RESEARCH ARTICLE

Paclitaxel loaded poly(sebacic acid-co-ricinoleic ester anhydride)-based nanoparticles

Santosh B Totiger, Jagadeesh G Hiremath[1]

Department of Pharmacology, NET, Pharmacy, College, Raichur, Karnataka, India, [1]Department of Pharmaceutics, School of Pharmacy, University of Nizwa, Birkat Al Mouz, Sultanate Oman

The main objective of the present study was to prepare paclitaxel (PTX)-loaded poly(sebacic acid-co-ricinoleic ester anhydride) (poly (SA-RA) 70:30 w/w)-based nanoparticles (NPs). PTX- Poly (SA-RA) NPs were prepared by solvent displacement technique. The prepared formulations were characterized in terms of particle size and distribution, surface morphology using Malvern laser analyzer, scanning electron microscope (SEM). Drug physical and chemical state were determined by Fourier transform infrared spectroscopy (FTIR), X-ray diffractometry (XRD) and differential scanning calorimetry. The particles obtained were spherical in shape with a smooth surface and mean particle size in the range of 443-436 nm. The entrapped PTX within the polymer matrix was in the form of amorphous state.

Key words: *Paclitaxel (PTX), poly (sebacic acid-co-ricinoleic ester anhydride), nanoparticles*

INTRODUCTION

Nanotechnology has been regarded as one of the most promising approaches to deal with cancer, which remains a leading cause of death all over the world.[1,2] Liposomes and polymeric nanoparticles are utmost investigated nanocarriers for anticancer drug delivery. Liposomes have been widely used as a drug delivery vehicle due to high biocompatibility, favorable pharmacokinetic profile, and ease of surface modification. However, liposomes have limitations for drug delivery including insufficient drug loading, fast release of hydrophobic drugs, and instability.[3] Polymeric nanoparticles were featured by their small size, ability to encapsulate drug of poor solubility and permeability, controlled drug release manner, and long circulation half-life.[4,5] It has been reported that polymeric nanoparticles are preferentially accumulated in tumor after intravenous (i.v.) administration due to the enhanced permeability and retention effect, known as "passive" targeting.[6,7] Although this passive manner of drug delivery omits the targeting group at the surface of polymeric nanoparticles, it is quite effective to deliver drugs to the tumor sites. To achieve effective tumor accumulation of polymeric nanoparticles through enhanced permeability and retention effect, the nanoparticles should stay long time in blood circulation. A number of approaches for prolonging the blood circulation and accumulating in tumor tissues of nanoparticles have been reported, including control of size, surface potential as well as surface hydrophilicity.[8,9]

Paclitaxel (PTX) is highly lipophilic and insoluble in water (<0.03 mg/mL). The compound does not contain any functional groups that can be ionized by altering pH or that allow salt formation to increase its solubility.[10] PTX is a mitotic inhibitor used in cancer chemotherapy especially breast and ovarian cancer. PTX has been associated with severe side effects, including hypersensitivity reactions, nephrotoxicity, and neurotoxicity. The submicron size of NPs offers numerous advantages, such as being more stable during storage.[11] Biodegradable polyanhydrides are useful materials for controlled drug delivery. Poly(sebacic acid-co-ricinoleic-ester anhydride) is a biodegradable polyanhydride polymer for controlled drug delivery. The hydrolytically labile anhydride and/or ester placed in aqueous medium, it may hydrolyze to dicarboxylic acids and hydroxy acid monomers. The hydrolytic degradation of aliphatic polyesters and polyanhydrides depends on various physical, chemical, and biological

Address for correspondence:
Dr. Jagadeesh G Hiremath,
Assistant Professor in Pharmaceutics, College of Pharmacy and Nursing, University of Nizwa, P.O. Box 33, Post Code 616 Birkat Al Mouz, Sultanate of Oman.
E-mail: jagadeesh@unizwa.edu.om

Access this article online

Quick Response Code:

Website: www.asiapharmaceutics.info

DOI: 10.4103/0973-8398.97120

parameters, including hydrophobicity of monomers and polymer, crystallinity of polymer, water permeability of the polymer matrix, additives, and the degradation medium and conditions.[12] Hence, the attempt has been made to prepare PTX containing nanoparticles by using polyanhydride class of polymer. The objectives of the present study were to prepare drug loaded and drug free, polyanhydride-based nanoparticles and their *in vitro* characterization.

