGA) as biodegradable matrix. The formulations were then characterized with respect to size and its surface morphology, zeta potential, entrapment efficiency, *in vitro* drug release profile, sterility testing, stability studies and *in vivo* tissue distribution study. The formulated Etoposide-PLGA nanoparticles were spherical with a diameter ranging from 150 to 250 nm. Highest entrapment efficiency was found to be 73.83%. Highest cumulative percent drug release was observed with F-8 (83.50%) in 120 hrs. Formulation F-8 with optimal particle size, high entrapment efficiency and satisfactory *in vitro* release was selected for *in vivo* studies. The average targeting efficiency of drug loaded nanoparticles was found to be 27.23±0.126% % of the injected dose in liver, 41.72±0.415% in lungs, 10.63±0.269% % in kidney and 13.24±0.572% in spleen. The results revealed that, the drug loaded nanoparticles showed preferential drug targeting to lungs followed by liver, kidney and spleen. Stability studies indicated that 4^0C is the most suitable temperature for storage of PLGA nanoparticles. This drug delivery is endowed with several exclusive advantages and hence holds potential for further research and clinical application.

INTRODUCTION

Nanomaterials are at leading edge of the rapidly developing field of nanotechnology. Nanoparticles are solid colloidal particles ranging in size from10-1000nm. They are made of macromolecular material which can be of synthetic or natural origin. Depending on the process used for their preparation, two different types of nanoparticles can be obtained, nanospheres and nanocapsules. Nanospheres have a matrix type structure in which a drug is dispersed, whereas nanocapsules exhibits a membrane-wall structure with an oily core containing the drug because these systems have very high surface areas, drugs may also be adsorbed on their surface[1]. Their unique size-dependent properties make these materials superior and indispensable in many areas of human activity[2].

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In recent years, polymeric nanoparticles have received considerable attention as potential drug delivery devices in view of their applications in the controlled release of drugs, their ability to target particular organs/ tissues, as carrier of DNA in gene therapy, and their ability to deliver proteins, peptides and gene through a peoral route of administration[3]. Polymeric nanoparticles can have engineered specificity, allowing them to deliver a higher concentration of pharmaceutical agent to a desired location, this feature makes polymeric nanoparticles ideal candidate for cancer therapy, delivery of vaccines and delivery of targeted antibiotics [4]. They can also facilitate important advances in detection, diagnosis and treatments of human cancers and lead to a new discipline of nano-oncology[5]. It has been demonstrated that a significant improvement in drug specificity of action can be reached upon its incorporation into nanoparticles, this effect being mainly attributed to change in tissues distribution and pharmacokinetics. These modifications may consequently result in a reduction in the side

effects and toxicity of the drug and increases in its therapeutic efficacy [6]. Chemotherapy is an integral part of cancer treatment. Despite of advance in oncology drug discovery, conventional chemotherapeutic agents still exhibit poor specificity to target tumor tissue and are often restricted by dose-limited toxicity. The combination of nano-technology and targeted drug delivery may provide a more efficient and less harmful solution to overcome the drawbacks of traditional drug delivery and by delivering chemotherapeutics right to the target site without hurting to normal organs and tissues [7].

Cancer, also called malignant neoplasis refers to a group of more than 100 different diseases that are characterized by DNA damage that causes abnormal and keep divining when new cells are not needed, a mass of tissue forms. This mass of extra tissues, called a growth or tumor, can be being or malignant. Antineoplastic drugs (also known as cytotoxic drugs) are used in the treatment of malignant neoplasm when surgery or radiotherapy is not possible or has proved ineffective as an adjunct to surgery or radiotherapy, or as the initial treatment [8].

Etoposide is a semi-synthetic derivative of podophyllotoxin, which is derived from the American Madrake (Podophyllum peltatum). Etoposide has been shown to inhibit the entry of cells into mitosis[9]. Etoposide is an anticancer agent used in the treatment of a variety of malignancies including malignant lymphomas. It acts by inhibition of topoisomerase-II and activation of oxidation reduction reactions to produce derivatives that bind directly to DNA and cause DNA damage [10]. Etoposide has a short biological half-life (3-6 hours), and although intra-venous injection would result in initial high local tumor concentrations, prolonged exposure of tumor cells may not be possible. It is investigated that intra-venous delivery of etoposide through polymeric nanoparticles would be a better approach for effective treatment of peritoneal tumors[11].

