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COLLOIDAL DRUG DELIVERY OF BIODEGRADABLE POLY (LACTIDE-CO-GLYCOLIDE) (PLG) INJECTABLE NANOPARTICLES FOR ANTICANCER DRUG

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ABSTRACT

Purpose: *The present study was aimed at preparing and evaluating biodegradable nanoparticles of docetaxel (DTX). Method: Nanoparticles were prepared by emulsification solvent evaporation technique using polylactic-co-glycolide (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, stability studies and in vivo tissue distribution study.*

Results: *The formulated DTX-PLGA nanoparticles were oval with diameter ranging from 200 nm to 400 nm. The entrapment efficiency was found to be in the range 51.07% to 62.16%. Highest cumulative percent drug release was observed F-1 (49.24 %) and lowest F-4 (36.25%) in 48 h. Based on the highest regression values (R), all four formulations followed Peppas Korsmeyer model. Formulation F-4 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 11.23±0.126% of the injected dose in liver, 27.72±0.415% in lungs, 10.63±0.269% in kidney and 13.24±0.572% in spleen whereas accumulation of pure drug in liver was 7.93±.2.104%, in lungs it was 8.57±1.724%, in kidney it was 08.10±0.827% and spleen 11.35±0.503% of the injected dose.*

Conclusion: *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° 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.*

Keywords: *Docetaxel, PLGA, nanoparticles, emulsification solvent evaporation*

INTRODUCTION

The challenge of modern drug therapy is the optimization of the pharmacological action of drugs coupled with the reduction of their toxic side effects *in vivo*. Nanoparticles are colloidal polymeric drug carriers that hold promise for peroral drug delivery. Nanoencapsulation of drugs involves forming drug-loaded particles with diameters ranging from 1 to 1000 nm. They consist of macromolecular materials in which the active ingredient is entrapped, to which the active ingredient is adsorbed or attached [1, 2].

The worldwide burden of cancer is a major health problem, with more than 8 million new cases and 5 million deaths per year [3]. It is clear that the

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progress in cancer treatment has been slow and inefficient [4]. The ultimate goal of all cancer therapy is cure of cancer without damaging the rest of the body. It is not possible to achieve it always because of the propensity of cancer to invade adjacent tissue [5]. In all cases of cancer, the effectiveness of treatment is directly proportional to the treatments ability to target and kill the cancer cells without affecting healthy cells. The amount of change in the patient's quality of life is directly related to this targeting ability of the treatment. For most current cancer patients only selectivity in their treatment is related to the inherent nature of the chemotherapeutics drugs to work on a particular type of cancer cell more intensely than on healthy cell. However, by administrating bolus doses of these intense drugs systematically some side effects will always occur and sometimes are so intense that the patients must discontinue therapy before the drugs have a chance to eradicate the cancer [5].

Docetaxel belongs to the class of taxanes, which are microtubule-stabilizing agents. It promotes tubulin assembly in microtubules and inhibits their depolymerization. It induces a mitotic block in proliferating cells by acting as a mitotic spindle poison and causes cell cycle arrest in S phase [6, 7]. Docetaxel is more potent than paclitaxel in various tumor models of breast, lung, ovarian, colorectal cancer, melanoma and leukemia. Docetaxel is cell cycle specific in G2 and M phases [8, 9, 10].

In the polymer PLGA the first number is the molar percentage lactide and second number is the molar percentage of glycolide. Poly (lactide-co-glycolic acid) (PLGA), from the ester family, has been widely used in the biomedical industry as a major components in biodegradable sutures, bone fixation nails and screws. It is well characterized polymer, its degradation sub-products are non toxic, it provides controlled drug release profiles by changing the PLGA copolymer ratio which affects the crystallinity (low crystallinity, more amorphous polymer means more fast degradation) of PLGA. For these reasons, PLGA has been selected as the polymer of choice in the present research [11]. From the analysis of bile it became clear that docetaxel is extensively metabolized by the liver, however no metabolized could be found in plasma in early reported pharmacokinetic studies [12].

