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BIODEGRADABLE PEGYLATED POLY[D, L-LACTIDE-CO-GLYCOLIDE] NANOPARTICULATE CARRIER FOR DOCETAXEL : *IN VITRO* DEGRADATION MECHANISMS AND DRUG RELEASE KINETICS

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Abstract
This study investigates *in vitro* degradation mechanisms of Docetaxel [an antineoplastic agent] [DTX] loaded PEGylated poly[d,l-lactide-co-glycolide] [PLGA] nanoparticles formulations. The degradation patterns of biodegradable nanoparticles [NPs] formulations were investigated in phosphate buffer saline at pH 5.0 and 7.4 media up to 6 weeks. It was characterized by changes in particle size and media pH, generated monomers [lactic and glycolic acid], liberated poly ethylene oxide moieties, changes in molecular weight, and drug release. Also, the influence of PEG moiety and its chain length of NPs were evaluated during the degradation. It was found that the rate of NPs degradation and drug release were higher in acidic medium, and suggests that the NP formulations could provide greater therapeutic efficaciousness at tumor sites.

Keywords: - PLGA-mPEG; Nanoparticles; Docetaxel; Degradation kinetics

Introduction
Biodegradable and biocompatible poly[d, l-lactic acid] [PLA], poly[glycolic acid] [PGA] and their copolymer poly[d, l-lactic-co-glycolic acid] [PLGA] have been extensively used in controlled drug delivery and are approved by the US Food Drug Administration [1]. The introduction of hydrophilic poly[ethylene glycol] [PEG] segment into PLGA polymer provides a longer circulation and sustained delivery at the sites [2]. This amphiphilic [PEG-PLGA] micro/nano carrier have a number of desirable characteristics, including persistence in the blood stream and good encapsulation properties [2-4]. A wide variety of drug formulations, such as micro/nano-particles [5], micelles [6], hydrogels [7], and injectable drug delivery systems [8] have been developed using PLGA-PEG block copolymers.

The degradation of biodegradable PLGA occurs in two stages, after penetration of water into the device, a random cleavage of the ester bonds in the bulk degradation phase. When the degradation products, such as PLGA oligomers, lactic [LA] and glycolic acid [GA] become water-soluble, they are released from the device in the second phase, characterized by loss or erosion of the polymer. The breakdown products are finally eliminated from the body through the citrate cycle [9]. The degradation of the core material PLGA depends on several factors including the type of chemical bonds, mobility of water within the polymer, polymer crystallinity, and pH of the polymer solution [10]. The degradation media's pH is an important factor, which may affect polymer degradation rate. Most researchers have studied the degradation patterns of PLGA-PEG under physiological conditions [11-13]. Hence, the present work investigates degradation mechanisms of PLGA-PEG NP formulations in acidic environments, since the carrier preferentially degrade and release the drug at acidic tumor sites. It has been reported that highly selective delivery to specific tumor sites was achieved using polymeric micelles as carrier through a passive targeting mechanism [14]. In our previous studies, we described about the diblock copolymer synthesis, NPs

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preparation and drug release pattern [15] and recently the NP's long circulating characteristics and accumulation in tumor sites were effectively investigated by *in vivo* [16].

In this study, the degradation behavior of the PEGylated NP formulations was investigated in both acidic [pH 5.0] and physiological [pH 7.4] media up to 6 weeks. The degradation mechanisms, formation of monomers and the drug release were studied. Also, the influence of PEG moiety and its chain length of NPs were evaluated during the degradation.

Materials And Methods

Materials

d, l-Lactide and Glycolide were procured from Polysciences, Inc, Warrington, PA; Methoxy poly[ethylene glycol] [mPEG, molecular weight [MW] 5KDa & 2KDa, Purity >95%, dried under vacuum at room temperature before use], Stannous octoate, 4-[2-hydroxy ethyl] piperazine-1-ethane sulfonic acid [HEPES] buffer, Lactic acid, Glycolic acid and Cellulose membrane [MW cut-off 12,400] were purchased from Sigma, USA; Sodium cholate [99%] was procured from Otto, India; DTX was kindly supplied by Cipla, India; Acetonitrile [ACN], dichloromethane [DCM] and tetrahydrofuran [THF] of high performance liquid chromatography [HPLC] grade [Rankem, Mumbai] were used. All other reagents and solvents were of analytical grade.