The present study was aimed to prepare and evaluate polymeric biodegradable nanoparticles of Etoposide (ETP). Nanoparticles were prepared by modified spontaneous emulsification solvent diffusion method using polylactic-co-glycolic acid (PLGA) as biodegradable matrix. PLGA is highly biocompatible and biodegraddble synthetic polymer, widely used extensively for controlled drug delivery systems[12]. The prepared nanopartcles were then characterized with respect to size and its surface morphology, zeta potential, entrapment efficiency, *in vitro* drug release profile, sterility testing, stability studies and *in vivo* organ tissue distribution studies of the NPs in rats.

MATERIALS AND METHODS

Etoposide was procured from Getwell Pharma, Haryanna, India; PLGA (75:25) was obtained from Cipla Ltd., Mumbai, India. Polyvinyl alcohol (PVA) (12000 MW) was purchased from West Coast Laboratories., Mumbai, India. Dichlomethane (DCM), Methanol and Acetone are purchased from Merck Ltd., Mumbai, India. Deionized water was used throughout the experiment. All other chemicals used were of laboratory grade.

Preparation of Etoposide-PLGA Nanoparticles

Nanoparticles were fabricated by using one of the promising techniques that is spontaneous emulsification solvent diffusion (SESD) method. The method involves preparation of an organic phase consisting of polymer (PLGA) and drug (etoposide) dissolved in mixture of organic solvents, dichloromethane (DCM)/Ethanol/ Acetone, which was suitably stirred to ensure that all material are dissolved. The organic phase is slowly poured into the aqueous phase containing a poly vinyl alcohol (PVA) using high speed homogenizer (IKA, Ultra Turrax, USA) at 24,000 rpm for 15 minutes. After evaporation of the internal phase under stirring, the polymer precipitated and nanoparticles were isolated by using a centrifuge at 21,000 g for 20minutes. The pellets were washed three times, resuspended in deionized water and formulation was sterilized by membrane filtration (0.22µ) in aseptic condition and freeze dried to obtain lyophilized particles. Lyophilized formulations were stored in previously sterilized (autoclave) glass containers and all the preparations steps were carried out at room temperature (23-25^{0}C).The prepared formulations were stored in freeze [12]. Eight different batches were prepared and labeled as F-1 to F-8 with the following composition as shown in Table 1.

Evaluation of Etoposide Loaded Nanoparticles

Practical Yield

Percentage practical yield is calculated to know about the efficiency of any method, thus it helps in selection of appropriate method of production. Practical yield was calculated as the weight of

nanoparticles recovered from each batch in relation to the sum of starting material. The percentage yield of prepared nanoparticles was determined by using the formula.

$$\text{Percentage Yield} = \frac{\text{Practical Yield}}{\text{Theoretical Yield}} \times 100$$

Table 1: Various Formulations of Etoposide-Loaded Nanoparticles

Formulation	Drug (mg)	Polymer (mg)	PVA (%)	DCM/ACE/ETL (ml)
F1	20	100	1.0	15/25/10
F2	20	100	1.5	15/25/10
F3	20	100	2.0	15/25/10
F4	20	100	2.5	15/25/10
F5	20	150	2.0	15/25/10
F6	20	200	2.0	15/25/10
F7	20	250	2.0	15/25/10
F8	20	300	2.0	15/25/10

DCM- Dichloromethane; ACE- Acetone; ETL- Ethanol

Determination of Drug Content

The nanoparticles formulations (0.1 ml/ 1ml) was diluted to 1 ml with methanol/ chloroform (1:1) solution. Final volume is diluted with same solvent mixture. And etoposide contents were estimated in a UV-Visible Spectrophotometer at 286 nm against blank solvent system containing the same concentration of drug in the formulation [13].

Drug Entrapment Efficiency

The entrapment efficiencies of prepared systems were determined by measuring the concentration of free drug in the dispersion medium. The entrapped etoposide was determined by adding 1 ml of nanosuspension to 9 ml of methanol in order to dissolve the entrapped drug; the obtained suspension was centrifuged for 30 min at 5000 rpm. The supernatant was separated and then filtered through 0.45 µm millpore (Millipore Filter). The filtrate was diluted using methanol and measured specrophotometrically (Shimadzu, UV 1700, Japan). The amount of free drug was detected in the filtrate and the amount of incorporated drug was determined as a result of the initial drug minus the free drug[14]. The entrapment efficiency was calculated using the following equation.

$$\text{Entrapment Efficiency} = \frac{W_{\text{initial drug}} - W_{\text{free drug}}}{W_{\text{initial drug}}} \times 100$$

Where "$W_{\text{initial drug}}$" is the mass of initial drug used and the "W_{free} drug" is the mass of free drug detected in the supernatant after centrifugation of the aqueous dispersion.

