Research Article

Assessment of release kinetics of Docetaxel loaded PLGA nanoparticles

Nishi Mody, Rajeev Sharma, Suresh P. Vyas*

Drug Delivery Research Laboratory, Department of Pharmaceutical Sciences, Dr. Harisingh Gour Vishwavidyalaya, Sagar (M.P.) – 470003, India

Received: 11 February 2019 Revised: 28 March 2019 Accepted: 5 April 2019

Abstract

Objective: The objective of the present study was to formulate and investigate in vitro release kinetics profile of docetaxel loaded poly (lactic-co-glycolic acid) nanoparticulate drug delivery system in order to improvise the solubility of the drug as well as to attain a controlled release drug pattern in pursuit to reduce the toxicity and dosing frequency problems associated with the present marketed formulations. **Materials and methods:** Preformulation study of docetaxel such as physical properties (melting point, partition coefficient, and solubility), identification (UV and FTIR spectroscopy) and linearly regressed calibration curves for quantification were carried out prior to formulation development. Taking advantage of biodegradable and biocompatible, and FDA approved polymer "poly (lactic-co-glycolic acid)", docetaxel loaded nanoparticles were developed. by emulsification - solvent evaporation technique. The morphology of the docetaxel loaded nanoprticles was observed via scanning electron microscopy (SEM). Synthesized docetaxel loaded nanoprticles were also characterized with regard to their particle diameters, zeta potential, drug loading capacities, and drug release kinetics. **Results and conclusion:** A nanoparticle formulation of PLGA encapsulating docetaxel was prepared via emulsification - solvent evaporation technique under optimized conditions. The developed formulation showed a favorable particle size below 200 nm for higher retention at tumor site owing to enhanced permeability and retention (EPR) effect. Entrapment efficiency was recorded to be 64.34±1.53%. In vitro studies showed that the docetaxel loaded nanoparticles exhibited a sustained drug release which suggested that nanoparticles are likely to reduce dose and dosing frequency. We also studied release behavior of docetaxel in terms of mechanism and pattern so that these aspects may be of great utility to formulation developers for cancer targeting and NPs development of similar drug. Developed NPs still warrant further investigation for assuring ex-vivo and in-vivo potential.

***Keywords*:** PLGA; Docetaxel, nanoparticles, solid tumors, release kinetics

Introduction

Recent developments in nanotechnology offer opportunities to transform cancer therapeutics considerably. This technology has enabled the manipulation of the biological and physicochemical properties of nanomaterials to facilitate more efficient drug targeting and delivery. In recent years, researchers are continuously exploring the field of nanotechnology to develop suitable drug carrier system for effective delivery of loaded bioactive to cancer cells. It includes the use of nanocarriers to alter and improve the systemic circulation time, pattern of drug release as well as the site of delivery of the drugs i.e. pharmacokinetics and pharmacodynamics of the bioactive. A number of polymers are used to formulate nanoparticles to achieve the goal of altering drug pharmacokinetics and pharmacodynamics for minimizing the side effects and enhance the therapeutic benefits (Mohanraj and Chen, 2006; Sinha et al., 2006). Taxanes are one of the most commonly used classes of drugs in chemotherapy including paclitaxel and docetaxel as representative agents. Docetaxel shows high anticancer activity particularly in solid tumors including breast, cervical and ovarian cancers in combination with other chemotherapeutic agents. It possess poor aqueous solubility and is available in the market as taxotere® which includes the

Address for Corresponding Author:
Prof. Suresh P. Vyas
Drug Delivery Research Laboratory
Department of Pharmaceutical Sciences
Dr. Harisingh Gour Vishwavidyalaya, Sagar-470003 M.P. (India)
E-mail: spvyas54@gmail.com

DOI: https://doi.org/10.31024/ajpp.2019.5.5.24

use of polysorbate 80 as surfactant. The surfactant is reported to have several hypersensitivity reactions and shows incompatibility with common polyvinyl chloride intravenous administration sets (Gelderblom et al., 2001). Also the dosing frequency is high. In order to eliminate the cons associated with polysorbate 80-based vehicle and to decrease the dosing frequency by providing drug for a longer time in circulation a well as at cancer cell, docetaxel loaded nanoparticles have been designed using biodegradabe and biocompatible polymer poly (lactic acid-co-glycolic acid) (PLGA). Also the release kinetics of the drug from the nanoparticulate system is analyzed to identify the release pattern (Immordino et al., 2003; Alexopoulos et al., 2004; Mu and Feng, 2003).