Preparation and Characterization of PEGylated PLGA Nanoparticles formulations

Firstly, PLGA-mPEG diblock copolymers were synthesized by ring opening polymerization reaction as reported by us [15]. Briefly, monomers of d,l-lactide and glycolide were dissolved with or without mPEG in freshly distilled toluene in the presence of stannous octoate [0.03% w/w] as a catalyst. The reaction was carried out at 110°C for 6 h under continuous stirring at 250 rpm under nitrogen atmosphere. The solvent was evaporated, the residue was dissolved in DCM, filtered and a solid product was recovered against hot water. The product was purified with excess of methanol and dried under vacuum at 40°C for 48 h. $PLGA_{[75:25]}$ [P-1], $PLGA_{[75:25]}$-$mPEG_{[2KDa]}$ [CP-1] and $PLGA_{[75:25]}$-$mPEG_{[5KDa]}$ [CP-2] copolymers were synthesized. The structure and percentage of the actual molar compositions were determined by ^1H NMR [Avance-300 Bruker, Switzerland] and FT-IR using KBr disc [Thermo Nicolet Nexus 670, USA]. Molecular weight and polydispersity index of the copolymer was determined by gel permeation chromatography [GPC] using Perkin Elmer Series-200 instrument equipped with the Refractive Index detector. For molecular weight determination, tetrahydrofuran [THF] was the mobile phase at a flow rate of 1ml/min and a temperature of 30°C. PLGA-PEG diblock copolymer was dissolved in THF, filtered, and then injected into column of PL Gel 5 micron, 300 × 7.5 mm. Average molecular weights was calculated using polystyrene standard.

Lastly, The NPs were prepared by the emulsion solvent evaporation [o/w emulsification] technique [15]. Briefly, the copolymer was dissolved with or without drug in DCM, vortexed and emulsified with aqueous phase containing 0.1% sodium cholate in a sonicator [Sonics-Vibra Cell, BC-130, Ultra sonic processor, CT, USA] at 20 W output for 1 min. The organic solvent was evaporated and the NPs were then recovered by centrifugation [35,000g, 20 min, 4°C; Remi, India], washed twice with water and lyophilized. The dried NPs were stored in a refrigerator at 4°C. Each sample was prepared in triplicate. P-1, CP-1 and CP-2 copolymers were formulated and the NP formulations named asCND [non-PEGylated], CND-2 [PEGylated; mPEG MW 2KDa] and CND-5 [PEGylated; mPEG MW 5KDa], respectively. The placebo NPs [without DTX] were also prepared as CNE, CNE-2 and CNE-5 formulations.

The amount of DTX in NPs was estimated by validated HPLC method [17]. Briefly, the NPs were dissolved in DCM and added mixture of a ACN:water [50:50, v/v] and DCM was evaporated under nitrogen. The aliquot was analyzed by using the following experimental conditions: Hypersil C_{18} column [150 mm × 4.6 mm i.d., 5 µm]; the mobile phase, ACN:water [50:50, v/v]; flow rate, 1.0 ml/min; and a detection wavelength of 227 nm using a UV detector. The recovery efficiency factor of the extraction procedure was calculated by using known amount of DTX whereby 98.2% recovery was achieved. The percentage drug content and drug entrapment efficiency were calculated. The individual values for triplicate determinations and their mean values are reported.

The particle size and surface charges were performed using a Malvern Zetasizer 1000HS analyzer [Malvern Instruments, UK] which evaluates mean diameter, size distribution profiles, and surface charge. The percentage intensity of NPs was measured by light scattering based on laser diffraction. The NPs were immersed in aqueous medium [pH 7.4] prior to the measurements at 25°C. Quadruplicate measurements

were performed for each sample. PEG surface density of PEGylated NP formulations was also calculated and as reported by us [16].

In vitro degradation studies

The degradation profiles of placebo NPs were studied in phosphate buffer saline [PBS] at pH 5.0 and 7.4 media for a period of 6 weeks. It was characterized by the changes in the particles size, decrease in the pH value of degradation media, and generated monomers of LA and GA as well as liberated PEO units and MW changes.

Briefly, the lyophilized placebo NPs were redispersed in 10 ml of buffer solutions separately in screw capped tubes, and preserved with 0.1% (w/w) sodium azide. The sample tubes were placed in orbital shaker bath, which was maintained at 37°C and shaken continuously. At defined time intervals, the media pH and the particles size were measured and subsequently the NPs suspension was centrifuged at 35,000g, 20 min, 4°C. The supernatant was processed, each monomer (LA, GA and PEO) was separately analyzed. Furthermore, the NPs pellets were dried under vacuum and subjected to GPC for MW changes.

At defined time intervals, the incubation media were withdrawn and changes in the pH were noted by pH-meter (361, Systronics, India) equipped with a glass electrode. The particle size was also measured as described above. The generated monomers of LA and GA were quantitatively estimated by HPLC system using Hypersil C_{18} column (150 mm × 4.6 mm i.d., 5 µm); the mobile phase, 2% (v/v) methanol in phosphate buffer (0.025 M, pH 7.4) containing 0.002 M tetra butyl ammonium iodide; flow rate, 0.6 ml/min; and detection wavelength: 210 nm with UV detector. The degradation profiles of PLGA were defined as the percentage ratio of LA and GA weight present in solution, at a given time, to the initial weight of the polymer placed in incubation media. The NPs were dissolved in DCM (2 ml), and 0.05N KOH-methanol solution (3 ml) was added to the solution. The resultant was incubated at room temperature for 3 h to hydrolyze the polymer into the monomers. The solvent was removed under nitrogen at 50°C. The monomers were assayed as LA and GA by the HPLC method (18). The liberated PEO moieties from matrix were measured colorimetrically and the resulting supernatant was diluted with iodine solution and the absorbance was read at 525 nm (13). The MW and PI were determined during the incubation period. The NPs MW changes were determined by GPC as described above. All the values reported are the mean±SD of at least three different batches of each NP formulations (n=3).