Particle Size

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. Particles suspended in a dispersing fluid are subject to random collisions with the thermally excited molecules of the dispersing fluid resulting in Brownian motion. The velocity and direction of the resulting motion are random but the velocity distribution of a large number of mono-sized particles averaged over a long period will approach a known functional form, in this case the size distribution of the particles were analyzed by Nanotrac.

Shape and Surface Morphology

Shape and surface morphology of nanoparticles was done by Scanning Electron Microscopy (JSM-T330A, JEOL). The three dimensional information about macro (0.1-10 mm) meso (1-100 µm) and microstructure (10-1000 nm), is often found within the same micrograph. SEM has been used to determine particle size distribution, surface topography, texture and to examine the morphology of fractured surface. Small volume of nanoparticulate suspension was placed on an electron microscope brass stub. The stubs were placed briefly in a drier and then coated with gold in an ion sputter. Pictures of nanoparticles were taken by random scanning of the stub. The shape and surface morphology of the nanoparticles was determined from the photomicrographs of each batch.

Zeta Potential

The surfaces of particles in suspension develop a charge due to adsorption of ions or ionization of surface groups and the charge is correspondingly dependent on both the surface chemistry and environment of the particles. Zeta potential was measured by using zeta potentiometer (Zeta 3.0+ meter, USA). Sample was filled into the cell; electrodes are inserted, placed under the microscope, and connected them to the Zeta-Meter 3.0+ unit. Electrodes energized and the colloids watched to move across a grid in the microscope eyepiece. Track one by simply pressing a "track" button and holding it down while the colloid traverses the grid. When the

"track" button released, the Zeta-Meter 3.0+ instantly calculates and displays the colloid's zeta potential (or electrophoretic mobility). The thumb rule describes the relation between zeta potential determination responses of the suspension being tested, particularly hydrophobic colloids.

In vitro Drug Release

The *in vitro* drug release of the formulation was studied by using dialysis membrane and modified apparatus. The dissolution medium used phosphate buffer of pH 7.2. Dialysis membrane, previously soaked overnight in the dissolution medium was tied to one end of a specially designed glass cylinder (open at both ends). 5ml of formulation was accurately placed into this assembly. The cylinder was attached to a stand and suspended in 150 ml of dissolution medium maintained at 37± 5 ^{0}C so that the membrane just touched the receptor medium surface. The dissolution medium was stirred at low speed using magnetic stirrer. Aliquots, each of 5 ml volume were withdrawn at various intervals of time over a period of 120 h. The aliquots were suitably diluted with receptor medium and analyzed by UV-Vis Spectrophotometer at 286 nm. The quantity of drug equivalent to 20 mg of Etoposide was taken for dissolution study.

Sterilization

Sterilization of etoposide Nanoparticles was carried out by membrane filtration (Mechanical method) using membrane filter with pore size of 0.22 µm in an aseptic condition using laminar air flow. All glass containers were sterilized by autoclave, 115-120 ^{0}C at 30 min (Moist heat Sterilization).

Test for Sterility

It is carried out to ensure the sterility of finished product. Since it is administered by parenteral route, direct inoculation method was preferred to carry out sterility testing. In this method the specified quantity of sample under test was drawn aseptically from the containers and transferred into a vessel of culture medium (Agar). Mixture of nanoparticles with the medium was incubated for not less than 14 days and observation for growth of any microorganisms in the medium was made[15].

***In Vivo* Drug Targeting Studies**

Nine healthy adult Sprague Dawley rats weighing 200-250 g (approved by Animal Ethical Committee, K.L.E.S's College of Pharmacy, Belgaum) were selected, a constant day and night cycle was maintained and they were fasted for 12 h. The animals were divided into 3 groups, each containing 6 rats. Group I received nanoparticles equivalent to 900 µg/kg of etoposide intravenously in the tail vein after redispersing them in sterile phosphate buffer saline solution, F-8 (optimized) batch were selected for the study. Group-II rats received 900 µg/ kg of pure etoposide intravenously. Group-III rats were treated as solvent control and were injected intravenously with sterile phosphate buffer saline solution After 24 h, the rats were sacrificed and their liver, lungs, spleen, kidney, heart and brain were isolated. The individual organs of each rat were homogenized separately by using a tissue homogenizer and the homogenate was centrifuged at 17609 x g for 30 min. The supernatant was collected and filtered through 0.22 µm filters and analyzed by UV Spectrophotometer at 286 nm after dilutation with phosphate buffer [16].