Materials and methods

Materials

Docetaxel was obtained as a gift sample from SPARC Vadodara, Gujrat, India and PLGA (50:50) was purchased from Sigma, India. All other chemical reagents were of analytical grade and were used as received.

Preformulation studies of Docetaxel

Preformulation studies may be described as the process of identifying and screening of those physical and chemical properties of drug and excipients which are considered important in formulation of a stable, effective and safe dosage form. Thus, preformulation studies are performed in order to establish optimum conditions for developing desired drug delivery system.

Physical properties

The physical examination of the drug was performed in day-light and drug was observed for its appearance (Huynh et al., 2009; Huynh-Ba, 2008).

Melting point

Melting point was determined in a melting point apparatus (Superfit, Mumbai, India). The drug filled capillary was placed in the apparatus and the temperature was raised gradually and temperature was recorded.

Partition coefficient

The partition coefficient is defined as the ratio of unionized drug distributed between the organic and aqueous phase at equilibrium. It can be calculated using the formula: $P_{o/w} = [C_{org}/C_{aq}]$ equilibrium. Accurately weighed ten mg of docetaxel was transferred in a 25 mL volumetric flask containing 10 mL each of two immiscible phases, n-octanol and aqueous phase (PBS pH 7.4 or distilled water). The vials were placed on a wrist action shaker (Yorco, New Delhi, India) for 24 h. Phases were separated in a separating funnel and aqueous phase (PBS 7.4 or distilled water) was analyzed spectrophotometrically at λ_{max} 230 nm (Cintra 10 GBC UV Visible spectrophotometer, Japan) for the amount of drug after making aliquot of suitable dilution. The concentration in the n-octanol phase was determined by difference and partition coefficient was calculated using the above formula (Table 1) (Dhanikula et al., 2007).

Solubility studies

Solubility is defined in quantitative terms as the concentration of solute in a saturated solution at a certain temperature and in qualitative terms it may be defined as the spontaneous interactions of two or more substances to form a *v/v* homogeneous molecular dispersion (Martin et al., 2011; Singh, 2006). Solubility of docetaxel was determined in water, phosphate buffer saline (PBS), methanol, ethanol, acetone, chloroform and dichloromethane (Table 2).

UV spectroscopy

The UV scan was performed in the photometric mode in a Cintra 10 GBC UV Visible spectrophotometer, Japan in ethanol. Accurately weighed 10 mg of drug was dissolved in minimum quantity of ethanol and volume was made up to 10 mL using PBS (pH 7.4): ethanol (7:3) and scanned after appropriate dilutions between 200 to 400 nm (Figure 1).

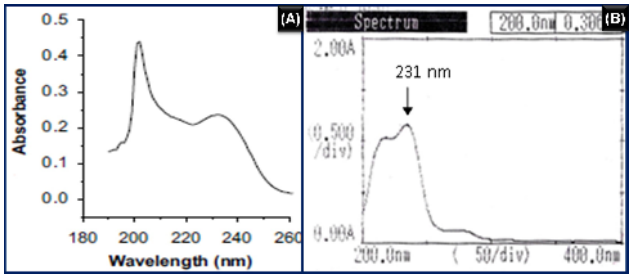

Figure 1. UV- Scans of Docetaxel: (A) Reference and (B) Sample in PBS (pH 7.4): ethanol (7:3)

Table 1. Partition Coefficient of Docetaxel

Medium	Concentration		Partition Coefficient (O/W)	Reference Value
	Aqueous Phase	Organic Phase		
PBS (pH 7.4)	1.4274 ± 0.05	3.5726 ± 0.09	2.50 ± 0.04	2.4-2.5 (HMDB; Drug Bank)
Distilled Water	1.3840 ± 0.04	3.6160 ± 0.08	2.61± 0.03	Not Available