In vitro drug release

In screw-capped tubes, drug loaded NP formulations were suspended in PBS (pH 5.0 and 7.4) media. The NPs suspensions were placed in an orbital shaker bath (GFL 1086 shaker, Burgwedel, Germany), which was maintained at 37°C and shaken continuously. At defined time intervals, the NPs suspension was centrifuged at 35,000g, for 20 min at 4°C after which the supernatant was withdrawn and replenished with an equal volume of fresh release medium. To amount of drug was determined as described above and the release profiles for each preparation were determined in triplicate.

Results And Discussion

Characterization PEGylated NP formulations

The mean diameter of the NPs and the PI of all samples were measured and exhibited a narrow size distribution and monodisperse unimodal pattern. Higher negative values of zeta potentials were obtained for CND formulation (-36.9±0.6 mV) due to the presence of uncapped end carboxyl groups. The values of the zeta potential were affected by the presence of mPEG chains because the coating layers shield the surface charge and move the shear plane outwards from the particle surface (19). Higher mPEG content in the copolymer leads to zeta potentials that are closer to zero (13). Similar changes in zeta potential were observed in case of drug loaded NP formulations (Table I). These results indicate that the DTX was not adsorbed on the surface of the NPs which was covered by PEG chains.

Table I: Characterization of PEGylated NP formulations

Formulations	Particle Size[a] (nm), (Polydispersity)	Drug entrapment[b] (%)	Zeta Potential (mV)[c]		δ^d PEG/nm^2
			Drug free	Drug Loaded	
CND	107 ± 0.21 (0.21 ± 0.03)	75.7 ± 0.8	-36.9 ± 0.6	-36.7 ± 0.4	-
CND-2	106 ± 0.11 (0.13 ± 0.02)	72.5 ± 0.5	-6.8 ± 0.4	-6.6 ± 0.3	0.43
CND-5	108 ± 0.18 (0.11 ± 0.02)	85.8 ± 0.4	-6.1 ± 0.3	-6.4 ± 0.7	0.61

(a) Mean diameter and Polydispersity of the NPs measured by light scattering based on laser diffraction
(b) Drug Entrapment (%) was determined by HPLC, (c) Surface charge measured in 1 mM HEPES buffer using Malvern Zetasizer, (d) PEG surface Density (δ)

***In vitro* degradation of NP formulations**

During the incubation period, changes in the particle size and variation in the media pH were monitored. In the first week, both media pH did not show considerable differences but later on medium pH was gradually decreased that it may be due to the presence of carboxylate ester in the media (12). The degradation media showed the decline in pH during 6 weeks (data not shown).

The NPs size did not show considerable difference in either media at first week, but later on both the PEGylated NPs (CNE-5 and CNE-2) in pH 7.4 media and the NPs in pH 5.0 media showed a slight variation. The slight variation in the NPs size may be due to liberated PEG chains from the matrix, which accumulated over NPs, but the integrity of the matrix is retained.

Initially, the rate of degradation was slow in both media, may be due to the hydroxide ions from the outside and hydronium ions generated by the initial matrix hydrolysis cannot diffuse in and out of the matrix freely (10). The degraded monomers and oligomers diffused out at a faster rate from pH 5.0 medium than at pH 7.4. The carboxylic terminated oligomers did not induce hydrolysis but the NPs were degraded homogeneously over time without auto catalysis, as reported earlier (11). In overall, the degradation rate of all NPs was higher at pH 5.0 than at pH 7.4 media. The yields of total LA and GA monomers generated from NPs in both media are a function of incubation time. Monomers generated over 6 weeks were 48.0±0.52% in pH 5.0 and 40.4±0.34% in pH 7.4 media of CNE-5 NPs. From CNE-2 NPs, 46.1±0.41% and 37.4±0.27% of monomers were generated in pH 5.0 and pH 7.4 media, respectively. In case of CNE NPs, monomers generated only 43.5±0.37% and 33.6±0.28% in pH 5.0 and pH 7.4 media, respectively. The total monomers of LA and GA generated from NPs from PBS (pH 7.4 and 5.0) are shown in Figure 1.

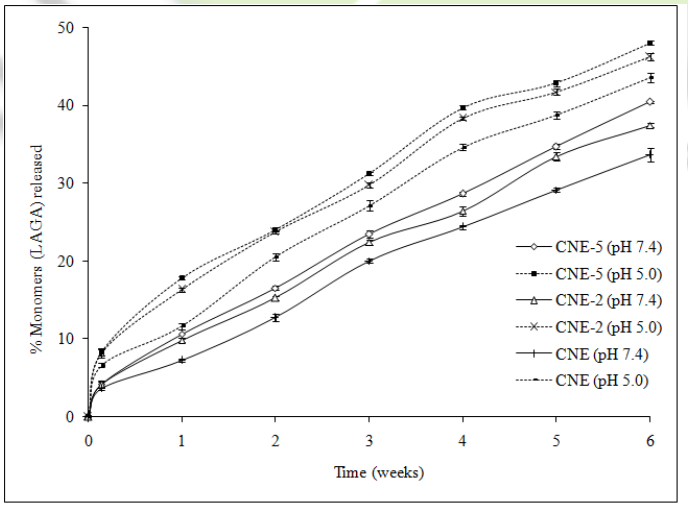

FIGURE 1: The total monomers (%) of LA and GA generated from PEGylated NPs in PBS (pH 7.4 and 5.0).