Stability Studies

Stabilities studies of the prepared nanoparticles were carried out, by storing formulation F-8 at 4 ^{o}C ± 1 ^{o}C and 30 ^{0}C $\pm$ 2 ^{0}C / 65 % $\pm$ 5 % RH in humidity control oven for thirty days. The samples were analyzed for drug content, entrapment efficiency and *in vitro* drug release profile (ICH Q1A (R_2) 2003).

RESULTS AND DISCUSSION

Percent Practical Yield

The results of percent practical yield are shown in Table 2. Percent practical yield depends on the concentration of polymer added. It increases with increase in concentration of polymer added to the formulation. Maximum percent practical yield was found to be 73.43% for F-8.

Particle Size

The size distributions along the volume mean diameter of the nanoparticles were measured by Dynamic Light Scattering Particle Size Analyzer (Nanotrac Particle Size Analyzer). Particle sizes of all eight batches are shown in Table 2.

Particles of all formulations were in nanosize having smooth spherical surface. Addition of a small volume of ethanol to the mixture of acetone/ DCM leads to smaller Etoposide nanoparticles and this alteration can prevents the aggregation of particles effectively. Lowering

the amount of acetone or ethanol resulted in production of larger particles. The higher the amount acetone in the internal phase, the smaller was the particle size. The amount of surfactant is crucial as it prevents the coalescence of the droplets and protects them. When polymer concentration was kept constant at 100 mg with surfactant i.e. PVA increase in concentration of 1.0 %, 1.5 %, 2 % and 2.5 % for formulations F1, F2 F3 and F4 the particle size was found to be 221.8 nm, 180.3 nm, 159.9 nm and 153.7 nm respectively. As the surfactant concentration increased there was a decreased in particle size may be because of stabilizing effect of the surfactant, thereby preventing the aggregation of particles.

Table 2: Characteristic Parameters of the PLGA Loaded Etoposide Nanoparticles

Formulation	% Practical Yield	Particle Size (nm)	Entrapment Efficiency (%)	Cumulative % Drug Release (After120 h)
F-1	55.10	221.8	58.22	43.51
F-2	59.16	180.3	62.58	48.27
F-3	63.33	159.9	65.76	53.26
F-4	65.80	153.7	66.78	58.04
F-5	65.88	158.0	67.05	63.94
F-6	69.09	154.7	69.91	70.58
F-7	71.48	163.	72.21	77.09
F-8	73.43	150.0	73.83	83.50

*Each value represents mean (n=3)

Drug Entrapment Efficiency

The entrapment efficiency of eight batches of etoposide nanoparticles are recorded in Table 2. As the polymer concentration was increased from 100-300 mg the encapsulation efficiency was increased. When the polymer concentration was maintained constant of 100 mg, the PVA concentration was varied as 1.0 %, 1.5 %, 2.0 % and 2.5 %, the encapsulation efficiency was slightly increased. The result indicates the polymer concentration plays a major role in drug entrapment efficiency rather than the PVA concentration. The maximum entrapment efficiency was found in F-8 with 73.83 % (Figure 1). The preparation parameters, such as PVA concentration and Etoposide /PLGA ratios were modified to obtain nanoparticles with higher entrapment efficiency.

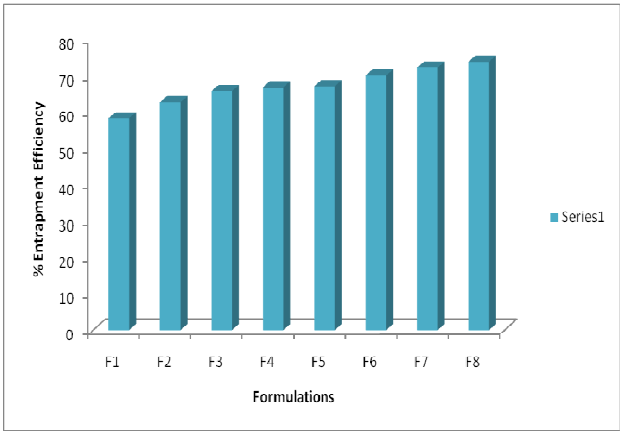

Figure 1: Drug Entrapment Efficiency, F-1 to F-8

While the increase in the concentration of surfactant from 1 % to 2.5 %, prevented the coalescence of the fine droplets during stirring.