Table 2. Solubility of Docetaxel in various solvents

Solvent	Solubility
Distilled Water	----
PBS (pH 7.4)	----
Methanol	++++
Ethanol	++++
Dichloromethane	+++
Chloroform	+++
Acetone	++

---- Insoluble, ++++ freely soluble +++ very soluble ++ soluble

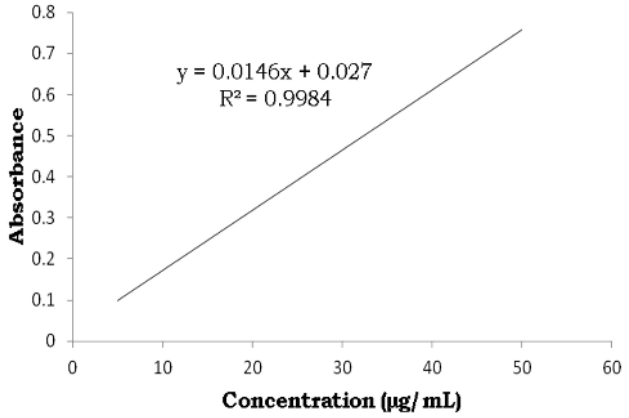

Figure 3. Standard Curve of Docetaxel in PBS (pH 7.4): ethanol (7:3)

FTIR spectroscopy

FTIR spectrum of docetaxel was obtained using FTIR spectrophotometer (Perkin Elmer-3600, U.S.A.) by KBr pellet method and the obtained peaks in spectrum were interpreted and compared with standard characteristic peaks. FTIR peaks of the docetaxel matched with the reference (Cheng et al., 2009). The results of interpretation are shown in figure 2.

Quantitative estimation

A spectrophotometric method based on UV-visible absorption provided convenient, precise and accurate mode to estimate the drug concentration in the microgram range. Estimations were carried out in PBS of pH 7.4.

Accurately weighed 10 mg of docetaxel was transferred into a clean and dry 100 mL volumetric flask, and the volume was made up to 100 mL with PBS (pH 7.4): ethanol (7:3) (Qiu et al., 2008). Proper aliquots were prepared so as to obtain the range of 5-50 µg/mL. The absorbance of each concentration was determined spectrophotometrically at λ_{max} 230 nm (Cintra 10 GBC UV Visible spectrophotometer, Japan) against PBS (pH 7.4): ethanol (7:3) as blank. The standard curve procedure was repeated three times and observations are recorded (Figure 3).