Materials and Methods

2.1 Materials

Docetaxel and Polylactic-co-glycolic acid (PLGA) 75:25 M ratio, Mw 34,000 Da was obtained as gift sample from Cipla Ltd. Mumbai, (India); Poly vinyl alcohol 12000 MW (PVA) was obtained from West Coast Laboratories, Mumbai,; Dichloromethane was supplied by S.D. Fine Chemicals Ltd. Mumbai, (India); Methanol was obtained from Merck Ltd, Mumbai; Acetone and Tetrahydrofuran (THF) was supplied by Ranbaxy Fine Chemicals Ltd., New Delhi, (India). All other chemicals were of the best quality commercially available.

2.2 Formulation of Docetaxel-PLGA nanoparticles

Nanoparticles were prepared by using emulsification by sonication-solvent evaporation. The method involves preparation of an organic phase consisting of polymer (PLGA) and drug (docetaxel) dissolved in organic solvent, dichloromethane (DCM). The organic phase is added to an aqueous phase containing a surfactant poly vinyl alcohol (PVA) to form an emulsion. This emulsion is broken down into nanodroplets by stirring and these nanodroplets form nanoparticles upon solvent evaporation. Once the colloidal suspension of nanoparticles is prepared using the above method, the free drug is removed by using extraction method to obtain the final nanoparticulate suspension [11, 13, 14].

2.3 Freeze-drying of nanoparticles

Usually, nanoparticles may subject to a series of stability problems such as aggregation, fusion and

leakage of the encapsulated drugs in to the storage medium. One of the approaches to resolve this kind of problems is to freeze-dry the nanoparticles. A freeze-dried product offers the advantages of improved stability, dosing accuracy and sterility. The nanoparticulate formulations were dispensed in glass containers rapidly frozen at -40ºC, freeze-dried for approximately 14 h under vacuum in a freeze dryer (Heto Freeze-dryer, UK). The drug encapsulated nanoparticles was taken in a 100 ml round bottom flask. The shelves and samples were cooled to -40ºC and maintained at -40ºC for 2 h. Primary drying was performed at –20 ºC for 4 h. The secondary drying phase was carried out by increasing the temperature to – 4 ºC for 10 h. After freeze-drying, the lyophilized product was obtained in the form of dry mass. This mass was broken gently using a glass rod to obtain a free flowing powder of lyophilized product. Lyophilized product was packed in glass vial and preserved in freeze until its further use [15, 16].

2.4 Practical Yield

Percentage particle yield is calculated to know about percentage yield or efficiency of any method, thus it helps in selection of appropriate method of production. Particle yield was calculated as the weight of nanoparticles recovered from each batch in relation to the sum of starting material [15].

2.5 Determination of drug content

10 mg of lyophilized docetaxel nanoparticles were dissolved in 100 ml of tetrahydrofuran and centrifuged at 17609 x g. The supernatant was collected after centrifuge and measured by UV spectrophotometer at 227 nm [15].

2.6 Shape and surface morphology

Shape and surface morphology of nanoparticles was done by Scanning Electron Microscopy (JSM-T330A, JEOL). 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 (Polaron SEM coating system). 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 [11, 17].

2.7 Particle size and size distribution

The particle mean diameter and size distribution were determined using Scanning Electron Microscopy (SEM) technique. The diameter of about 100 nanoparticles was measured from the photomicrographs of each batch. Finally, average mean diameters were obtained [11, 19].

2.8 Zeta potential

The electrophoretic mobility and zeta potential were measured using a zeta potentiometer (Zeta Meter 3+, USA). To determine the zeta potential, nanoparticles sample were diluted with KCl (0.1 Mm) and placed in the electrophoretic cell where an electric field of 15.2 V/cm was applied. Each sample was analyzed in triplicate [18].