The monomers LA and GA couldn't be detected in initial days because it requires a considerable lag-time to generate monomers. From the first week on, the formation of GA was higher than LA of all the NPs in pH 5.0 medium. The monomers were generated at higher rate in high mPEG and glycolide content NPs in both PBS media. The result suggests that higher amount of monomers were generated from the NPs composed higher amount of mPEG content that is the PEO chains enhanced the NPs degradation rate. The relative amounts of LA and GA are corresponding to the percentage of monomeric units initially present in the NPs. The generated LA and GA monomers were quantified by HPLC whereas the PLGA oligomers interference was not observed and which was eluted later. The results are consistent with previous reports on similar systems (20-22) and demonstrate that the changes in lactide:glycolide ratio can be used to manipulate drug release rates.

From the first day on, the liberated PEG chains from both the media were detected due to the rapid hydrolysis of ester bonds connecting PLGA. The PEO moieties were liberated at higher rate from PEGylated NPs matrix from pH 5.0 medium and observed considerable differences between mPEG 5 and 2KDa conjugated copolymeric NPs. Initially, the higher amount of PEO was detected in CNE-5 NPs but later on gradually reduced and attained CNE-2 NPs pattern. The detachment of PEG chains from the solid matrix was not complete even after 6th week of incubation time. The liberated PEG units correspond to the percentage of PEG units initially present in the NPs. PEO monomers released over 6 weeks were 88.0±0.18% in pH 5.0 and 81.3±0.28% in pH 7.4 media of CNE-5 NPs. From CNE-2 NPs, 76.0±0.22% and 72.5±0.43% of monomers were released in pH 5.0 and 7.4 media, respectively. The amount of PEO liberated (%) from PEGylated NPs in PBS (pH 7.4 and 5.0) are shown in Figure 2.