MATERIALS AND METHODS

Materials

PTX was obtained as a gift sample from Naprod Life Sciences. Pvt. Ltd. (Mumbai, India) Poly (SA-RA) 70:30 w/w (weight-average molecular weight = 21,000; number-average molecular weight = 10,000) was synthesized as previously reported.[13] Briefly, poly (SA-RA) was prepared in a one-pot reaction in which poly(sebacic anhydride) was reacted with ricinoleic acid (70:30 w/w ratio) at 120°C for 2 haves followed by anhydride polycondensation at 130°C under vacuum (0.1 mm Hg) using acetic anhydride for activation of the carboxylic acid end groups. The formed polymers were used without further purification. Tween-80 (poly-oxyethylene sorbital monooleate), ethanol, glucose, sodium dihydrogen orthophosphate, and potassium dihydrogen orthophosphate were supplied by SD Fine Chem. Ltd. (Mumbai, India). Acetone and sodium chloride were purchased from Thermo Fisher Scientific India Pvt. Ltd. (Mumbai, India).

Methods

Preparation of PTX-loaded nanoparticles

PTX- Poly (SA-RA) NPs were prepared by solvent displacement method.[14] PTX (10 mg) was dispersed in 5 mL of acetone containing 100 mg poly(sebacic acid-co-ricinoleic ester anhydride) 70:30 previously dissolved. The mixture was magnetically stirred for 15 min at room temperature. Ethanol/water mixture (1:1, 20 mL) introduced into the organic phase and solution was stirred for 20 min at 1000±5 rpm. Obtained solution was stabilized for 30 min. Organic phase from the obtained tri-phasic system was removed by rapid evaporation with the aid of vacuum (Superfit Pvt. Ltd., Bangalore, India). For NPs, aqueous phase was freeze-dried (Christ alpha 1-4 LD Plus lyophilizer) with glucose as cryoprotector added prior to lyophilization. Drug free NPs were prepared in the same manner without adding PTX. The quantity of polymer and drug used for the preparation of NPs are illustrated in Table 1.

Characterization of PTX-Poly (SA-RA) NPs

Percentage yield

The lyophilized NPs from each formulation were weighed and the respective percentage yield was calculated using the following Equation 1:

$$\text{Percentage yield} = \frac{\text{Wt of NPs obtained}}{\text{Wt of drug, polymer and glucose used}} \times 100 \quad (1)$$

Determination of drug loading and entrapment efficiency

The average drug content was determined by extracting the drug with mixture of methanol and phosphate buffer saline pH 7.4 (9:1 v/v) as solvent. Accurately weighed NPs (~10 mg) were dissolved in 25.0 ml of solvent. After filtration (0.22 µm, Nylon membrane, Millipore, India) and suitable dilutions, amount of PTX was analyzed using double beam UV-spectrophotometer at 230 nm. All the readings were carried out in triplicate. Determination of drug loading using Equation 2.

$$\text{Drug loading (\%)} = \frac{\text{Weight of drug in nanoparticles}}{\text{Weight of nanoparticles taken}} \times 100 \quad (2)$$

Entrapment efficiency of poly (SA-RA)-based nanoparticles were determined by analyzing the un-entrapped drug. Suspension of 10.0 mg of NPs in 10.0 ml PBS pH 7.4 was subjected to cold centrifugation at ~4°C with 14,000 rpm for 15 min. Aliquot from the supernatant solution in which free drug was suspended was withdrawn and suitably diluted with methanol. The resulting solution was analyzed by UV-spectrophotometrically at 230 nm. The entrapment efficiency is defined as the ratio of amount of entrapped drug to that of the drug used for the NPs preparation. Entrapment efficiency was determined using Equation 3.

$$\text{Entrapment efficiency (\%)} = 1 - \frac{\text{Free drug}}{\text{Theoretical drug loaded}} \times 100 \quad (3)$$

Scanning electron microscopy (SEM analysis)

The shape and surface characteristics of NPs were visualized using scanning electron microscopy (JSM-848, Joel, Japan). The NPs were first dried under vacuum and were glued to aluminum stab and gold coated under argon atmosphere. The coated NPs were finally characterized for surface morphology (scale bar was 25000x magnification).