2.9 In vitro drug release study

The *in vitro* release of drug from the nanoparticulate formulations was determined using dialysis cassettes (Slide-A-Lyzer®3.5K, Pierce, U.S.A). The suspension of nanoparticles were taken in the dialysis tube, which was immersed in a beaker containing 200 ml of pH 7.4 phosphate buffer as the diffusion medium and was stirred with magnetic stirrer. The time at which diffusion was initiated was noted and 5 ml of diffusate was withdrawn with pipette at various time intervals of 1, 2, 4, 6, 8, 12, 24, 36 and 48 h, and these samples were filtered through 0.22 µm membrane filter (Minisart, Germany) and extracted three times with 5 ml of dichloromethane. The

extraction solvent was evaporated and docetaxel residue was solubilized in tetrahydrofuran. The obtained solution was analyzed spectrophotometrically (Shimadzu UV/Vis spectrophotometer, Japan) at 227 nm after suitable dilution if necessary, using appropriate blank [16].

2.10 In vivo tissue distribution studies

This study was carried out after obtaining the due permission for conduction of experiments from relevant ethics committee (K.L.E.S's College of Pharmacy, Belgaum) which is registered for "Teaching and Research on Animals" by committee for the purpose of control and supervision of experiments on animal, Chennai (Registration number 221/CPCSEA).

Dose of docetaxel to be administered to rats was calculated according to body surface area ratio of human being. Nine healthy adult *Sprague dawley* rats weighing 200-250 g 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 3 rats. Group I received nanoparticles equivalent to 300 µg/kg of docetaxel intravenously in the tail vein after redispersing them in sterile phosphate buffer saline solution, F-4 (optimized) batch were selected for the study. Group-II rats received 300 µg/ kg of pure docetaxel 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 0.22µ filters (Minisart, Germany) and analyzed by UV Spectrophotometer at 227 nm [16, 20].

2.11 Stability Study

Stability tests are the series of tests designed to obtain information on the stability of the pharmaceutical product in order to define its shelf life and utilization period under specified packaging and storage conditions. The purpose of stability testing is to provide information on how the quality of a drug product varies with time under the influence of variety of environmental factors such as temperature, humidity and light, and to establish a shelf life for the drug product at recommended storage conditions.

From the four batches of docetaxel-loaded nanoparticles, formulation F-4 was tested for stability studies. Formulation F-4 was divided into 3 sample sets and stored at: $4^{o} \pm 1^{o}C$, $25^{o} \pm 2^{o}C$ and 60% ± 5% RH. and $37^{o} \pm 2^{o}C$ and 65% ± 5% RH. After one month, the drug release of selected formulation (F-4) was determined by the method discussed previously in in-vitro drug release studies and percentage drug content was carried out for the same formulation. [21]

RESULT AND DISCUSSION

The results of percent practical yield are shown in Table 1. 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 73.4 % for F-4.

To investigate the possible morphological changes of nanoparticles on loading process, samples of DTX nanoparticles were observed under the scanning electron microscope. Scanning electron photomicrographs of all the four formulations are taken, Formulation (F4) result is shown in Fig. 1. Magnification of 7,500- 20,000 X was used while taking these photographs. Average particle size of nanoparticles of docetaxel was 283 $\pm$ 0.013 nm, 358 $\pm$ 0.081 nm, 304 $\pm$ 0.093 nm, 395 $\pm$ 0.078 nm for formulations F1, F2, F3 and F4 respectively. Particles of all formulations were in nanosize having smooth

surface. The results of particle size data are shown in Table 1 for formulation F-1, F-2, F-3 and F-4.

The % drug content in docetaxel nanoparticles was found to be 62.38%, 69.47%, 65.71% and 73.65% for formulation F-1, F-2, F-3 and F-4 respectively. The entrapment efficiency in four batches of docetaxel nanoparticles was studied. The result for entrapment efficiency is shown in Table 1. It was observed that the entrapment efficiency increases with the increase in concentration of PLGA in the formulations and also it increases with increase in the concentration of PVA in internal phase. The maximum entrapment was found in F-2, and F-4.