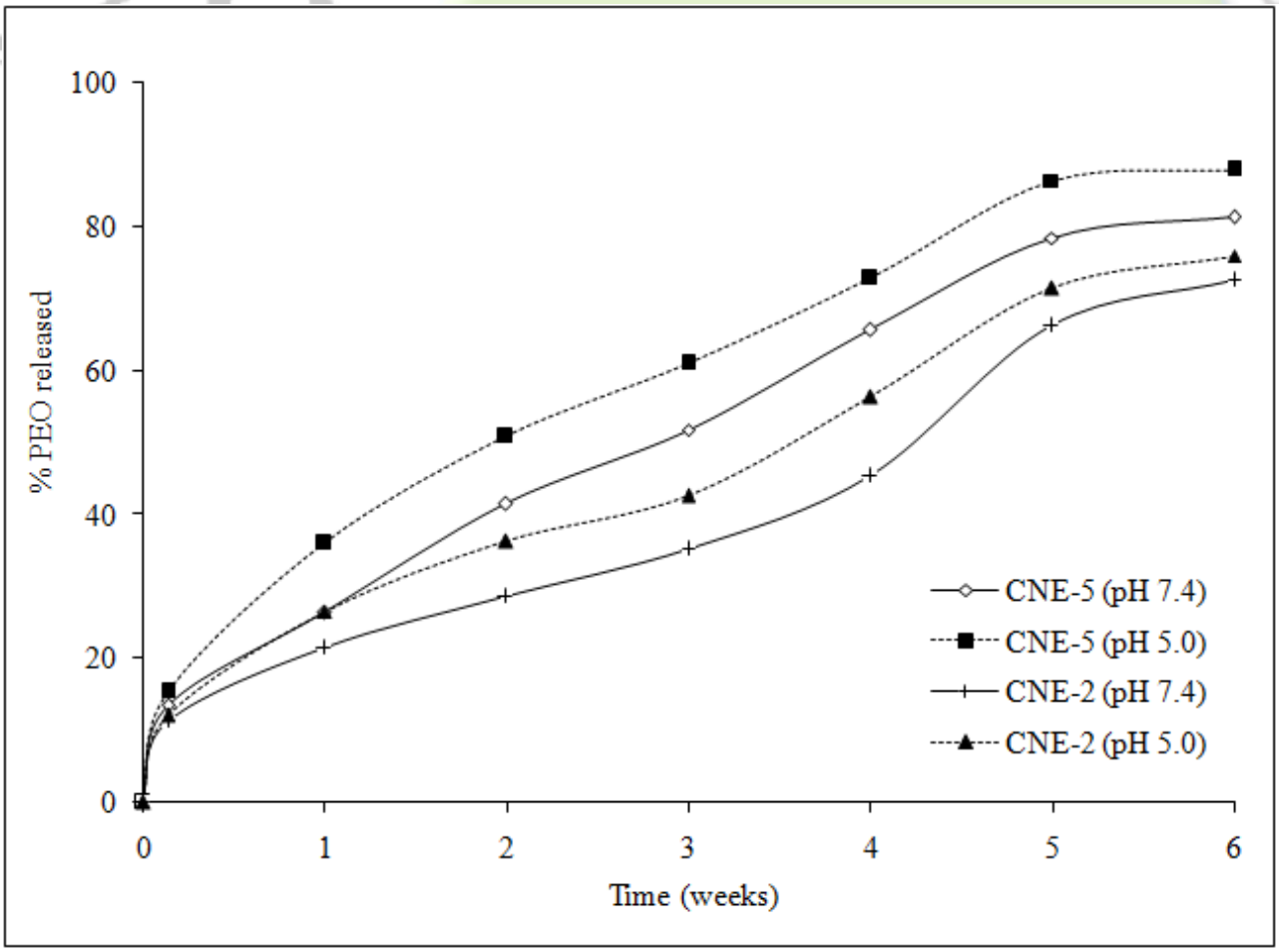

FIGURE 2: The PEO liberated (%) from PEGylated NPs in PBS (pH 7.4 and 5.0)

The degradation rate of PEGylated copolymers is higher than that of PLGA copolymers due to increased hydrophilicity. Although, the rate of *in vitro* degradation of the PEGylated NPs depended on their composition, increasing when the mPEG content of the NPs increased. The higher degradation rate of the NPs with a high mPEG content may be attributed to their increased hydrophilicity, which apparently overrides their decreased content of cleavable ester bonds. During NPs degradation, the generation of mPEG precedes faster than the generation of LA and LA-oligomers. This could be that the mPEG part of the PEGylated NPs is located on the surface and the lactic-mPEG ester bonds are easily accessible to the water surrounding the NPs, whereas the PLGA part of the copolymer is mainly located in the relatively hydrophobic core of the NPs and the LA-LA (or LA-GA) bonds are less easily accessible to the water (13). Also the degradation profiles indicated that the ester link between PLGA and PEG was more stable in pH 7.4 than 5.0 media (11). The degradation profiles suggested that all the NPs exhibited higher degradation rate at acidic environments and the generated monomers did not interfere the degradation rate, especially the PEO moieties.

Degradation of the diblock copolymers depends on several factors including, type of chemical bonds, mobility of water within the matrix, and pH of the polymer solution. Moreover, each block length, determined by the ratio change in mPEG and PLGA block segments, could affect physicochemical properties of degradation (10, 23). Thus, the degradation behavior of the NPs was studied by determining the average MW of the polymer of the NPs at different period of incubation in both PBS media at 37ºC. When the degradation of the polymers of the NPs was examined the MW was found to decrease with time. The ratio of the MW at time and the initial MW of the polymers of the NPs indicated the capability of degradation of each type of NPs.

PEGylated NPs and pH 5.0 medium containing NPs showed a largest decrease in MW and significant differences were obtained between mPEG 2 and 5KDa conjugated PEGylated NPs. At the end of 6th week incubation time, the average MW in pH 5.0 medium of CNE-5 NPs (mPEG 5KDa conjugated) showed a largest decrease of 46% than pH 7.4 medium of 37%, respectively. The average MW in pH 5.0 medium of CNE-2 NPs (mPEG 2KDa conjugated) decreased as 46% than pH 7.4 medium as 41%, respectively. In CNE NPs, the average MW in pH 5.0 medium decreased 38% than pH 7.4 medium of 33%, respectively.