Particle size analysis

Average particle size and distribution of the blank and PTX-Poly (SA-RA) NPs were measured by laser scattering light analyzer (Malvern Laser Analyzer Instruments). Drug loaded lyophilized NPs were dispersed in ultrapure water according to concentration and completely sonicated before measurement. The average particle size was expressed as the value of average size of three replicate samples. The width of the size distribution was indicated by the polydispersity index.

Fourier transform infrared analysis (FT-IR)

FT-IR spectral measurement for pure PTX, poly (SA-RA) 70:30,

Table 1: Composition of PTX-Poly (SA-RA) NPs

Formulations	Drug paclitaxel (mg)	Polymer poly (SA-RA) 70:30 w/w (mg)	Cryoprotector glucose (7% w/w) (mg)
F1	10	190	14
F2	20	180	14

physical mixtures of PTX and poly (SA-RA) (1:1ratio) and PTX-Poly (SA-RA) NPs were taken at ambient temperature using FT-IR spectrophotometer (Thermo Nicolet, Japan). Samples were mixed with KBr and vaccuum packed to obtain pellets of the material. All the spectra acquired were scanned between 500 and 4000 cm^{-1} at a resolution of 4 cm^{-1}.

Differential scanning calorimetric analysis

The physical status of the pure PTX, poly (SA-RA) 70:30 and mixture of PTX and poly (SA-RA) (1:1, ratio) as well as the drug encapsulated in the PTX- Poly (SA-RA) NPs formulations were investigated by employing the Differential Scanning Calorimetric (DSC) method (DSC 822e, Mettler Toledo, Switzerland). The samples were purged with liquid nitrogen at a constant flow rate. The temperature was ramped at a rate of 10°C/min, from 10 to 230°C.

X-ray diffraction analysis

X-ray diffraction patterns of pure PTX, poly (SA-RA) 70:30, physical mixtures of PTX and poly (SA-RA) 70:30 and PTX- Poly (SA-RA) NPs were determined using a X-ray diffractometer (AXS D8 Advance, Bruker) equipped with a rotating target X-ray tube and a wide-angle goniometer. The X-ray source was K_{α} radiation from a copper target with graphite monochromater. The X-ray tube was operated at a potential of 40 kV and a current of 30 mA. The range (2θ) of scans was from 0 to 70° and the scan speed was 0.04° per min at increments of 0.02°.

In vitro drug release studies

The *in vitro* release studies of PTX- Poly (SA-RA) NPs were carried out at 37±2°C in phosphate buffer saline (PBS) pH 7.4 media for a period of 90 haves. A horizontal water bath shaker was used to conduct *in vitro* release studies. NPs were suspended in 20 ml of release medium (PBS pH 7.4 containing 0.1% w/v Tween-80) in amber colored screw-capped bottles. Temperature was constantly maintained at 37±2°C and the platform was mechanically vibrated in horizontal axis at an average speed of 75 rpm. At predetermined time intervals, samples were withdrawn and replenished with fresh medium to provide the necessary sink condition. The samples were further suitably diluted with methanol, filtered by 0.22 µm Nylon membrane (Millipore, India) and analyzed using UV-spectrophotometer for the estimation of amount of PTX released. The cumulative percentage drug release was calculated to establish the drug release profile of the PTX-loaded NPs. Data of release studies were fitted into various kinetic models to understand the release mechanism of the from drug. Kinetic equations used were zero order equation, first order equation, Higuchi model, and Korsmeyer-Peppas model.[15]

Stability studies

PTX-Poly (SA-RA) NPs were stored at elevated temperatures with relative humidity (25±2°C/60%±5% RH, 40±2°C/75%±5% RH) in a stability analysis chamber (GMG, India) for a period of 3 months.[16] Freshly prepared PTX-Poly (SA-RA) NPs, stored at 5±3°C were used as control. Samples were kept for 3 months for stability analysis and at interval of 15, 30, 60, and 90 days, PTX content of NPs was estimated by using UV- spectrophotometrically and compared with those of the control formulations.