Surface Morphology

Shape and surface morphology of nanoparticles was studied by Scanning Electron Microscopy (SEM) (JSM-T330A, JEOL). SEM photographs of all formulations were shown in Figure 2. Etoposide nanoparticles have shown smooth and spherical shape with different sizes depending on the ratios of the surfactant and polymer used.

Zeta Potential

The stability study of the nanoparticles was evaluated by measuring the zeta potential of the nanoparticles by the zeta meter. Zeta potential of all formulated nanoparticles was in the range of –25.16±0.243 to – 32.71±0.964 mV which indicates moderate stability with no agglomeration.

Compatibility

From the I.R. spectral analysis it was found that I.R. spectrum of etoposide with PLGA 75:25 showed all characteristic peaks in combination with no significant changes as shown in Figure 3.

In Vitro Drug Release Study

Cumulative percentage drug released for formulations F-1 to F-8 after 120 hours was observed (Figure 4). It was observed that the drug release from the formulations increases as the polymer concentration increases and all the eight formulations showed a biphasic release with initial burst effect. The mechanism for the burst release can be attributed to the drug adsorbed on the nanoparticles or due to leakage of drug from nanoparticles.

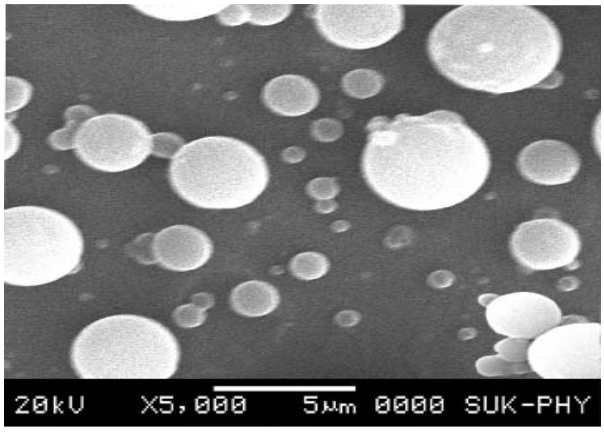

Figure 2(A)

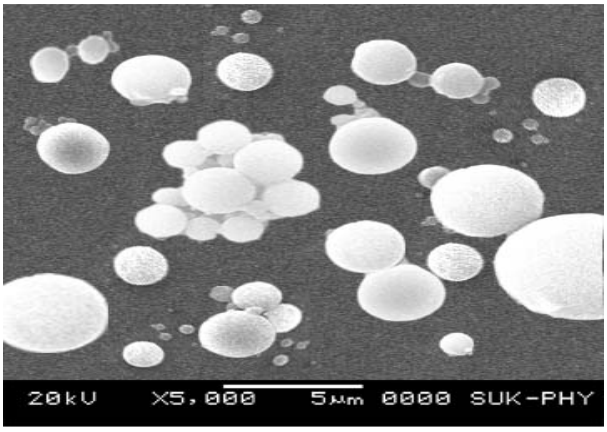

Figure 2(B)

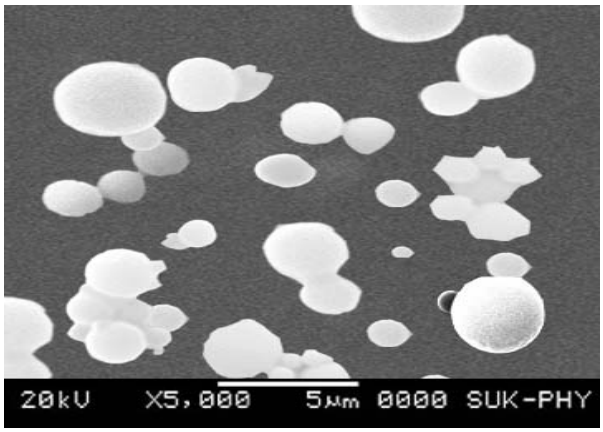

Figure 2(C)

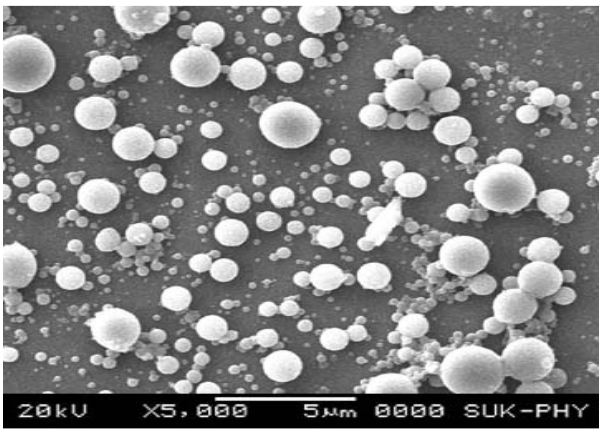

Figure 2(D)