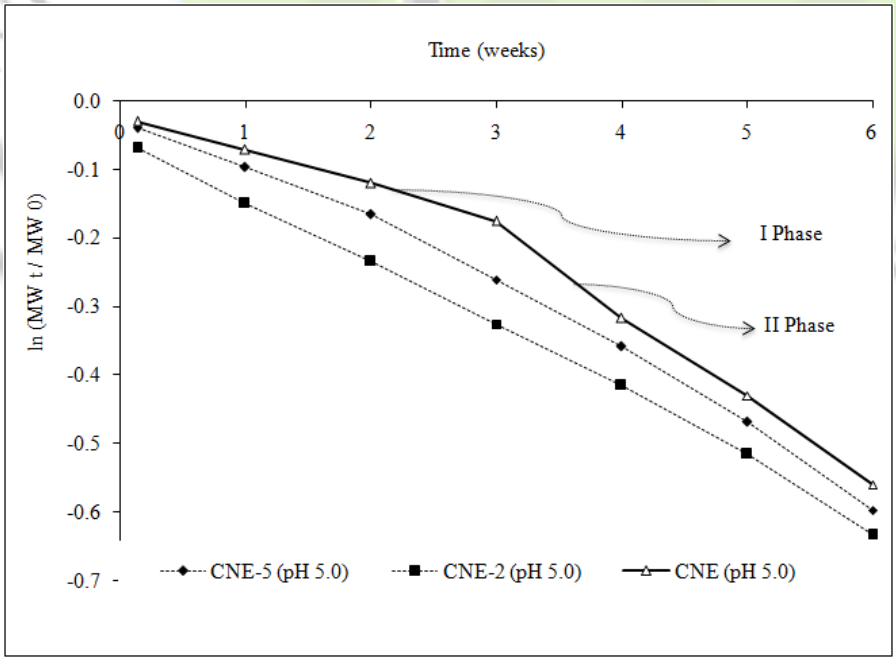

Figure 3 A

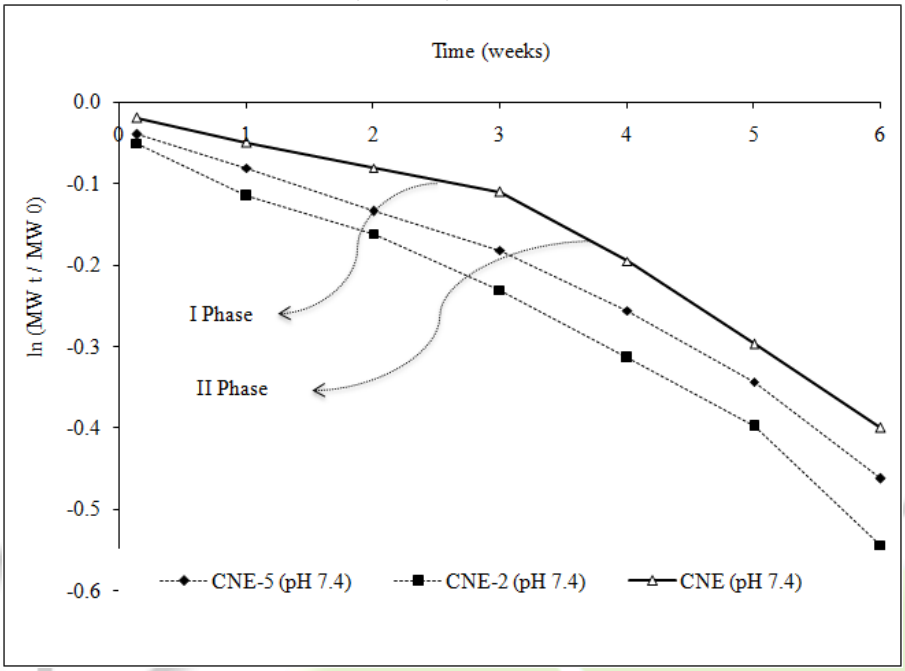

Figure 3 B

FIGURE 3: Logarithmic plots of ratio (MW^t / MW^0) versus time for PEGylated NPs and PLGA NPs incubated in PBS (A: pH 7.4; B: pH 5.0)

Figure 3 A & B shows the logarithmic plots of ratio (MW at time "t" / MW at initial time "0") versus time for PEGylated NPs (mPEG 5 and 2KDa) and PLGA NPs incubated in both media, respectively. The phases of the degradation process and their duration were shown, and the degradation rate constants (k), half-life times (**t½**) and regression coefficient (r^2) of the NP formulations (Table II) were obtained (24).

$$k = \frac{\ln (MW^t/MW^0)}{t}$$

$$t½ = \frac{\ln 2}{k}$$

Table II: Degradation rate constants (k), half-life times (t½) and regression coefficient (r^2) of PEGylated NPs at the end of 6th week incubation time

Type of NPs	k (Day^{-1})	t½	r^2	
			I phase	II phase
CNE-5 (pH 7.4)	11.0	63.0	0.9254	-
CNE-5 (pH 5.0)	14.2	48.0	0.9726	-
CNE-2 (pH 7.4)	12.9	53.7	0.9254	-
CNE-2 (pH 5.0)	15.0	45.9	0.9942	-
CNE (pH 7.4)	9.5	72.8	0.9726	0.9999
CNE (pH 5.0)	11.3	61.3	0.9956	0.9559

All the PLGA NPs exhibited two phases of degradation; the largest decrease in MW took place in the second phase of degradation, which agrees with a first order kinetic. This second phase began the week 3 of incubation of PLGA NPs, in contrast the both PEGylated NPs showed single phase linear degradation pattern (Figure 3 A & B). Even after 6 weeks of incubation period, the second phase was not detected for PEGylated NPs. However, the variation in the degradation rate constant of the first phase of the process as a function of polymeric composition of the NPs was in accordance with a first order plot, which seemed to indicate that the degradation pattern of PEGylated NPs was different from that of PLGA NP formulations.

In case of PEGylated NP formulations, the average MW was rapidly decreased during first 2 weeks due to rapid of ester bonds connecting PEG in core material and formation of oligomers. After 2 weeks, the MW of all the NPs was

reduced gradually, due to the formation of monomers LA, GA and PEO. The degradation rate was faster in acidic medium hence, the elimination half-life time of NPs shortened.

The initial slow degradation in the MW of the PLGA part can be attributed to the compact structure of the NPs matrix, leading to slower diffusion of the water. An accelerated MW loss was observed to be proportional to increasing the relative mPEG block in diblock copolymer, indicating that the incorporation of the mPEG segment as a hydrophilic part into the hydrophobic PLGA part led to faster erosion of the PLGA part due to better accessibility of water to the ester bonds of PLGA block. This result strongly indicates that the increased mPEG induced a faster incoming of water into the matrix due to swelling of the mPEG segment, resulting in the faster PLGA degradation.