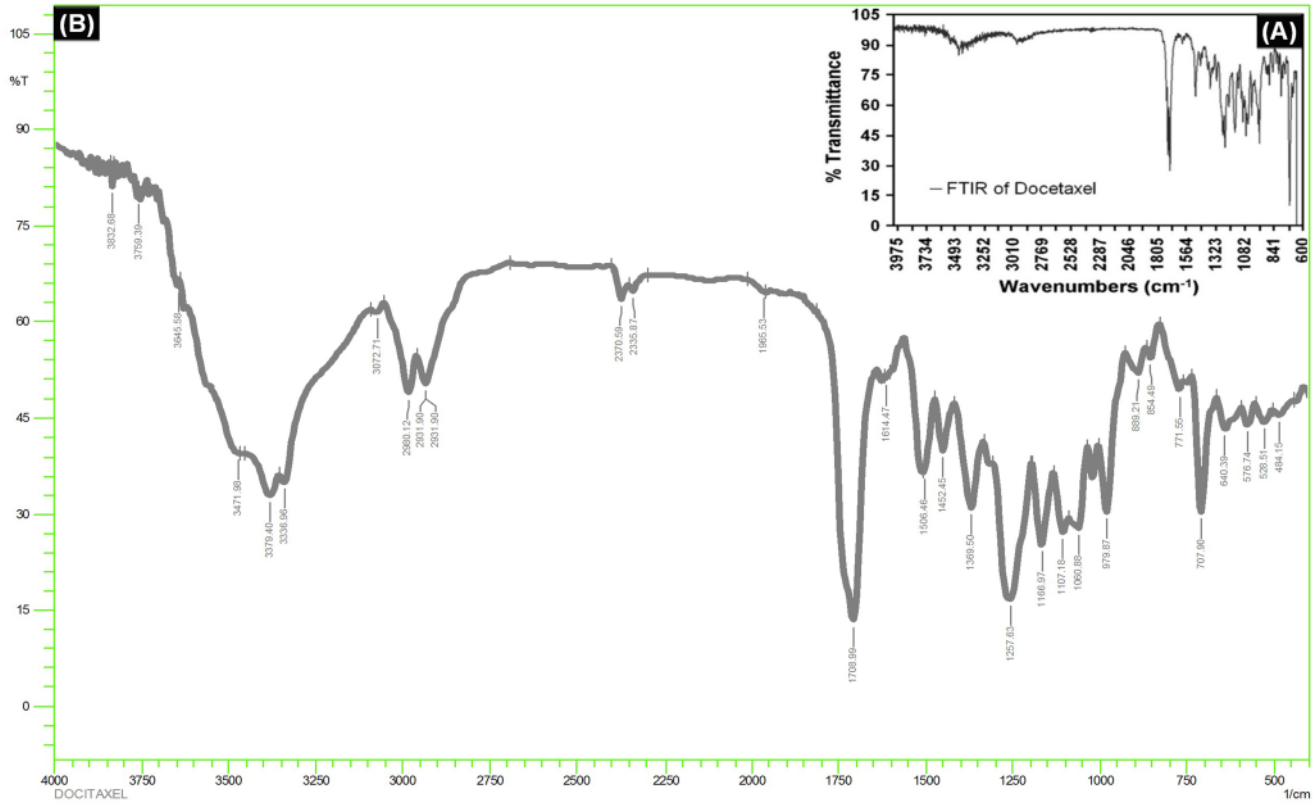

Figure 2. FTIR Spectra of Docetaxel (A) Reference, (B) Sample

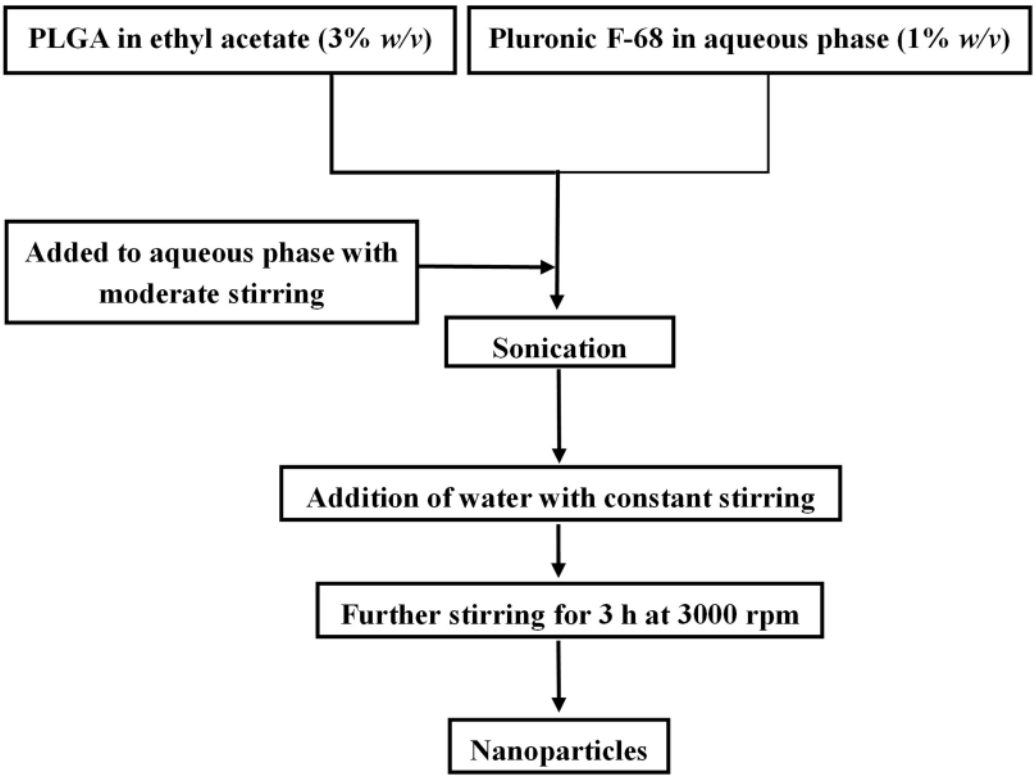

Figure 4. Schematic representation of preparation of PLGA nanoparticles

Preparation of nanoparticles

PLGA nanoparticles (PLGA NPs) were prepared by emulsification - solvent evaporation technique as previously reported by Mody et al. (2014) with slight modifications (Mody et al., 2014). An organic phase was prepared by dissolved polymer (PLGA) in ethyl acetate (3% *w/v*). This phase was added to an aqueous phase (Pluronic F-68, 1% *w/v*) to form an emulsion. The obtained emulsion was undergone sonication (Soniweld, Mumbai, India) to break down emulsion into nanodroplets that resulted in formation of nanoparticles upon solvent evaporation by addition of deionized water with constant stirring on a magnetic stirrer (Remi, Mumbai, India). Further stirring (3000 rpm) at room temperature for 3 h was carried out to obtain stable dispersion of NPs (Figure 4).

Characterization of prepared PLGA nanoparticles

Particle Size and Zeta potential Analysis

Lyophilized Docetaxel nanoparticles were suspended in phosphate buffer saline to make a 1% solution. Then particle size, polydispersity and zeta potential were measured by Malvern Zetasizer (Malvern, USA) (Das et al., 2005).

Particle shape and surface morphology

Scanning electron microscopy (SEM) analysis of the prepared Docetaxel nanoparticles was carried out to study the morphological properties. The dried samples were mounted on brass specimen studies, using double sided adhesive tape. Gold-palladium alloy of 120˚A knees was coated on the sample using sputter coating unit (JEOL-JSM- 6490LV SEM analyzer, USA) in Argon at ambient of 8-10 Pascal with plasma voltage about 20 MA. The sputtering was done for nearly 5 min.

Entrapment efficiency