The possible effects of surface charge may affect the *in vivo* life span of the natural drug delivery system. The surface charge on the microscope particle produced a difference in the electric potential in mV between the surface of each particle and bulk of the suspending liquid. That difference is called as zeta potential. It is easily measured because the charge of the potential will move as the suspension is placed between the two electrode that have D.C. voltage across them and the velocity will be proportional to the zeta potential of the particle. The technical term for this is electrophoresis. The electric charge present on the nanoparticles was evaluated by measuring the zeta potential by the zeta meter. Zeta potential of all formulated nanoparticles was in the range of -25.16 to – 32.71 mV which indicates moderate stability with no agglomeration.

All the four formulations of prepared nanoparticles of docetaxel were subjected to *in vitro* release studies. These studies were carried out by using magnetic stirrer with diffusion technique in phosphate buffer pH 7.4. The results obtained for *in vitro* release studies were five models of data treatment as shown in Table 2. Cumulative percentage drug released for F-1, F-3 after 48 h was more than cumulative release of F-2, and F-4 (Fig. 2). It was observed that the drug release from the formulations decreases as the polymer concentration increases and all the 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. The Peppas model is widely used when the release mechanism is not well known or when more than one type of release phenomenon could be involved. 'n' value could be used to characterize different release mechanisms.

Formulation F-4 with optimal particle size, high entrapment efficiency and satisfactory *in vitro* release was selected for in vivo drug targeting studies. The comparison between the amount of drug targeted from nanoparticles and free drug in various organs are presented in Fig.3. The average targeting efficiency of drug loaded nanoparticles was found to be 11.23±0.126% of the injected dose in liver, 27.72±0.415% in lungs, 10.63±0.269% in kidney and 13.24±0.572% in spleen whereas accumulation of pure drug in liver was 7.93±.2.104%, in lungs it was 8.57±1.724%, in kidney it was 08.10±0.827% and spleen 11.35±0.503% of the injected dose. These results revealed that, the drug loaded nanoparticles showed preferential drug targeting to lungs followed by liver, kidney and spleen. 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.

Stabilities studies of the prepared nanoparticles were carried out, by storing formulation F-4 at $4^{o} \pm 1^{o}C$, $25^{o}\pm 2^{o}C$ 60% RH ± 5% RH and $37^{o}\pm 2^{o}C$ 65% RH ± 5% RH in humidity control oven for 30 d. Two parameters namely residual percent drug content and *in vitro* release studies were carried out. *In vitro* dissolution for the same formulation is also shown in Table 3. These results indicate that the drug release from the formulation stored at $4^{o}\pm1^{o}C$ was lowest followed by formulation stored at $25^{o}\pm2^{o}C$; 60%±5% RH and $37^{o}\pm2^{o}C$; 65%±5% RH. It was also revealed that F-4, the one stored at $4^{o}\pm1^{o}C$ showed maximum

drug content followed by that stored at 25°±2°C; 60%±5% RH and 37°±2°C; 65%±5% RH.

CONCLUSION

PLGA nanoparticles were successfully formulated, characterized and evaluated *in vitro*. The formulated Docetaxel-PLGA nanoparticles were of optimum particle size, the entrapment efficiency and satisfactory cumulative percent drug release. The average targeting efficiency of drug loaded nanoparticles was found to be in liver followed by in lungs, kidney and spleen respectively. Stability studies indicated that 4°C 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.