***In vitro* drug release**

The drug release appeared to have two hydrolysis exponential phase. On the 1st day, the formulations components: a rapid initial burst followed by a slower an exhibited initial burst release that slowed down gradually and remained constant up to 4 weeks. The rapid initial release may be attributed to the fraction of drug content, which was close to the surface of the NPs and higher surface area-to-volume ratio. The high surface area of small size NPs enhances drug exposure to the aqueous media, resulting in a larger initial burst and enhanced NPs degradation. DTX fraction was diffused rapidly into the surrounding liquid and accounting for the rapid initial part of the release profiles (13).

DTX released over 4 weeks was 77.5±1.2% in pH 5.0 and 69.0±1.5% in pH 7.4 media of CND-5 formulation. From CND-2 formulation, 72.4±2.4% and 64.2±2.1% of DTX was released in pH 5.0 and 7.4 media, respectively. In case of CND formulation, drug released only 44.1±2.1% and 36.9±2.7% in pH 5.0 and pH 7.4 media, respectively. The amount of drug release was affected by the composition of the NPs, increasing when the mPEG content of the NPs increased. The *in vitro* release profiles of DTX-loaded NP formulations in PBS pH 7.4 and 5.0 media at 37ºC are shown in Figure 4.

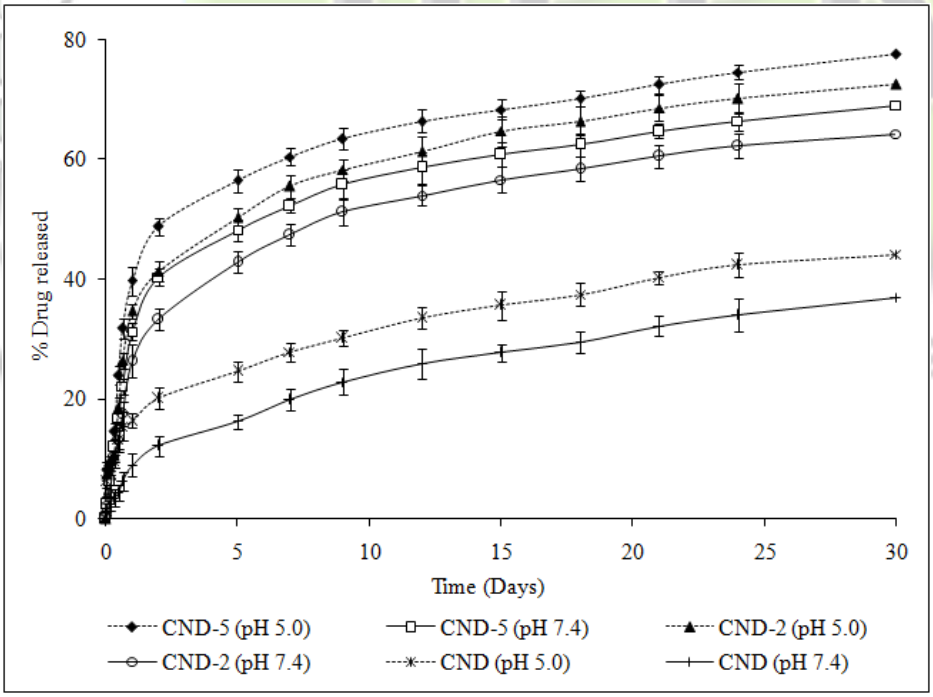

FIGURE 4: The *in vitro* release profiles of DTX-loaded PEGylated NP formulations in PBS pH 7.4 and 5.0 media at 37ºC

The drug release mechanism from PLGA-based matrices have been described by several authors (23, 25). Drug release from NPs matrix system is generally governed by both the diffusion of drugs in the matrix as well as the matrix erosion resulting from degradation and dissolution of smaller MW polymer at the surface. The mechanism indicates that the drug release may be influenced by physicochemical properties of the polymer and the drug, such as polymer MW, composition of lactide and glycolide, drug solubility, drug loading as well as NPs fabrication (20, 23). The results indicate that the release of DTX from these matrices is consistent with a diffusion mechanism; the result also confirms that the matrix integration does not change appreciably during the drug release. Generally, zero-order release of drug is desirable for a long term releasing formulation (22). However, the NPs prepared from the diblock copolymers showed a bi-phasic release behavior, i.e., an initial burst, a lag time, and a subsequent steady release. During the NPs preparation, drug can be deposited at the oil/water interface as the solvent evaporates. Enhanced drug mobility can increase surface drug disposition, resulting in a greater burst release. Also, more polymer chain mobility of low MW PLGA enhances aqueous diffusion into the NPs, thereby improving polymer permeability and degradation, which in turn increase drug release (22).