Figure 2: Scanning Electron Microscopy of Etoposide Loaded Nanoparticles

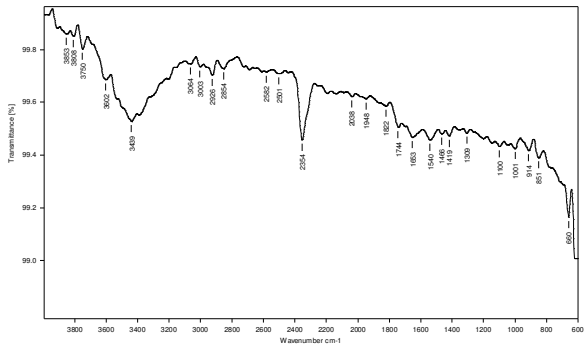

Figure 3(A): IR Spectra of pure PLGA

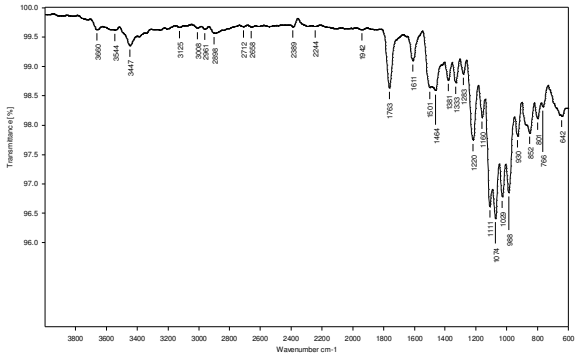

Figure 3 (B): IR Spectra ETOPOSIDE

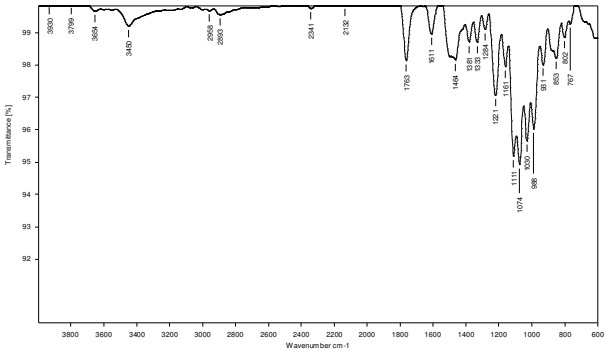

Figure 3 (C) : IR Spectra of ETOPOSIDE+PLGA

Figure 3: IR Spectra for compatibility study

The release is gradually decreased and remained constant even after 120 h. In fact, etoposide has good solubility in acetone which by diffusing towards the aqueous phase, not only enhances the drug leakage from nanoparticles significantly, but also promotes its distribution in proximity of the oil-water interface. The enhancement in the release of etoposide from nanoparticles may be supported by the hydration process which occurs very rapidly due to the smaller size of nanoparticles. Afterwards, the matrix materials would require time to erode in the aqueous environment.

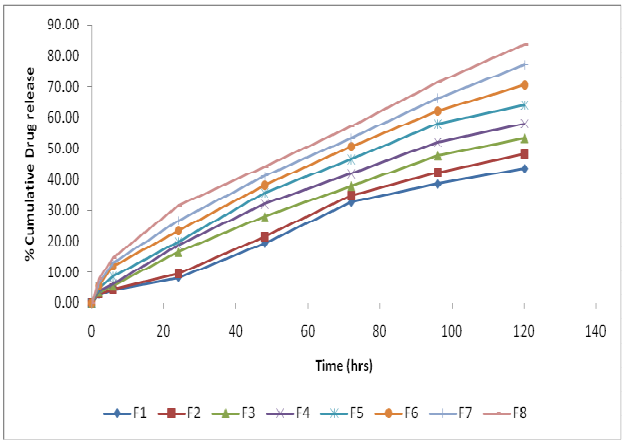

Figure 4: *In Vitro* Drug Release Profile of Etoposide Loaded Nanoparticles, F-1 to F-8.