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Table 1: Physicochemical characteristics of PLGA loaded Docetaxel nanoparticles

Formulations	Percentage Yield	Particle Size (nm)	Drug Entrapment Efficiency (%)
F1	61.6	283±0.013	51.07
F2	70.1	358±0.081	57.45
F3	66.4	304±0.093	56.36
F4	73.4	395±0.078	62.18

Table 2: Model Fitting analysis of Formulated Docetaxel Nanoparticles by Using Different Mathematical Models

Formulation	First Order	Zero Order	Higuchi's Matrix	Peppa's Plot	Crowell Hixson	'n' value	Best fit model
F1	0.823	0.748	0.913	0.962	0.81	0.857	Peppa's model
F2	0.842	0.770	0.929	0.967	0.829	0.913	Peppa's model
F3	0.801	0.737	0.893	0.943	0.786	0.976	Peppa's model
F4	0.781	0.721	0.934	0.984	0.767	0.965	Peppa's model

Table 3: *In Vitro* Release Profile after Stability of Selected Formulation F-4

Time	Cum % Drug Release at 4° ± 1°C After 30 Days	Cum % Drug Release at 25° ± 2°C / 60 % ± 5% After 30 Days	Cum % Drug Release at 37°± 2°C / 65 % ± 5% After 30 Days
1	2.89	4.89	8.24
2	5.25	8.61	13.51
4	10.25	13.87	19.46
6	16.25	20.31	26.13
8	21.25	26.49	33.13
12	26.95	35.88	38.26
24	33.25	46.28	51.71
36	36.25	48.03	52.12
48	37.25	49.30	52.49