RESULTS AND DISCUSSION

The PTX-Poly (SA-RA) NPs were prepared by solvent displacement method using biodegradable poly (SA-RA) 70:30 w/w polyanhydride polymer. Poly (SA-RA) is a hydrophobic polymer built of natural fatty acids.[17] Temperature during organic phase evaporation was maintained ~40°C to avoid degradation in polymer property. Glucose, natural saccharides was used as a cryoprotector. Glucose prevents the shrinkage of particles during lyophilization and facilitates the particle formation.

The entrapment efficiency and drug loading of PTX in the poly (SA:RA) 7:3 based nanoparticles was found in the range of 86-90%. The percentage yield of developed nanoparticulate formulations were obtained in range of 61-64% w/w [Table 2]. The loss of yield might be due to recovery problem and adherence of formulation due to the sticky nature of polymer. The amount of PTX loaded in NPs and entrapment efficiency are shown in Table 2. The entrapment efficiency was decreased with increase of drug-polymer ratios. Large PTX entrapment in polymer matrix was probably due to hydrophobicity of drug and insolubility in water, which leads to minimum partition of drug into the external water phase.

The particles of size range in between 436-443 nm [Table 2] were obtained after lyophilization. Polydispersity of NPs was found within the range of 0.300-0.499 [Table 2]. Particle size was decreased slightly with increasing PTX content. Increase in particles size with increased polydispersity index was observed. The variation in particle size distribution (mean diameter) observed was might due to the variations in drug and polymer concentration and also the method of preparation.

Table 2: Results of nanoparticles yield, drug loading, entrapment efficiency, mean particle size and polydispersity index

Formulations	Yield (% w/w)[a]	Drug Loading (% w/w±SD)[a]	Entrapment efficiency (%±SD)[a]	Mean Particle size (nm±SD)[a]	Pdl[b]
Blank				489±0.085	0.0501± 0.046
F1	60.7	4.77±0.10	89.94±1.65	443±0.015	0.300±0.019
F2	63.9	9.18±0.47	89.16±1.30	436±0.033	0.499±0.026

[a]Average of three determinations, [b]Polydispersity index

Figure 1 shows typical scanning micrographs of blank (A) and PTX-Poly (SA-RA) NPs (B). Spherical particles with varying sizes and smooth surface were obtained. The particles are observed aggregated, probably due to the fatty acid nature of polymer.[17]

FT-IR spectra of pure PTX, poly (SA-RA) 70:30, physical mixture of PTX, and poly (SA-RA) 70:30 and PTX-Poly (SA-RA) NPs, F1 and F2 are shown in [Figure 2]. The spectrum of PTX shows characteristics absorption bands at 3066 cm^{-1} (–CH sp^3 stretching), 1733 cm^{-1} (C=O stretching) of amide group, 1640 cm^{-1} (N-H bending), 1444 cm^{-1} (C=C ring stretching). Poly (SA-RA) absorption bands were obtained at 2933 cm^{-1} (C-H stretching) and 1698 cm^{-1} (C=O stretching) and 1248 cm^{-1} (C-O bending) of anhydride group. Physical mixture shows absorption bands for both drug and polymer, indicated that there was no chemical or physical interaction between drug and polymer. While in case of formulations F1 and F2, only the characteristics bands of polymer was obtained at 2934, 1699, 1299 cm^{-1}, which indicated that the drug was effectively entrapped within the polymer matrix.

DSC analysis was performed to determine the physical state of drug in NPs, which is reported to be involved in the endothermic or exothermic process. DSC thermograms of pure PTX, poly (SA-RA) 70:30, physical mixture, and PTX loaded F1 and F2 are shown in [Figure 3]. The melting endothermic peak of pure PTX appeared at 220.0°C. A sharp and broadened endothermic peak of poly (SA-RA) 70:30 and physical mixture were observed at 120.0 and 122.5.0/227.8°C, respectively. In case of PTX-Poly (SA-RA) NPs formulations F1 and F2, broadened endothermic peaks of only polymer were observed at 123.0°C and 122.0°C, respectively, which indicated that PTX in the formulation was in amorphous form.