Based on the highest regression values (r), the best–fit model for formulation F-1 and F-2 was Higuchi Model, and for formulations F-3, F-4, F-6, F-7 and F-8 was Hixson Crowell Model. The drug release from nanoparticles may be due to mechanism such as diffusion and dissolution of drug molecules from polymeric matrix of nanoparticles and the biodegradation of polymeric matrix of nanoparticles. On the basis of results, for formulations F-1 and F-2 it was found that the best fitting was obtained with Higuchi Model. Higuchi Model of drug release which describes the mechanism largely on the basis of diffusion. PLGA degradation in neutral medium is very slow, and no degradation of PLGA in the buffer medium is expected to be occurred within the period of release experiments. For formulations F-3, F-4, F-6, F-7 and F-8 it was found to be Hixson Crowell Model, results indicated that the release rate was limited by the drug particles dissolution rate and erosion of the polymer matrix. The hydrophilic nature of the matrix caused by the surfactants may accounts for a more rapid entry of water into the nanoparticles, eventually accelerating the release of etoposide in formulation when higher amount of the surfactants is used.

Sterility Test

In order to ensure the sterility of the finished products, the optimized formulation F-8 along with F-3, F-5 was subjected to sterility test. Selected formulation was incubated with agar medium for a period of 14 days. The results obtained after 14 days of incubation showed no growth of organisms on the culture medium. This indicates that, the formulation is sterile and passes the test for sterility.

***In Vivo* Tissue Distribution Study**

The average targeting efficiency of drug loaded nanoparticles was found to be 79.61±0.126% of the injected dose in liver, 37.09±0.415% in spleen, 35.23±0.269% in kidney and 31.41±0.572% in lung, whereas accumulation of pure drug in liver was 18.57±.2.104%, in lungs it was 8.90±1.724%, in kidney it was 07.10±0.827% and spleen 7.93±0.503% of the injected dose. (Figure 5)

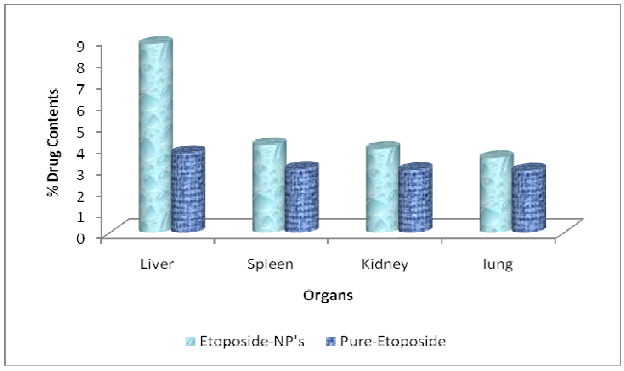

Figure 5: *In Vivo* Tisuue Distribution Studies of Etoposide Loaded Nanoparticles, F-8

These results revealed that, the drug loaded nanoparticles showed preferential drug targeting to liver followed by spleen, kidney and lungs. It was also revealed that, as compared to pure drug, higher concentration of drug was targeted to the organs like lungs and liver after administering the dose in the form of nanoparticles.

Stability Studies

Stabilities studies of the prepared nanoparticles were carried out by storing formulation F-8 at 4 ^{o}C ± 1 ^{o}C and at 30 ^{0}C $\pm$ 2 ^{0}C / 65 % $\pm$ 5 % RH in humidity control oven for 90 days. Three parameters namely percent drug content, entrapment efficiency and *in vitro* drug release studies were carried out.

Table 3: Drug Content and Entrapment Efficiency After 90 Days Storage of Formulation (F-8)

	Evaluation Parameters			
Time	**% Drug content at 4 ± 1°C**	**% Drug entrapment efficiency at 4 ± 1°C**	**% Drug content at 25° ± 2°C / 60 ± 5 % RH**	**% Drug entrapment efficiency at 25° ± 2°C / 60 ± 5 % RH**
30 Days	76.38	71.56	73.37	43.37
60 Days	77.74	69.37	71.83	22.71
90 Days	78.03	70.81	69.09	08.33

Table 4: *In Vitro* Drug Release after 90 Days Stability Study of Formulation (F-8)

Time (h)	% Cumulative Drug Release					
	At 4 ± 1°C			At 30°C ± 2°C / 65% ± 5% RH		
	30 Days	60 Days	90 Days	30 Days	60 Days	90 Days
0	0	0	0	0	0	0
2	6.21	7.8	8.9	26.08	40.60	58.50
6	13.68	15.66	16.33	45.34	78.00	99.16
24	30.34	34.78	35.67	72.71	99.18	0.00
48	39.04	46.76	53.9	99.97	0.00	0.00
72	50.45	58.98	67.8	0.00	0.00	0.00
96	71.22	75.67	80.41	0.00	0.00	0.00
120	84.31	86.04	88.36	0.00	0.00	0.00