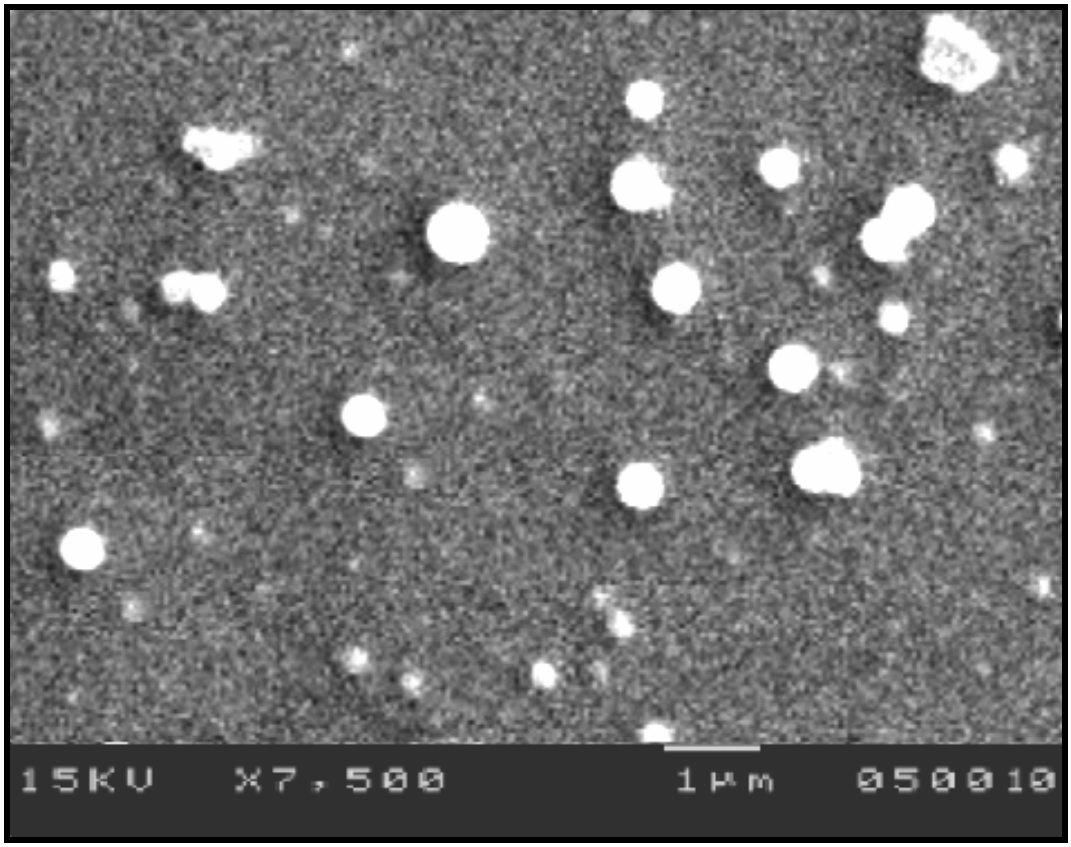

Fig. 1: Scanning Electron Photomicrograph of Formulation F-4

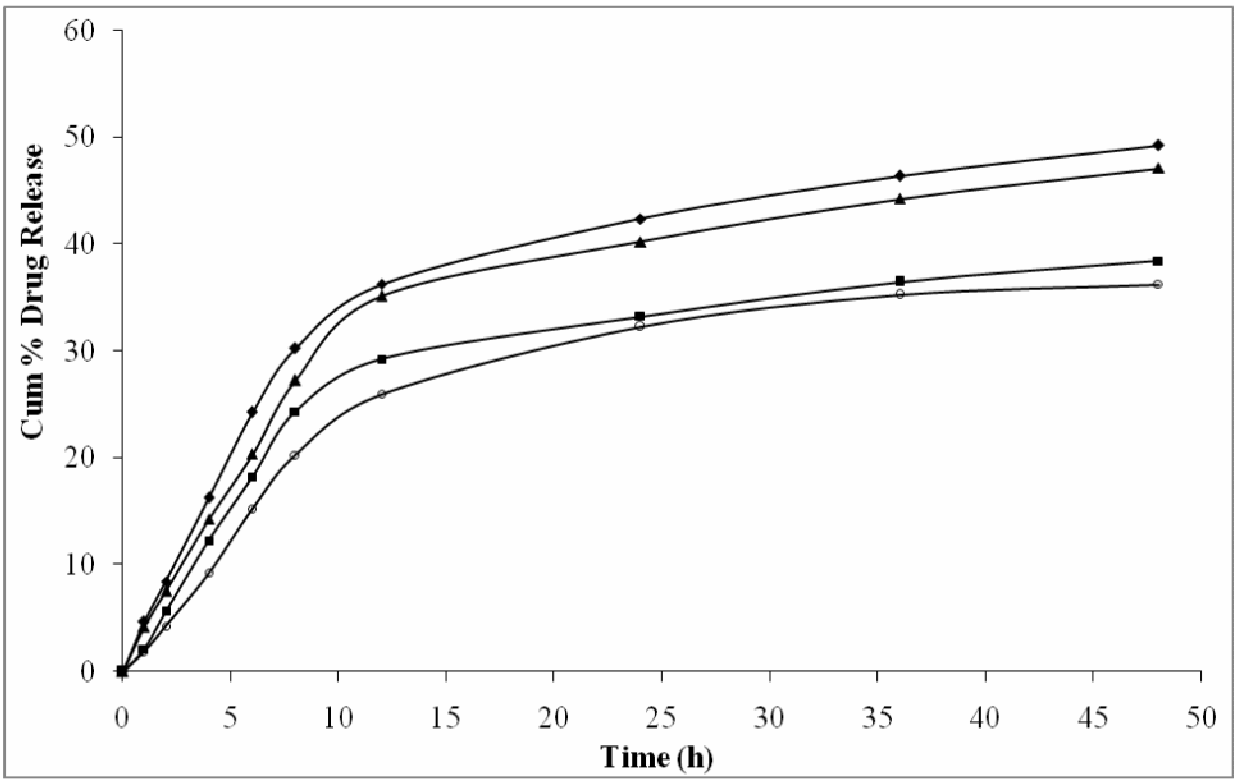

Fig. 2 Comparative in vitro Release of Docetaxel Nanoparticle According to Zero Order Kinetics. Data represents the mean ± S.E. (n=3).
Formulation F-1 (♦ - ♦), F-2 (■ - ■), F-3 (▲-▲) and F-4 (○ - ○)