The amorphous state of drug within the NPs was further confirmed by X-ray diffraction patterns. The diffraction peaks of PTX were observed at 10.82, 12.90, 14.96, 17.97, 25.08, 43.744° (2θ) [Figure 4a]. In case of pure polymer, diffraction peaks were observed at 5.27, 20.22, 21.73, 25.3° (2θ) [Figure 4b]. Physical mixture of drug and polymer when subjected for X-ray diffraction analysis (XRD), same prominent diffraction peaks of drug and polymer were observed in mixture at 7.61, 17.76, 19.58, 21.91° (2θ) [Figure 4c]. The drug peaks did not appear in the formulations F1 and F2, while only polymer characteristic peaks were appeared at 5.12, 21.80, 23.63, 25.86° [Figure 4d], and 5.83, 20.43, 21.64, 23.82, 25.86° [Figure 4e], respectively. Absence of drug diffraction peak in the formulations, reveals the amorphous state of the PTX.

The *in vitro* release profile of PTX- Poly (SA-RA) NPs was investigated in PBS pH 7.4 with 0.1% v/v of tween-80. Figure 5 shows the cumulative percentage release of PTX from polymeric NPs. Tween-80 was added to increase the PTX solubility in water. At the initial stage, burst effect related to the drug entrapped near the surface of the

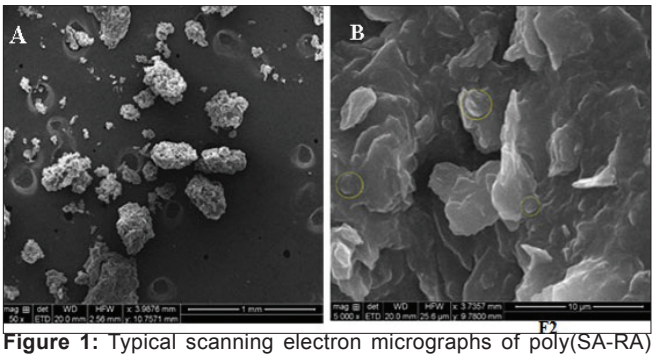

Figure 1: Typical scanning electron micrographs of poly(SA-RA) 70:30nanoparticles: (A) blank NPs and (B) PTX loaded NPs (F2)

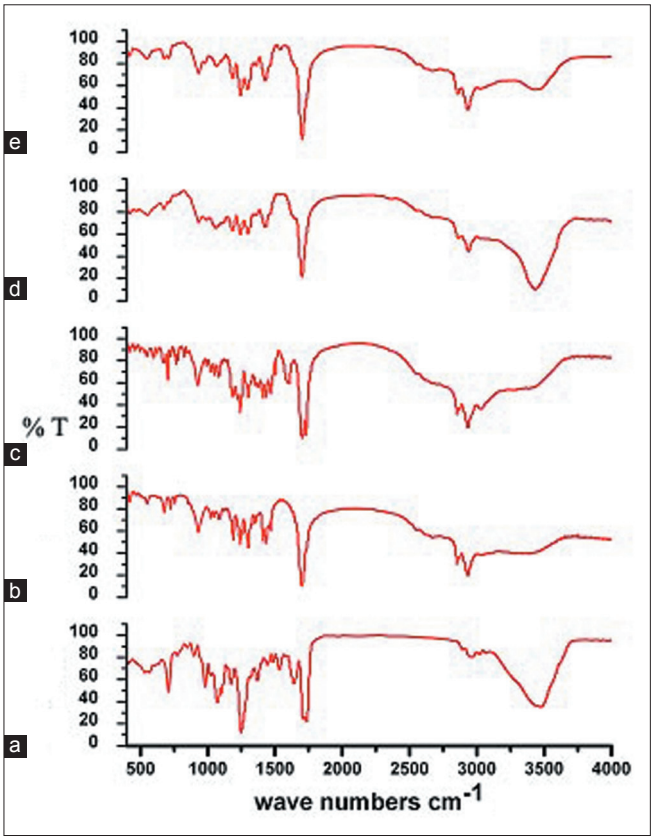

Figure 2: Transmission FT-IR spectra of pure PTX (a), pure poly(SA-RA) 70:30 (b), physical mixture of PTX and pure poly(SA-RA) 70:30 (c), F1 (d) and F2 (e)