The results of drug content and entrapment efficiency after 90 days of stability testing at different storage conditions are shown in Table 3. By comparing this data with previous data it was observed that there is overall decrease in % entrapment efficiency. *In vitro* drug release for the same formulation is also shown in Table 4. On comparing this data with the % cumulative drug release data of F-8, it was observed that there is overall increase in the drug release. These results may be attributed to bulk erosion of nanoparticles to some extent during storage.

CONCLUSION

The result of the present investigation proposes a novel formulation of Etoposide nanoparticles by application of a high speed homogenization-solvent diffusion method using a single O/W emulsification. It was found that nanoparticles with desirable particle size and high entrapment efficiency can be produced by adjusting the process parameters. The particle size and drug entrapment as well as the drug release kinetics can be optimally controlled. The *in vivo* results revealed that, the drug loaded nanoparticles showed preferential drug targeting to liver followed by spleen, kidney and lungs.

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REFERENCES:

[1] Mu L, Feng SS. A novel controlled release formulation for the anticancer drug paclitaxel (Taxol): PLGA nanoparticles containing vitamin E TPGS. J Control Rel. 2003; 86:33-48.

[2] Salata OV. Applications of nanoparticles in biology and medicine. [Online]. 2004 [Cited 2006 January 6];[6Screens]. Available from;URL:http://www.jnanobiotechnology.com/content2/1/3

[3] Soppimath K, Aminabhavi TM, Kulkarani AR, Rudzinski WE. Biodegradable polymeric nanoparticles as drug delivery devices. J Control Rel. 2001; 70: 1-20.
[4] Michale S. Biodegradable polymeric nanoparticles in drug delivery. [Online].2006 [Cited 2006 May 12]; [2 Screens]. Available from: URL:http// www.arches.uga.edu/sjay/intro.html
[5] Patlolla RR, Vobalaboina V. Pharmacokinetics and tissue distribution of etoposide delivered in parentral emulsion. J Pharma Sci. 2005; 94(2): 437-45.
[6] Fonseca C, Simoes S, Gaspar R, Paclitaxel-loaded PLGA nanoparticles: preparation, physicochemical characterization and in vitro antitumor activity. J control Rel. 2002; 83: 273-86.
[7] Illum, L. Nasal drug delivery: new development and strategies. Drug Discovery Today. 2002; 7: 1184-9.
[8] Tripathi KD. Essentials of medical pharmacology 5th ed. New Delhi: Jaypee Brothers; 2003. 769-82.
[9] Hande KR, Topoisomerase II inhibitor. Updates on cancer therapeutics. 2006: 3-15.
[10] Reddy LH, Adhikari JS, Dwarakanath BSR, Sharma RK, Murthy RR. Tumoricidal effects of etoposide incorporated into solid lipid nanoparticles after intraperitoneal administration in dalton's lymphoma bearing mice. AAPS Journal. 2006; 8(2): E254-E262.
[11] Couvreeur P, Dabernet C, Puisieux F. Controlled drug delivery with nanoparticles: current possibilities and future trends. Eur J Pharm Biopharm. 1995(41):2-13.
[12] Esmaeili F, Atyabi F Dinarvand R. Preparation and Characterization of estradiol-loaded PLGA nanoparticles using homogenigation- solvent diffusion method. Daru. 2008; 16(4):196-202.
[13] Patlolla RR, Vobalaboina V. Pharmacokinetics and tissue distribution of etoposide delivered in parentral emulsion. J Pharma Sci 2005;94(2):437-45.
[14] Gadha A, Raina HF. Diazepam loaded solid lipid nanoparticles: Design and characterization. AAPS pharma Sci Tech. 2009; 10(1):221-19.
[15] Clyde M, Ofner III, Karen P, Bill J, Bowman, Chao-Sheng Chen. Growth inhibition, drug load, and degradation studies of gelatin/methotrexate conjugates. Int J Pharm. 2006; 308: 90–99.
[16] Doijad RC, Manvi FV, Godhwani DM, Joseph R, Deshmukh NV. Formulation and targeting efficiency of cisplatin engineered solid lipid nanoparticles. Indian J Pharm Sci. 2008;70(2):203-7