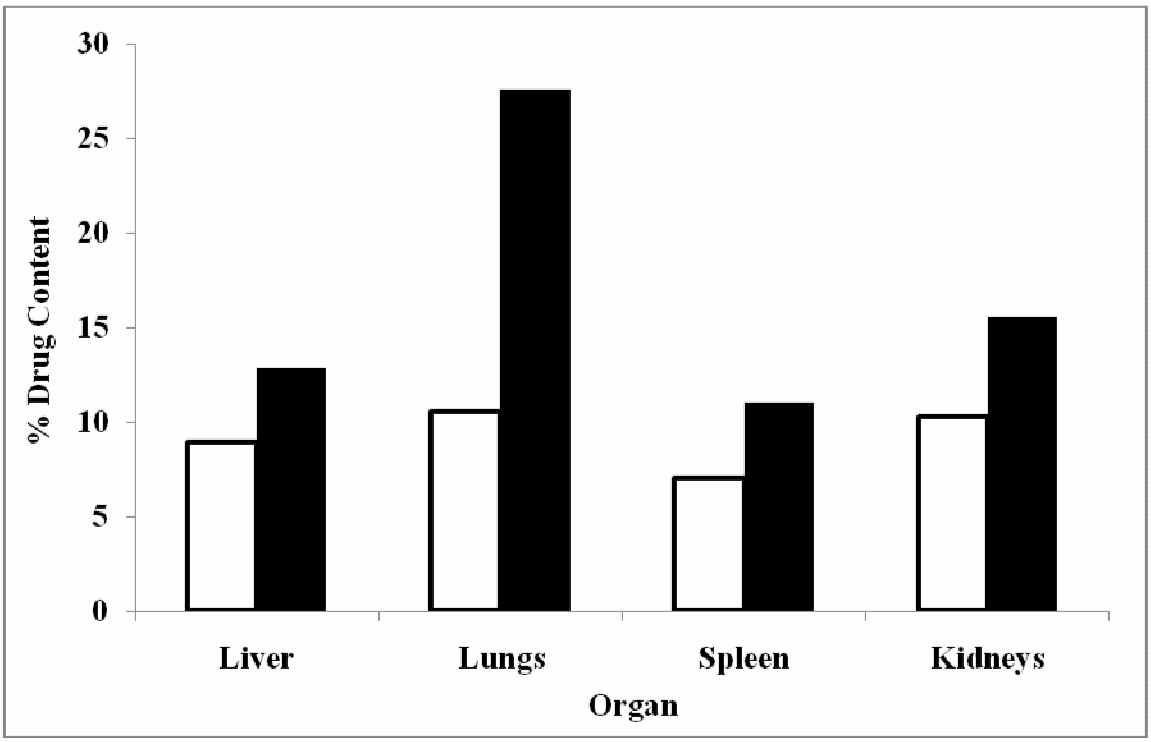

Fig. 3: Comparative *in vivo* Tissue Distribution Studies of Docetaxel nanoparticles (F-4) and docetaxel pure drug. Data represents the mean ± S.E. (n=3). Docetaxel nanoparticles (■),docetaxel pure drug (□)