DTX from PEGylated NP formulations showed higher release rate in pH 5.0 than pH 7.4 media. The results demonstrated that PEGylated NPs showed greater release rate of DTX than PLGA NPs. The drug release rate is totally depending on degradation of NPs. At the end of 4 weeks the PEGylated NPs released DTX from CND-5 formulation was 77.5% in acidic medium, at the same time 37.8%, 10.4% and 72.9% of LA, GA and PEO monomers were generated, respectively. The total LA and GA monomers of 48.2% were generated simultaneously 77.5% DTX (hydrophobic) was released from the matrix. PEGylated NPs were degradated with elimination half-life time of 48 days. The drug release could be altered including by changing the composition of PLGA and mPEG and method of preparation. PEGylated NP's degradation pattern at the end of 4 weeks incubation time in PBS media (pH 7.4 & 5.0) (Figure 5)

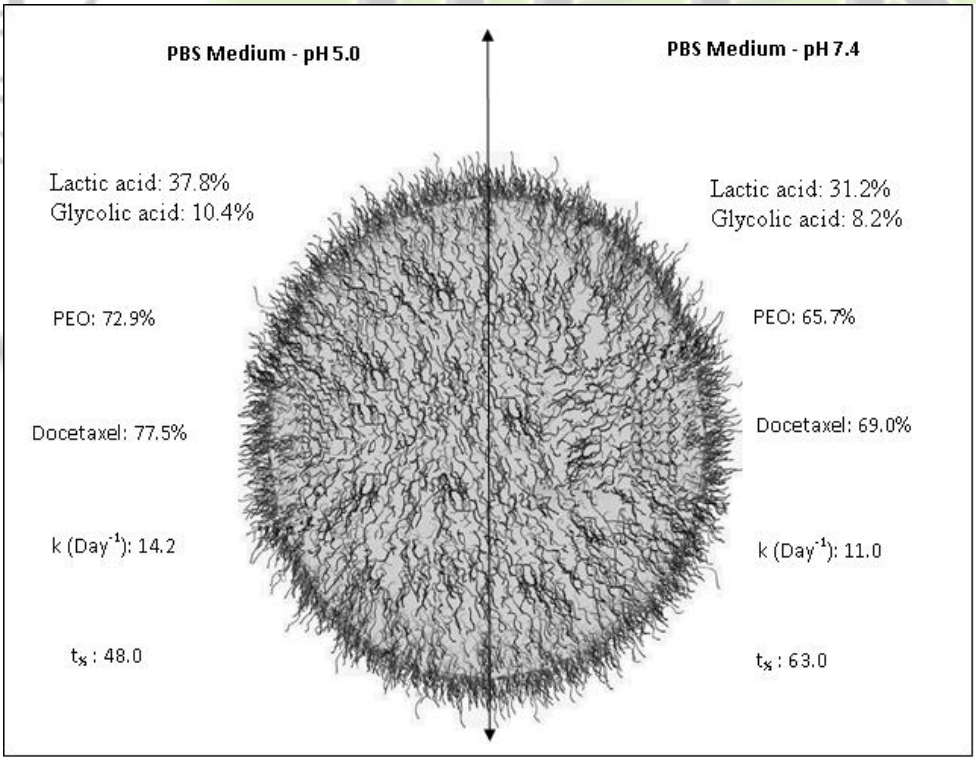

FIGURE 5: PEGylated NPs degradation pattern at the end of 4 weeks incubation time in PBS media (pH 7.4 & 5.0).

Higher the drug release could be higher mPEG content composition that may be attributed to their increased hydrophilicity, which apparently overrides their decreased content of cleavable ester bonds. This could be explained by the degradation mechanisms; the PEG chains enhance the water penetration in to the matrix and the NPs were degraded homogeneously over time. However, the release of DTX depends on several factors including the drug: polymer ratio, drug solubility inside the matrix and the interaction between the core and the drug. This interaction kept the DTX undissolved in the matrix during the release studies and also did not shield the polymer terminal carboxyl residues, resulting in lower matrix erosion. Absence of strong ionic interaction between the DTX and polymer might cause a faster diffusion of drug from the matrix and yielded crystals in the aqueous medium. DTX did not affect the matrix erosion and hence the solubility of drug in the hydrated matrix became the predominant parameter affecting drug diffusion also obtained similar results by Miyajima et al. 1998 (26).

Drug release from PEGylated NPs is biphasic (initial 'burst', followed by a zero- order drug release phase) and is predominantly controlled by drug diffusion. In PEGylated NPs, DTX is continuously released over 3 days and the release pattern revealed almost pseudo-zero-order kinetics. Gref et al. 1994 (3) reported that crystallization of hydrophobic drug occurred inside the NPs and, especially, at the higher drug loading contents, a phase separation occurs, leading to the crystallization of part of the drug in NPs. Then, hydrophobic drugs loaded into NPs release more slowly at higher drug contents, differing from hydrophilic water-soluble drugs. On the other hand, at the low drug contents, DTX might be relatively present as a molecular dispersion inside the NPs. The crystallized drug should dissolve and diffuse more slowly into the outer aqueous phase than that of molecular dispersion state.

Conclusion

PEGylated NP formulations were showed higher degradation as well as release rate in pH 5.0 medium, wherein the NPs formulation are to degrade and subsequently release the drug in the acidic tumor environments rather than systemic circulation. The degradation profiles and drug release at acidic environments showed the potential of the NPs formulation to provide a sustained drug delivery system. The study found that the drug release could be triggered within the acidic intracellular environments from biodegradable PEGylated NPs at controlled manner, anticipated a promising carrier for effective cancer chemotherapy.

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