Docetaxel nanoparticles were separated from the aqueous suspension medium by ultra-centrifugation at 8000 rpm for 30 min. The amount of free docetaxel was determined by UV quantification method as reported earlier. The percentage entrapment for the formulation was calculated by the following equation (Bhandari and Kaur, 2013):

Cumulative percent drug release

In vitro release of Docetaxel from the PLGA nanoparticles was evaluated using dialysis bag diffusion method. First, 10 mg of lyophilized Docetaxel-loaded nanoparticles was suspended in PBS (pH 7.4). The solution was then placed into a dialysis bag and immersed into PBS, at 37°C with gentle agitation. The incubation medium was sampled at various time points to monitor the docetaxel release rate. After sampling, equal volume of fresh PBS was immediately added back to keep the constant volume of the incubation medium. Drug released was estimated using UV method as reported in earlier section. The concentration of

docetaxel released from the nanoparticles was expressed as a percentage of the total Docetaxel in the nanoparticles and plotted as a function of time.

Kinetic treatment of dissolution data

In order to describe the kinetics of the release process of drug from the different formulations, models were fitted to the dissolution data of formulations using non-linear regression analysis. *In vitro* release data was fitted to various release models i.e.: Peppas, Hixon and Crowell, Higuchi Square Root Time and First Order. The determination coefficient (R^2) and Sum of Squares of Residuals (SSR) were used as the indicators of the best fit to release data for each model.

Statistical analysis

In vitro drug release kinetics model fitting was carried out using Sigma Plot for Windows Version 11.0 (wpcubed GmbH, Germany). A difference with $p \leq 0.05$ (i.e. 5% level of significance) was considered to be statistically significant.