Reference

1) *J. Kreutor, Nanoparticles in encyclopedia of pharmaceutical technology, in: Swarik J editor, Marcel Dekker Inc., New York, USA. 1994, pp. 165-90.*
2) *G.C.S. Rao, M.K. Satish, N. Mathivanan, et al. Advances in nanoparticulate drug delivery systems. Indian drugs. 41 (2004) 389-390.*
3) *S.S. Feng, et al. Chemotherapeutic engineering: Application and further development of chemical engineering principles for chemotherapy of cancer and other diseases. Chem Eng Sci., 58 (2003) 4087-4114.*
4) *Cancer [Online], [Cited 2009 Sept 17]. Available from: URL: http:// www.cancer.org, 2006.*
5) *A. Pokale, Inorganic and organic hybrid nano-capsules based anticancer drug delivery, Cranfield University, Cranfield health MSc Clinical Research Academic Year, 2006/2007.*
6) *A. Suzuki, T. Kawabata, M. Kato, Necessity of interleukin-1beta converting enzyme cascade in taxotere-initiated death signaling, Eur. J. Pharmacol., 343 (1998) 87-92.*
7) *R. Schimming, Lack of correlation between mitotic arrest or apoptosis and antitumor effect of docetaxel, Cancer Chemoth Pharm, 43 (1999) 165-172.*
8) *Baorui Liu, Mi Yang, Rutian Li, et al, The antitumor effect of novel docetaxel-loaded thermosensitive micelles, Eur. J. Pharm. Biopharm., 69 (2008) 527–534.*
9) *M.C. Bissery, G. Nohynek, G.J. Sanderink, et al, Docetaxel (Taxotere): a review of preclinical and clinical experience. Part I: Preclinical experience, Anticancer Drugs., 6 (1995) 339-8.*
10) *Wikipedia the free encyclopedia, Docetaxel [ONLINE], cited [2009 Sept 08], Available From: URL:http://en.wikipedia.org/wiki/Docetaxel.*
11) *A. Budhian, J.S. Siegel, K.I. Winey, Haloperidol-loaded PLGA nanoparticles: Systemic study of particle size and drug content. Int. J. Pharm., 336 (2007) 367-375.*
12) *H. Rosing, V. Lusting, F. P. Koopman, et al, Beinen Bio-analysis of Docetaxel and hydrotaxel and hydroxylated metabolites in human plasma by high-performance liquid chromatography and automated solid-phase extraction, J Chromatogr., B 69 (1997), 689-698.*

13) *P.C. Reis, R.J. Neufeld, A.J. Ribeiro, et al, Nanoencapsulation: methods for prerparation of drug-loaded polymeric nanoparticles, Nanomedicine: Nanotechnology, Biom M., 2 (2006) 8-21.*

14) *Nanoparticulate formulations of docetaxel and analogues there of, [Online], cited [2007 Nov 20]; Available from: URL: http://www. freepatentsonline. com/ 20060188566.html*

15) *L. D. Hu, X. Tang, F.D. Cui, Solid lipid nanoparticles to improve oral bioavailability of poorly soluble drugs, JPP., 15 (2004) 1527-1535.*

16) *F. Quaglia, L. Ostacolo, A. Mazzaglia, et al, The intracellular effects of non-ionic amphiphilic cyclodextrin nanoparticles in the delivery of anticancer drugs, Biomaterials., 30 (2009) 374–382.*

17) *D.D. Magharla, R. Rajagopalan, S. Devrajan, et al, Biodegradation and biocompatibility of contraceptive-steroid-loaded poly (DL-lactide-co-glycolide) injectable microspheres: in vitro and in vivo study, Contraception., 74 (2006) 148-56.*

18) *H. Tajerzadeh, M. Hamidi, A. Dehpour, et al, In vitro characterization of human intact erythrocytes loaded by Enalaprilat, Drug Delivery., 8 (2001) 223-230.*

19) *E.I. Shabouri, Positively charged nanoparticles for the oral bioavailability of cyclosporine –A, Int. J. Pharm., 249 (2003) 101-108.*

20) *L. Zhao, Y.M. Wei, X.D. Zhong, Pharmacokinetics and tissue distribution of docetaxel in rabbits after I.v. administration of liposomal and injectable formulations, J Pharmaceut Biomed (2008), doi:10.1016/j.jpba.2009.01.016.*

21) *ICH Q1A (R2), Stability testing guidelines: Stability testing of new drug substances and products, The European agency for the evaluation of medicinal products, CPMP/ICH/2736/99 (2003) 4-20.*

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