NPs was remarkably small, and it was followed by a slow controlled release of drug. Such a small initial burst is an especially interesting phenomenon, which was due to the un-entrapped drug on the surface of the NPs. Controlled prolong release was a result of low permeability of water in poly (SA-RA) 70:30, a hydrophobic polyanhydride polymer.[17] After 90 haves, the amount of cumulative PTX release for F1 and F2 formulations was 89.73±0.95% and 97.17±1.8%, respectively. Release of PTX was observed to be decreasing with increase in PTX content. Zero order and Korsmeyer-Peppas model gave a good fit for the drug release profiles of all NPs formulations with greater regression coefficients in comparison to other models. For F1 and F2, regression

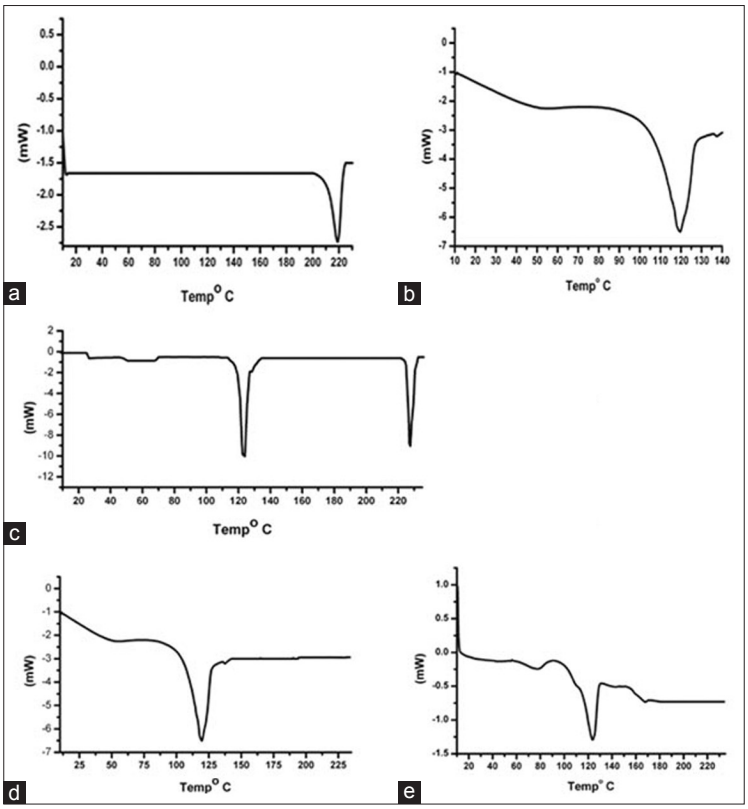

Figure 3: DSC thermograms of pure PTX (a), pure poly(SA-RA) 70:30 (b), physical mixture of drug and polymer (c), F1 (d) and F2 (e)