Results and discussion

Docetaxel was obtained as white crystalline, odorless powder. The physical appearance, color and crystalline nature of the drug were found to be similar to that reported in official literature. The melting point of docetaxel was found to be 188±1°C that was in the range mentioned in Drug Bank. The log P values observed were found to be 2.5 and 2.61 for docetaxel in PBS (pH 7.4), and distilled water, respectively suggesting hydrophobic nature of the drug. On UV scan a sharp peak of drug was obtained at 230 nm in PBS (pH 7.4): ethanol (7:3). The data matched with the standard λ_{max} as reported in I.P. 2010. The peaks in IR spectrum of drug were in accordance with that of reported literature (Cheng et al., 2009). The standard curves of the drug were prepared using UV absorption method at λ_{max} 230 nm in PBS (pH 7.4): ethanol (7:3) using the stock prepared in ethanol. A straight line was obtained in all the cases in the range of 5 to 50 µg/mL with R^2 values greater than 0.99. This confirms that Beer Lambert's law was followed in the used range in UV spectroscopy. It can be concluded that the gift sample of docetaxel was authentic and results of this study were in conformity with the standards given in official monographs. Particle size, size distribution and surface charge of nanoparticles were determined using Zetasizer (Malvern ZS 90, UK). The average size of the PLGA nanoparticles (PLGA-NPs) was found to be 147±2.7 nm. Zeta potential is one of the most important indices to evaluate nanoparticulate suspension stability. The value of the zeta potential was found to be -8.7±0.54 mV for PLGA-NPs due to the presence of terminal carboxylic groups in the polymer. SEM image of nanoparticles showed that particles were spherical in shape and do not show considerable variation in shape as evident from figure 5. The results are in accordance with previous report (Moosavian et al., 2016; Pillai et al., 2015; Yamashita et al., 2018).

Percent drug entrapment was calculated using Sephadex G-50 column.Entrapment efficiency of the nanoparticles was found to be 64.34±1.53%. *In vitro* release behavior in media of Phosphate buffer of pH 7.4 was also determined. A biphasic drug release pattern was observed with Drug released from PLGA nanoparticles as compared to plain drug solution (Figure 6) which confirms the time dependent slow and sustained release behavior of drug from nanoparticles. In physicochemical characterization, PLGA NPs were of nanometric size (147±2.12 nm) with narrow PDI (0.178±0.021). Zeta potential and %EE of these NPs were -8.7±0.54 mV and 64.34 ± 1.53%, respectively.

Mathematical models help predict the drug release rates and diffusion behavior from these systems by the selection of the best fit model. It is reported for spherical particles $n \leq 0.5$– Fickian diffusion, n = 0.5-0.85 – Anomalous (non-Fickian) diffusion, n = 0.85 – Case II transport, and n > 0.85 – Super-

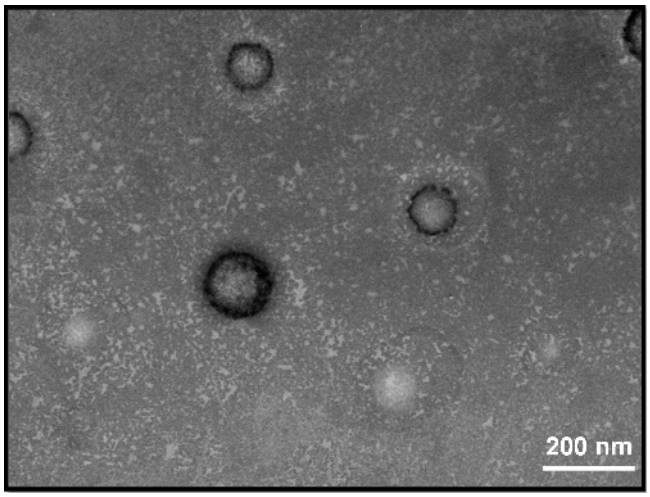

Figure 5. Scanning electron microscope image of docetaxel loaded poly(lactic-co-glycolic acid) nanoparticles

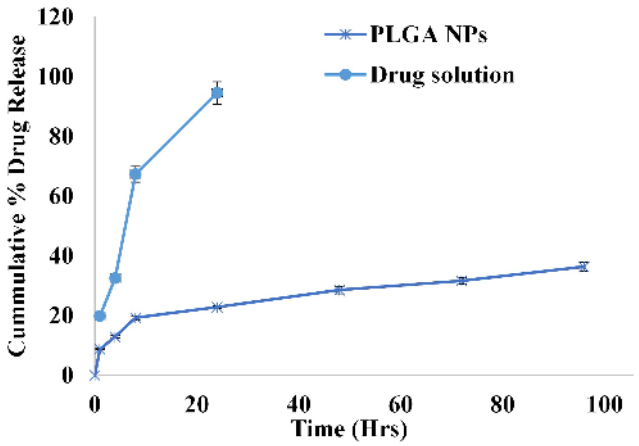

Figure 6. Cumulative percent drug release in PBS (pH 7.4)

Table 3. Release model curve fitting for various PLGA Nanoparticles

Controlled release kinetics model	Equation	Parameter Estimates (p < 0.0001)		R^2	SSR	Statistical Tests (α = 0.0500)				
		Parameter	Coefficient			Normality Test (Shapiro - Wilk)		W Statistic	Constant Variance Test	
Peppas	F=k*t^n	k	4.8389	0.9913	9.8744	Passed	P = 0.7530	0.9516	Passed	P = 0.0600
		n	0.4987							
Hixon and Crowell	F=100*(1 -(1-k*t)^3)	k	0.0023	0.7934	235.4039	Passed	P = 0.8165	0.9596	Passed	P = 0.0600
Higuchi, Square Root Time	F=k*sqrt(t)	k	4.8135	0.9913	9.8787	Passed	P = 0.7171	0.9471	Passed	P = 0.0600
First Order	F=100*(1 -exp(-k*t))	k	0.0077	0.8304	193.1739	Passed	P = 0.9206	0.9744	Passed	P = 0.0600

*Abbreviations: SSR, Sum of Squares of Residuals; α, level of significance; all other symbols are test statics.

case II transport (Jain and Jain, 2016b). In all cases, n was found to be around 0.5 which indicated Fickian diffusion and biphasic release pattern (Table 3). The kinetic treatment of *in vitro* release data revealed the biphasic drug release pattern. The biphasic release comprises of two phases i.e. immediate release phase and sustained release phase (Jain and Jain, 2016a). The immediate release phase is indicated by the initial burst effect showed during drug release at 8thhr. The initial burst effect could be due to dug near or on the surface of the nanoparticles. The drug continued to release in sustained manner till 24thhr indicating the sustained release phase of biphasic release pattern (fickian diffusion) which is characteristic of Higuchi release model.

Conclusion

Docetaxel is hydrophobic in nature and it is insoluble in water. It has partition coefficient (n-Octanol/water: 2.5) which indicates challenge for formulation development and unfavorable pharmacokinetic performance. Developed PLGA NPs were nanometric in size (below 200 nm) and were negatively charged with a zeta potential about -8.7±0.54 mV. NPs showed good entrapment efficiency of 64.34±1.53%. There was sustained release of drug at physiological pH i.e. percentage cumulative drug release around 40% at the end of 24 hours. More importantly, the release kinetics suggested fickian release (release exponent, n < 0.5) following Higuchi's model that is best suited model for sustained drug delivery to achieve desired therapeutic outcomes. Preliminary results open new passages for bring advance development in PLGA nanoparticles as considerable formulation for sustained delivery of docetaxel and another drug having similar physiochemical properties.

Acknowledgement

Nishi Mody is grateful to DST for providing financial assistance as DST-INSPIRE fellowship.

Conflicts of interest

Authors report no conflict of interest.

References

Alexopoulos A, Karamouzis M, Stavrinides H, Ardavanis A, Kandilis K, Stavrakakis J, Georganta C, Rigatos G. 2004. Phase ii study of pegylated liposomal doxorubicin (caelyx®) and docetaxel as first-line treatment in metastatic breast cancer. Annals of Oncology, 15 (6): 891-895.

Bhandari R, Kaur IP. 2013. Pharmacokinetics, tissue distribution and relative bioavailability of isoniazid-solid lipid nanoparticles. International Journal of Pharmaceutics, 441 (1-2): 202-212.

Cheng H, Liu H, Zhang Y, Zou G. 2009. Interaction of the docetaxel with human serum albumin using optical spectroscopy methods. Journal of Luminescence, 129 (10): 1196-1203.

Das S, Banerjee R, Bellare J. 2005. Aspirin loaded albumin nanoparticles by coacervation: Implications in drug delivery. Trends Biomater Artif Organs, 18 (2): 203-12.