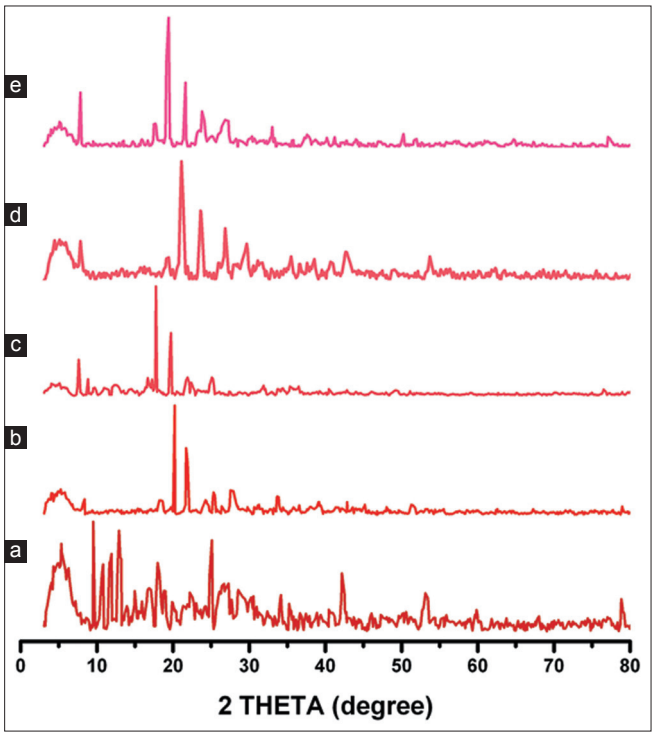

Figure 4: XRD patterns of pure PTX (a), poly(SA-RA) 70:30 (b), physical mixture (c), F1 (d) and F2 (e).The experiment was carried with crimped aluminum pans and a heating rate of 10°C/min; the samples were scanned at 10°C/min from 10 to 230°C

values in case of zero order 0.967 and 0.959, respectively, and in Korsmeyer-Peppas models were 0.970 and 0.965. Since

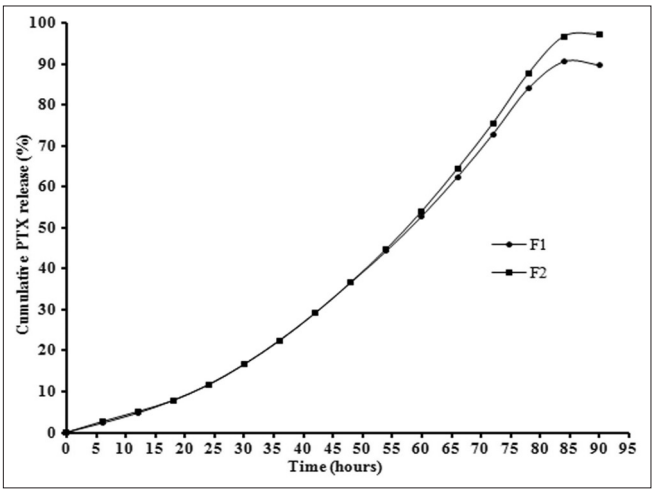

Figure 5: *In vitro* release profile of PTX- Poly(SA-RA) NPs. Dissolution studies were performed in PBS pH 7.4: F1 (●) and F2 (■)

the value of n was in between 1.300-1.314, it indicates super case II transport mechanism.[15]

Drug stability in the PTX- Poly (SA-RA) NPs formulations was assessed by comparing the stored formulations with the control of its drug loading [Table 3]. There was no change in the PTX content in the formulations stored at 25°±2°C/60%±5% RH at the end of 90 days. However, in the samples kept at 40°±2°C/75%±5% RH, significant reduction in amount of PTX was determined at the end of 90 days in each formulation. This might be due to the degradation of both drug and lipid

Table 3: Stability studies data of PTX nanoparticles

Time (days)	Formulations	Drug loading (% w/w)		
		Control 5±3°C	25±2°C / 60±5% RH	40±2°C / 75±5% RH
Initial	F1	4.77	4.77	4.77
	F2	9.18	9.18	9.18
15 days	F1	4.76	4.69	4.50
	F2	9.15	9.10	9.02
30 days	F1	4.71	4.59	4.09
	F2	9.10	8.97	8.84
60 days	F1	4.62	4.31	3.77
	F2	8.97	8.75	8.49
90 days	F1	4.51	3.78	3.15
	F2	8.89	8.59	8.01

polymer having a low glass transition temperature and low melting point. It also suggested that method of preparation and polymer used did not affect the drug chemical stability.

CONCLUSION

PTX- Poly (SA-RA) NPs were prepared and characterized. However, the continuous release of a PTX from a controlled delivery device for prolong period is usually required for effective treatment of cancer. The obtained results indicated that the potential use of NPs using poly (SA-RA) 70:30 for sustained release of hydrophobic drug such as PTX. *In vitro* toxicity and *in vivo* studies may be future subjects.