Dhanikula AB, Khalid NM, Lee SD, Yeung R, Risovic V, Wasan KM , Leroux J-C. 2007. Long circulating lipid nanocapsules for drug detoxification. Biomaterials, 28 (6): 1248-1257.

Drug Bank. Docetaxel [Online]. Available: https://www.drugbank.ca/drugs/DB01248 [Accessed 18 April 2019].

Gelderblom H, Verweij J, Nooter K , Sparreboom A. 2001. Cremophor el: The drawbacks and advantages of vehicle selection for drug formulation. European Journal of Cancer, 37 (13): 1590-1598.

Hmdb. Docetaxel (hmdb0015378) [Online]. Available: http://www.hmdb.ca/metabolites/HMDB0015378 [Accessed 18 April 2019].

Huynh-Ba K. 2008. Handbook of stability testing in pharmaceutical development: Regulations, methodologies, and best practices, Springer Science & Business Media.

Huynh NT, Passirani C, Saulnier P, Benoit JP. 2009. Lipid nanocapsules: A new platform for nanomedicine. International Journal of Pharmaceutics, 379 (2): 201-9.

Immordino ML, Brusa P, Arpicco S, Stella B, Dosio F, Cattel L. 2003. Preparation, characterization, cytotoxicity and pharmacokinetics of liposomes containing docetaxel. Journal of Controlled Release, 91 (3): 417-429.

Jain A, Jain SK. 2016a. In vitro release kinetics model fitting of liposomes: An insight. Chemistry and Physics of Lipids, 201: 28-40.

Jain A, Jain SK. 2016b. Multipronged, strategic delivery of paclitaxel-topotecan using engineered liposomes to ovarian cancer. Drug Development and Industrial Pharmacy, 42 (1): 136-149.

Martin AN, Sinko PJ, Singh Y. 2011. Martin's physical pharmacy and pharmaceutical sciences: Physical chemical and biopharmaceutical principles in the pharmaceutical sciences, Lippincott Williams & Wilkins.

Mody N, Tekade RK, Mehra NK, Chopdey P, Jain NK. 2014. Dendrimer, liposomes, carbon nanotubes and plga nanoparticles: One platform assessment of drug delivery potential. AAPS PharmSciTech, 15 (2): 388-399.

Mohanraj V, Chen Y. 2006. Nanoparticles-a review. Tropical Journal of Pharmaceutical Research, 5 (1): 561-573.

Moosavian SA, Abnous K, Badiee A, Jaafari MR. 2016. Improvement in the drug delivery and anti-tumor efficacy of pegylated liposomal doxorubicin by targeting rna aptamers in mice bearing breast tumor model. Colloids and Surfaces B: Biointerfaces, 139: 228-236.

Mu L, Feng S. 2003. A novel controlled release formulation for the anticancer drug paclitaxel (taxol®): Plga nanoparticles containing vitamin e tpgs. Journal of Controlled Release, 86 (1): 33-48.

Pillai JJ, Thulasidasan AKT, Anto RJ, Devika NC, Ashwanikumar N, Kumar GV. 2015. Curcumin entrapped folic acid conjugated plga–peg nanoparticles exhibit enhanced anticancer activity by site specific delivery. RSC Advances, 5 (32): 25518-25524.

Qiu Y, Gao Y, Hu K, Li F. 2008. Enhancement of skin permeation of docetaxel: A novel approach combining microneedle and elastic liposomes. Journal of Controlled Release, 129 (2): 144-150.

Singh Y. 2006. Martin's physical pharmacy and pharmaceutical sciences. New Jersey: Department of Pharmaceutics Ernest Mario School of Pharmacy Rutgers, The State University of New Jersey.

Sinha R, Kim GJ, Nie S, Shin DM. 2006. Nanotechnology in cancer therapeutics: Bioconjugated nanoparticles for drug delivery. Molecular Cancer Therapeutics, 5 (8): 1909-1917.

Yamashita S, Katsumi H, Hibino N, Isobe Y, Yagi Y, Tanaka Y, Yamada S, Naito C, Yamamoto A. 2018. Development of pegylated aspartic acid-modified liposome as a bone-targeting carrier for the delivery of paclitaxel and treatment of bone metastasis. Biomaterials, 154: 74-85.