ACKNOWLEDGEMENTS

The authors also thank Naprod Life Science Pvt. Ltd., Mumbai, India for providing the gift sample of PTX and IISc, Bangalore, India, for providing SEM, particle size analysis facilities.

REFERENCES

1. Jemal R, Siegel E, Ward YP, Hao JQ, Xu T, Murray MJ. Cancer statistics. CA Cancer J Clin 2008;58:71-96.
2. Farokhzad OC, Langer R. Impact of nanotechnology on drug delivery. Nano Lett 2009:3;16-20.
3. Rai S, Paliwal R, Gupta PN, Khatri K, Goyal AK, Vaidya B, *et al*. Solid lipid nanoparticles (SLNs) as a rising tool in drug delivery science: one step up in nanotechnology. Curr Nanosci 2008;4:30-44.
4. Tong R, Cheng JJ. Anticancer polymeric nanomedicines. Polymer Rev 2007;47:3450-81.
5. Zhang LF, Gu FX, Chan JM, Wang AZ, Langer R, Farokhzad OC. Nanoparticles in medicine: Therapeutic applications and developments. Clin Pharmacol Ther 2008;83:761-9.
6. Kwon G, Suwa S, Yokoyama M, Okano T, Sakurai Y, Kataoka K. Enhanced tumor accumulation and prolonged circulation times of micelle-forming poly (ethylene oxide-aspartate) block copolymer-adriamycin conjugates. J Control Release 1994;29:17-23.
7. Cabral H, Nishlyama N, Kataoka K. Optimization of (1, 2-diamino-cyclohexane) platinum (II)-loaded polymeric micelles directed to improved tumor targeting and enhanced antitumor activity. J Control Release 2007;121:146-55.
8. Owens DE, Peppas NA. Opsonization, biodistribution and pharmacokinetics of polymeric nanoparticles. Int J Pharm 2006;307:93-102.
9. Li SD, Huang L. Pharmacokinetics and biodistribution of nanoparticles. Mol Pharm 2008;5:496-504.
10. Singla AK, Garg A, Aggarwal D. Paclitaxel and its formulations. Int J Pharm 2002;235:179-92.
11. Brigger C, Dubernet P, Couvreur. Nanoparticles in cancer therapy and diagnosis. Adv Drug Deliv Rev 2002;54:631-51.
12. Katti DS, Lakshmi S, Langer R, Laurencin CT. Toxicity, biodegradation and elimination of polyanhydrides. Adv Drug Deliv Rev 2002;54:933.
13. Krasko MY, Shikanov A, Ezra A, Domb AJ. Poly (ester anhydride) prepared by the insertion of ricinoleic acid into poly (sebacic acid). J Polym Sci Part A Polym Chem 2003;41:1059-69.
14. Agueros M, Ruiz-Gaton L, Vauthier C, Bouchemal K, Espuelas S, Ponchel G, *et al*. Combined hydroxypropyl-e-cyclodextrin and poly (anhydride) nanoparticles improve the oral permeability of paclitaxel. Eur J Pharm Sci 2009;38:405-13.
15. Costa P, Lobo JM. Modeling and comparison of dissolution profiles. Eur J Pharm Sci 2001;13:123-33.
16. Sahana K, Santra S, Mukherjee B. Development of biodegradable polymer based tamoxifen citrate loaded nanoparticles and effect of some manufacturing process parameters on them: A physicochemical and *in vitro* evaluation. Int J Nanomedicine 2010;5:621-30.
17. Shikanov AE, Domb AJ. Poly (sebacic acid-co-ricinoleic acid) biodegradable carrier for paclitaxel-effect of additives. J Control Release 2005;10:552-67.

How to cite this article: Totiger SB, Hiremath JG. Paclitaxel loaded poly(sebacic acid-co-ricinoleic ester anhydride)-based nanoparticles. Asian J Pharm 2011;5:225-30.

Source of Support: Nil. **Conflict of Interest:** None declared.